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Integrated Fruit Protection in Fruit Crops

Use of Pheromones and other Semiochemicals in Integrated Control

Editors:

Jerry Cross and Claudio Ioriatti

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"Use of pheromones and other semiochemicals in integrated control"

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Strategies to control apple proliferation disease in Trentino and in Germany

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Abstract: Since the late 1990ies a serious epidemics of apple proliferation (AP) disease is developing in Trentino (northern Italy) threatening the whole apple production. Therefore, an interdisciplinary project has been started in 2001 to establish control strategies for AP in Trentino. The disease development in Southwest Germany was studied for comparison. In a first step, the spread of the disease in Trentino was monitored by different diagnostic methods. The incidence of the disease is high in specific regions (Val di Non, Val di Sole) and reach up to 75% for certain cultivars. Two control strategies were adopted: as a short-term solution the control of the insect vectors and as a durable long-term solution the development of plant material resistant to the pathogen, the AP phytoplasma. As a prerequisite for the establishment of efficient control strategies the population dynamics of the psyllid vectors *Cacopsylla picta* (syn. *C. costalis*) and *Cacopsylla melanoneura* were studied, the biological life cycle of these species was investigated, the infection rate of the insects with AP phytoplasma was tested by PCR and their transmission efficiency was verified in transmission trials. Although the population densities of *C. picta* and *C. melanoneura* were dramatically different between Trentino (very high) and Germany (very low), the transmission efficiency of *C. picta* was high in both regions. *C. melanoneura* individuals were found infected by PCR but an AP phytoplasma transmission could only be achieved once. Different insecticides were tested for their effect on the psyllids and different application programs were conducted. Up to now, a satisfying control of the disease spread could not be obtained by these treatments. Therefore, the development of AP-resistant rootstocks was investigated in parallel. This strategy is based on the natural resistance found in wild, apomictic *Malus* species and the observation that AP phytoplasmas are eliminated each year in the upper parts of the tree during renewal of the phloem. Breeding programs were started to introduce the resistance into rootstock material of agronomic value. To speed up the breeding, SSR analysis of the progeny was employed, molecular markers for the resistance were looked for and an *in vitro* resistance screening system was established.

Key words: phytoplasma, psyllids, transmission trials, pathogen detection, disease control, resistance

Introduction

Apple proliferation (AP) occurs in all countries of central and southern Europe but its highest incidences are in the apple growing regions of Trentino and in southwestern Germany. The disease cause important economic losses due to small sized fruits with poor taste. All actually grown cultivars and rootstocks are susceptible to the disease and no curative treatments are applicable. A specific phytoplasma, the apple proliferation phytoplasma, is associated with the disease. The non-cultured, phloem-restricted phytoplasmas are transmitted by man through grafting and latently infected planting material. However, the natural spread by psyllid vectors is most important in the orchards. Two *Cacopsylla* species, *C. picta* and *C.*

melanoneura, have been identified as AP phytoplasma vectors in northern Italy (Frisinghelli *et al.*, 2000; Tedeschi *et al.*, 2002) and in Germany (Jarausch *et al.*, 2003).

AP phytoplasmas were first detected in the sieve tubes of infected trees by electron microscopy and then by fluorescence microscopy after DAPI staining. However, direct identification of the pathogen became only be possible with the establishment of AP phytoplasma-specific PCR assays (Jarausch *et al.*, 1994) and, most recently, the development of AP phytoplasma-specific monoclonal antibodies useful in ELISA (Loi *et al.*, 2002). The molecular characterisation of AP phytoplasma isolates from different geographic origin revealed the existence of three subtypes named AT-1, AT-2 and AP (Jarausch *et al.*, 2000). These subtypes can be distinguished by PCR-RFLP analysis.

Natural resistance to AP was discovered in wild, apomictic *Malus* species, namely in *Malus sieboldii* (Kartte & Seemüller, 1991). Crossings of these wild *Malus* species with *M. domestica* were carried out in the 1950ies and 1970ies in order to obtain apomictic rootstocks for apple amenable to seed propagation. Although the obtained progeny turned out to be to vigorous for modern apple culture a certain number of genotypes remained resistant to AP disease. These genotypes are now the basis for new breeding programs.

Micorpropagation of AP phytoplasma-infected apple has been established as a means to maintain the pathogen in the laboratory (Jarausch *et al.*, 1996). Based on these *in vitro* cultures *in vitro* grafting has been shown to be a very efficient tool to inoculate healthy apple under standardised conditions (Jarausch *et al.*, 1999). This is the prerequisite for the establishment of a rapid resistance screening system *in vitro*.

AP disease has been reported in Trentino already in the early 1950ies but a serious epidemics developed only since the late 1990ies. In 2001 a project called SMAP (Scopazzi del Melo – Apple Proliferation) was started aiming to follow three control strategies: 1) the production of healthy planting material, 2) control of the insect vectors and 3) development of resistant plant material. For this, four Italian and German research institutes (Istituto Agrario San Michele all'Adige, Istituto per la Ricerca Scientifica e Tecnologica Trento, Centrum Grüne Gentechnik Neustadt/W., Biologische Bundesanstalt für Obstbau Dossenheim) work together 1) to improve the diagnostic, identification and monitoring methods in order to monitor the disease spread in Trentino and to assure healthy planting material, 2) to study the epidemiology of AP in Trentino and southwestern Germany in order to control the disease spread by the psyllid vectors and 3) to develop AP-resistant plant material in order to find a durable solution. A part of these research activities will be presented here.

Results and discussion

The monitoring by visual inspection of AP disease was done by the Plant Protection Service (Ufficio Fitosanitario Provinciale, Trento) with the help of the Centre for Technical Assistance (Centro di Assistenza Tecnica, San Michele) for the whole province of Trentino. The results showed that the disease is unevenly spread in the province with high incidence in Val di Non and Val di Sole and low incidence in Val d'Adige. Apparent symptoms became visible only in the late 1990ies but then the disease spread was rapid. E.g., in an old orchard (plantation cv. Golden Delicious in 1980) from 3% infected trees in 1999 to 43% in 2002 whereas in the same period the spread in a young plantation (cv. Gala in 1999) was from 7% infected trees in 2000 to 10% in 2002. The high percentage of infected trees one year after plantation rose the question about the sanitary status of the planting material. Different diagnostic methods such as DAPI staining, ELISA and PCR, were tested for their use in routine detection of AP phytoplasmas in nursery material. As a result AP-specific ELISA according to Loi *et al.* (2002) proved to be suitable for AP phytoplasma detection in root phloem preparations of planting material in Trentino whereas PCR with universal primers U5/U3 (Lorenz *et al.*, 1995) and AP-specific primers AP5/AP4 (Jarausch *et al.*, 1994) was preferred in Germany. In the tests conducted in 2002 and 2003, one out of 647 samples tested in Trentino was found to be infected and in Germany one out of 486 samples was tested positive. These results indicate that latent infection of planting material occur occasionally but cannot account solely for the high contamination rate observed in young plantations. The disease spread due to the insect vectors seems to be much more important.

The genetic variability of the pathogen was assessed by determining the subtypes of AP phytoplasma present in the different regions in Trentino. Interestingly, the predominant subtype in Germany and France (subtype AP) was almost not detectable in Trentino (further data in: Cainelli & Grando, this issue)

The putative AP phytoplasma vectors *C. picta* and *C. melanoneura* were constantly found in AP-infected orchards in Trentino and in Germany. The population dynamics of these psyllids was studied in the project by regular captures in different orchards. The population dynamics were rather similar between Trentino and Germany but differed from year to year due to climatic influences. Overwintering adults of *C. melanoneura* migrated from winter hosts in February/March into the orchards whereas overwintering adults of *C. picta* did not appear before March/April. After egg deposition and larval development on apple the springtime generation of *C. melanoneura* is present in May/June on apple and the springtime generation of *C. picta* can be found in June/July. After these periods both species can no longer be captured from apple. Secondary host plants of these psyllids were looked for but the obtained data are not conclusive, yet. The population densities, however, varied dramatically between different regions in Trentino as well as between Trentino and Germany. Whereas a maximum of 700 overwintering adults of *C. melanoneura* per 100 branches were found in Val d'Adige only 10 and 6 individuals per 100 branches were captured in upper Val di Non and in Germany, respectively. Contrary, the population densities of *C. picta* were higher in Val di Non (90-300 overwintering adults per 100 branches) as in Val d'Adige (2 per 100 branches). Also in Germany the population densities of *C. picta* were constantly low (maximum 3 individuals per 100 branches).

AP phytoplasmas were detected by PCR with universal and AP phytoplasma-specific primers in pools of 10 individuals of both psyllid species in Trentino. In Val di Non, 36% and 32% of the samples of overwintering adults of *C. picta* were tested positive in 2002 and 2003, respectively. In the same region and the same years 4% and 20% of the samples of overwintering adults of *C. melanoneura* were AP phytoplasma-positive. In Germany, overwintering adults of both species were tested individually by PCR. Nine per cent of *C. picta* were found to be AP phytoplasma-positive but only 0,4% of *C. melanoneura*.

Transmission trials were conducted in Trentino and in Germany in order to prove the vectoring ability of both species. In four years of trials (1999 – 2002) the springtime generation of *C. picta* transmitted constantly the AP phytoplasma to healthy apple in cages. No transmission was obtained with the overwintering adults. Contrary, in transmission trials in Germany both generations of *C. picta* transmitted highly efficient the AP phytoplasma, e.g. in 33% of the trials with overwintering adults and in 14% of the trials with the springtime generation in 2003. However, no successful transmission could be obtained with *C. melanoneura* in Germany and only one trial within four years of experimentation with *C. melanoneura* yielded a transmission of AP phytoplasma by overwintering adults in Trentino. Bait plant trials with in total 1600 healthy apple seedlings were conducted in Trentino in four different orchards in 2002. Plants were changed bi-weekly from middle of February until the end of June. Two bait plants in an orchard in Val di Non were naturally infected in the periods of middle of June and end of June. These periods are the migration periods of the springtime generation of *C. picta*. In summary, the different transmission trials clearly indicate that *C. picta* is the main vector of AP phytoplasmas in Trentino and in Germany. In Trentino, the transmission by the springtime generation seems to be very efficient. The contribution of *C. melanoneura* to the spread of AP disease remains unclear and needs further investigation.

Up to now the control strategies against the psyllid vectors are focused on the prevention of egg deposition in the orchard and thus on the reduction of the springtime generation. The application of epofenprox before blossom proved to be efficient against the overwintering adults of *C. melanoneura* and *C. picta*. However, the main migration period of *C. picta* falls in the blossom period and, thus, control of *C. picta* is not possible. New products are currently tested for the control of the springtime generation after blossom. Further trials are needed to optimise these insecticide treatments. The control of the vector transmission is regarded as a transitory, short-term solution.

A durable, long-term solution is expected from the development of AP-resistant plant material. The resistance strategy is based on the natural resistance found in wild *Malus* species. This resistance shall be introduced into apple rootstocks. Phytoplasma-infections in the susceptible cultivar are eliminated each year during the renewal of the phloem in early spring. This phenomenon could be confirmed under Trentino conditions by studying the seasonal colonisation behaviour of AP phytoplasmas in infected trees. *Malus sieboldii* and its apomictic rootstock derivatives are currently used in new breeding programs in combination with dwarfing rootstock genotypes like M9. The objective is to develop AP-resistant rootstocks of agronomic value useful in modern apple orchard management. More than 2600 seedlings have been produced in crossings made in 2001, 2002 and 2003. These seedlings were examined by microsatellite (SSR) analysis in order to define genotypes which are recombinant and not apomictic (see Bisognin & Grando, this issue). All recombinant seedlings were experimentally graft-inoculated with AP phytoplasma to test for their resistance. As this resistance screening in the field is long-lasting an *in vitro* screening system has been established. In this system parental and interesting progeny genotypes were introduced into *in vitro* culture as described by Jarausch *et al.* (1996). Each genotype was graft-inoculated *in vitro* in repetitions under standardised conditions according to Jarausch *et al.* (1999). The resistance was evaluated by the transmission rates and by determining the phytoplasma titre in the inoculated plant by quantitative PCR (Jarausch *et al.*, 2004). The resistance of the parentals could be confirmed by this system. To further facilitate the resistance screening molecular markers are currently developed in the project.

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The third edition of the IOBC Basic Documents on Integrated Production

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Abstract: The development and implementation of ecosystem-based technologies in plant protection and plant production have always been important objectives of the IOBC since its foundation in 1956. As a consequence, a joint effort of IOBC/WPRS Executive Committee and Council, an *ad hoc* Panel of Experts that represented the horizontal IOBC Working Groups, and the Commission on Integrated Production led in 1993 to the first edition of the important document "Integrated Production. Principles and Technical Guidelines". Since then, the IOBC concept on Integrated Production (IP) has been the standard followed by most regional or national IP guidelines all over the world.

We present here the third edition of the document, which has been approved in spring 2004. The main ideas and principles remain unchanged, but more emphasis is done on concepts such as the total quality of the production in the frame of a sustainable agriculture, the need of the transparency of standards, and traceability. The concerns and needs of consumers, retailers, growers and food processors for safe food have generated a number of international standards in food industry that have also been taken into account. Therefore, a number of relevant elements, derived from published and internationally applied food safety schemes and Good Agricultural Practices (GAP) criteria, have been incorporated in this 3rd edition to increase the inspection compatibility. The previous editions took as granted (and hence they were omitted) many basic elements concerning legal compliance and GAP practices.

It is intended that the IOBC standards for Integrated Production be placed in the highest position of the Food Quality Pyramid.

Key words: Integrated Production Guidelines, total quality, food quality pyramid

Introduction

The concept and principles of Integrated Production as stated by IOBC/WPRS in the first edition of its document "Integrated Production. Principles and Technical Guidelines" (1993) are now widely accepted all over the world. At the same time, several international standards in food industry have been generated in recent years as an answer to the concerns and needs of consumers, retailers, growers and food processors for safe food of good quality. In consequence, they have been taken into account in the 3rd edition of IOBC/WPRS standards. The 3rd edition was prepared by members of the Executive Committee and the IP-Commission and has been recently published (Boller *et al.*, 2004). We focused in this paper in the most new aspects taken into account.

Standards in food industry are either defined by legal national or international governmental regulations or by voluntary agreements reached within the private sector. Most of them focus on product quality and on the food safety aspects. They implement, through their inspection schemes, a multitude of inspection criteria that address the safety of the fresh produce. Other standards expand the safety focus by agronomic components and define their specific interpretation of Good Agricultural Practice (GAP). A limited number of recent standards add items related to environment, animal welfare or fair trade and move into higher quality categories.

IOBC concepts and guidelines established since the early 1990s define the general and crop specific criteria of advanced sustainable production systems. Up to now these documents took as granted (and were hence omitting) many basic elements concerning legal compliance and Good Agricultural Practice. Nor did they elaborate in detail the specific requirements addressing external and internal product quality including food safety aspects.

Increasing international awareness about the transparency of standards, traceability, competitive benchmarking, certified inspection procedures etc. is also increasing the pressure on the farmer to comply with the prevailing technical standards on the market. IOBC has taken this development into account during the preparation of the 3rd edition of its normative documents. The "compatibilisation" of IOBC standards with prevailing market standards is not intended to erode and to lower the traditionally high IOBC standard by stating the obvious, but to assist the grower to minimise redundant and/or contradicting inspection activities on the farm. Therefore, a number of relevant elements derived from published and internationally applied food safety schemes and GAP criteria to increase this inspection compatibility have been incorporated in the 3rd edition.

Basic and relevant elements of GAP-standards and of food safety management procedures that are identified in internationally accepted standards, must also be taken into account in IOBC endorsed IP guidelines and must be listed in the respective inspection protocols (= checklists). The IOBC Technical Guideline II and the crop specific IOBC Technical Guidelines III do not and cannot mention all published "must" rules of Good Agricultural Practice and of Food Safety Management but will present selected requirements that seem to be of special relevance to Total Quality. It is strongly recommended that IOBC endorsed organisations discuss with their members and assist in the implementation of HACCP (Hazard Analysis Critical Control Points, as defined by the Codex Alimentarius).

Total Quality in Sustainable Production

Interpretation of the meaning of food quality has seen major changes due to food scandals and major protests within the consumer community. Perfect external food quality, acceptable taste, (internal food quality) and cheap price seem to be no longer the dominant yardsticks applied by the increasing number of critical consumers. The partial definition of food quality is replaced by a notion of total quality that reaches beyond conventional food quality aspects. Quality aspects become more and more linked to food safety and to an "added value" basket of indirect and invisible food quality criteria vaguely described as "healthy environment", "animal welfare" and "fair trade".

IOBC has always tried to maintain a holistic view of its activities and has perceived food quality always in a larger context, considering as well 4 additional but for the consumers largely invisible quality traits of products, production and/or processing procedures, and working conditions that provide the essential components of the overall quality of food and fibre:

- **Internal Product Quality** *(chemical, physical, organoleptic).*
- **Ecological Quality** *of production and processing.*
- **Ethical Quality** *of production, processing and conduct of people involved.*
- **Socio-economic Quality** *of production, processing and working conditions of people involved.*

The relationships among the consumer's food quality criteria and the components of the Total Quality are shown in Figure 1.

The product quality covers the internal and external traits of the product, including food safety.

The ethical quality addresses the ethical attitude with respect to the production procedures.

The ecological quality covers in a more precise form the general notion of environment protection and animal welfare.

The social quality addresses compliance with the basic rights, health and welfare of workers.

A pyramid whose height represents the total quality of the food obtained and whose width, the percentage of the food obtained that belongs to this particular quality level can be used to represent the concept of total quality (Food Total Quality Pyramid).

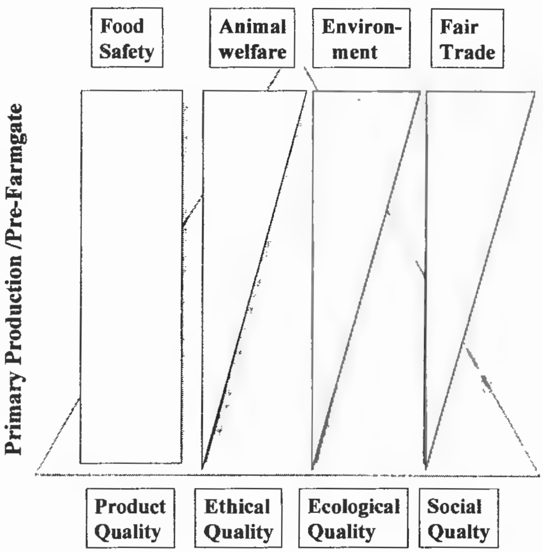

Figure 1. The relationships among the consumer's food quality criteria and the components of the Total Quality

The Food Total Quality Pyramid can be divided into different layers (Figure 2):

- "Low price" food at the bottom.
- Standard food or with "certified labels".
- "Premium Label" food with highest total quality at the top.

Figure 2 also shows the position of three international standards into the pyramid: the organic agriculture standards, that have a long tradition an a high reputation; the IOBC standards, that also look back to a long tradition now, and the more recent and very extended Level 3 of the International Farm Assurance Scheme (IFA) of Eurep-Gap.

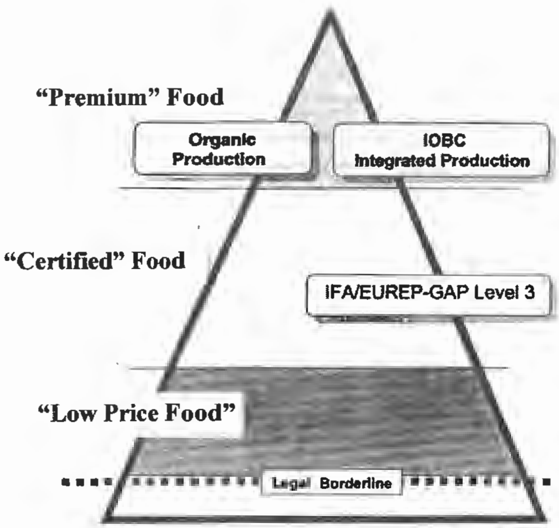

Figure 2. The Food Total Quality Pyramid and the position of three international standards

The IOBC standards are defined by the basic document "Integrated Production: Principles and Technical Guidelines", by the crop specific IP-guidelines and by the "Admission Criteria for Organisations seeking IOBC Endorsement". Whereas the "Admission Criteria" are positioned close to the interface of the "Label" and Premium-Label segment and function as first general entry scenario, a detailed analysis by crop specific evaluation tools will decide on the successful endorsement of the candidate organisation. During a 3-year experimental period the IOBC endorsed organisation has the opportunity to improve weak points of the program and eventually position the overall quality of its label in the Premium-Label segment.

With this clear policy established in the early 1990s IOBC has resisted in the past all attempts to lower its standards. IOBC will pursue this policy in the future to assist motivated farmers to develop their sustainable farming systems based on advanced technical knowledge. A high total quality standard must also be maintained to justify and to achieve added value generated at the farm level.

Green lists are more than pesticide lists

"Green", "yellow" and "red" lists of agrochemicals have a long tradition and became common tools in Integrated Production programs. However, the common practice to apply green and yellow categories exclusively to direct plant protection measures opens the door for misunderstandings. One is the wrong belief that the plant protection products and control procedures included in the green lists are *per se* the preferred plant protection program.

Taking into account the IP principles, the contents and functions of the green list must be broader. The green list of plant protection measures must be established annually for a given cropping system and for a defined geographic area with a comparable plant protection situation. It must be a technical document that covers all crop specific aspects necessary to plan and implement Integrated Plant Protection at the farm level, namely:

- the list of key pests, diseases; weeds and physiological disorders;
- the 2 most important antagonists;
- the list of preventive measures;
- the monitoring tools and economic thresholds;
- the list of indirect plant protection methods;
- the list of highly selective direct control measures (physical, biological, biotechnical, chemical) with no negative impact on human health, non-target organisms and environment.

A complementary "yellow list" should contain a critically selected group of plant protection products that do not qualify for the "green list" but should be available to the grower despite certain negative aspects. Reasons to consider the use of such products are aspects of resistance management or exceptionally difficult cases.

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Controlled Integrated Production of Fruit – A Comparison of Production Guidelines and Checking Procedures in Europe

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Abstract: The study evaluates and compares IP (Integrated Production) guidelines and related production systems in Spain, Italy, the Netherlands, France and Germany. Based on IOBC's endorsement system, a scheme was developed for the evaluation of IP guidelines for fruit and vegetables. Through data of exhibitors at the "fruit logistica 2003" and advertisements at the weekly journal "Fruchthandel", 20 to 30 labels could be identified for each country. The studies on the countries concerned show a dynamic development in Spain, federal structures in Italy, meeting the needs of international trade in the Netherlands, sectoral solutions in France and a phase of new orientation in Germany. The crop specific requirements show a significant reduction of the IP requirements from Integrated Production over Good Agricultural Practice to Quality Management.

Key words: Integrated Production, Good Agricultural Practice, Quality Management, fruit, guidelines, Spain, Italy, France, the Netherlands, Germany

Introduction

The European fruit and vegetables sector experienced profound changes in the past decades. Producers had to meet the challenges of a single European market, of global competition and of a strong concentration process of retailers. Integrated Production (IP) was introduced and product certification systems gained importance. IP and Quality Management (QM) Systems are communicated to retailers and consumers via certificates, labels, brands, quality signs etc.

The objective of this study was the evaluation and comparison of IP guidelines and organisations in Spain, Italy, the Netherlands and France. These countries are the four most important European nations exporting fruit and vegetables to Germany. About three quarters of all fruit (excluding citrus) and vegetables imported to Germany come from these countries (ZMP, 2002, ZMP, 2002a).

Material and method

Based on IOBC's endorsement system (IOBC, 2003, IOBC, 2003a), a scheme was developed for the evaluation of IP guidelines in the area of fruit and vegetables. The evaluation scheme has two parts.

Part one consists of 31 questions. It analyses general requirements and is concerned with the following three aspects: 1. IP organisation, 2. Inspection system and exclusion and 3. Ecological and agricultural requirements. The questions are answered on a simple yes or no basis.

Part two of the evaluation scheme asks 72 questions, concerning crop specific guidelines. The questions are structured into 13 topics, such as soil management, irrigation, integrated pest management etc. The evaluation is calculated and expressed in points (minus and plus). However, for 40 essential aspects of IP, non-compliance is not assessed by negative points but rather marked as an unacceptable point.

The analysis started with basic data collection by using data of exhibitors at the "fruit logistica 2003" as well as articles and advertisements in the weekly journal "Fruchthandel Magazin". A follow-up questionnaire collected information about the guidelines used in the respective countries.

In a second step, IP guidelines and QM systems for fruit and vegetables were evaluated. The investigation for fruit included 12 standards from Spain, Italy, the Netherlands, France and Germany.

Results and Discussion

Identification of labels

For each country, 20 to 30 IP and QM labels were identified. In total, 131 organisations were asked by a questionnaire about production guidelines and labels used. Answers were received from 37 (28%) organisations. For the evaluation, 12 guidelines for fruit and 11 guidelines for vegetables were chosen (Table 1).

Table 1. Contacted organisations and number of evaluated guidelines

Country	Organisations contacted	Organisations answered	Evaluated guidelines	
			Fruit	vegetable
Spain	40	8	3	2
Italy	28	7	2	2
The Netherlands	28	11	3	3
France	35	11	2	2
Germany	-	-	2	2
Total	131	37	12	11

Situation in the respective countries

Dynamic national development in Spain: Spain has the largest production of fruit and vegetables in Europe. The sector plays an important role in Spain's agriculture and is oriented towards international markets. Germany is the most important recipient of Spanish produce. IP is not yet widely practised but shows a fast growth in the past years, especially for export. The system EurepGap is also gaining in importance. In 2002, a new national law on IP (Real Decreto 1201/2002) was passed. It sets a minimum standard for the notion "*producción integrada*" which is also binding for existing and future regional regulations and private IP guidelines. The government publishes detailed guidelines for each crop.

Federal structures in Italy: In Italy, the fruit and vegetables sector is important and export oriented. About 50% of the national production is exported to Germany. Several provinces, such as South Tyrol or Emilia-Romagna, have regional IP laws. The specific guidelines are published by producer organisations (e.g. South Tyrol) or by the regional agricultural development service (e.g. Emilia-Romagna). In these areas, IP is widely practised. Only sparse information was available for Central and Southern Italy. The Italian retailers co-op Italia and Conad support Italian IP production through their IP brands "*Prodotti con Amore*" and "*Percorso Qualità*". Thus, IP is important for the domestic market and for export.

Importance of international trade in the Netherlands: The situation in the Netherlands is strongly influenced by the needs of international trade. The auctions (commercial enterprises) work according to internal quality management systems. These systems are rather flexible. New requirements of clients can be quickly incorporated in the QM systems. The international norm EurepGap is generally applied. IP as a concept is of no significance, the System "*Milieu Bewuste Teelt*" was abolished in 2001. Stifting Milieukeur started an own program for the sign "*Milieukeur*", which aims at reducing negative impacts to the environment. The guidelines were developed in a broad discussion with the government, scientists and relevant groups of society.

Sectoral solutions in France: France's diversified production of fruit and vegetables satisfies primarily the domestic market. Important for export are selected products such as apples, cauliflower or endives. Regarding IP, sectoral solutions can be observed. For example the national product branch for apples, *Section Nationale Pomme*, issued a national charter for IP. The label PFI "*Production Fruitière Intégrée – Working with Nature*" is clearly designed to reach customers outside of France. Charters of other product branches do not communicate IP through their labels. In 2002, the French government issued a new law on *Agriculture Raisonnée* which aims at replacing IP, apart from other objectives.

Phase of new orientation in Germany: In Europe, Germany imports the largest quantities of fruit. The IP of fruit in Germany is organised by regional organisations, each with its separate guidelines and respective labels. Retailers ask more and more for one general label. As an answer, producer organisations developed the so called QS label (*Qualität und Sicherheit*), which is from its structure and requirements a QM system. Now, a discussion started if the QS label is to be understood as the further development of IP or if the current IP schemes having more sophisticated requirements than QS should continue. EurepGap gains importance for retailers and producers in Germany.

Evaluation of guidelines

In total, 12 fruit guidelines were evaluated. The guidelines come from different production systems such as IP, Good Agricultural Practice (GAP) and QM (Table 2).

In the evaluation of the general requirements, the difference between all guidelines reaches from 55 to 77% of fulfilment. No guideline showed high scores of fulfilment in all three aspects. IP guidelines were strong in the part "ecological and agriculture aspects", GAP and QM systems received good results in the part "inspection systems and exclusion".

Looking at the crop specific requirements, the total of points and unacceptable points shows a clear decline of the IP requirements from IP over GAP to QM. Most visible is this pattern in the percentage of unacceptable points, which represent a minimum standard of IP requirements. These aspects are not necessarily totally neglected in GAP and QM guidelines but are very often mentioned as a recommendation and not as a requirement.

The variance between the strongest and weakest IP guidelines is quite high. This means that the notion IP as such has in different countries little meaning in itself. This could, however, be improved by creating an obligatory European IP standard, similar to (EEC) No 2092/91 for organic farming or a national solution like in Spain, which requires an obligatory minimum standard.

GAP and QM can supplement IP but not replace it. In the marketing efforts, GAP and especially QM will continue to gain importance. IP will keep its importance in extension services.

Table 2. Evaluated guidelines by country, region and production system

Country Organisation	General requirements, fulfilled (%)	Crop specific requirements, points (%)	Crop specific requirements, unacc. points (%)	Production system
Spain				
Region Murcia	58	36	28	IP
UNE Norm	77	49	38	GAP
Naturane	58	31	45	IP
Italy				
South Tyrol	77	58	8	IP
Emilia-Romagna	65	53	38	IP
The Netherlands				
The Greenery	55	18	63	QM
The Greenery EurepGap	68	24	58	GAP
Stifting Milieukeur	58	32	45	GAP
France				
Agriculture Raisonnée	55	23	45	GAP
PFI	77	39	35	IP
Germany				
Rhineland-Palainate	71	42	28	IP
QS-Label	58	14	68	QM
Range from - to				
IP-System	58 – 77	31 – 58	8 – 45	
GAP-System	55 – 77	23 – 49	38 – 58	
QM-System	55 – 58	14 – 18	63 – 68	

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A history and achievements of IOBC subgroup - Soft Fruits

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Abstract: Soft fruits crops are a highly diverse branch of fruit production with multifarious problems, perhaps more so than pome and stone fruit crops. A first overview on the most striking pests and diseases, availability of the control measures, and necessity of some research activities concerning soft fruit crops was given by Cravedi and Jörg in 1995 at Cedzyna (Poland) during IOBC International Conference on Integrated Fruit Production. At that time an idea of an elaboration of IP soft fruit guidelines was born as well. A realization of this project was begun at Vienna (Austria) in 1996. Then, a few experts defined a baseline for IP of the various soft fruit crops. One year later, again in Vienna, the "First Workshop on Problems in Integrated Production of Soft Fruits and on Guidelines for Integrated Production of Soft Fruits" was organized. As a result of this meeting a sub group "Soft Fruits", as a branch of the IOBC/WPRS working group" Integrated Plant Protection in Fruit Crops" was activated. Moreover, a draft version of technical guidelines for Integrated Soft Fruit Production was elaborated. These guidelines were published in IOBC/wprs Bulletin Vol. 23(5)2000 and completed the IOBC series defining Integrated Production for all fruit crops.

After the workshop in Vienna, three others were organized at two year interval, successively in Warsaw (Poland) in 1999, in Dundee (Scotland) in 2001, and in Conthey (Switzerland) in 2003.

In 2001 a survey of the status of Integrated Production of soft fruits in several European countries was also carried out. It proved that IP or quality assurance schemes were operated in most of the investigated countries. Unfortunately, the total production area of these crops included under IP rules was rather small. None of elaborated national (or regional) guidelines was fully in compliance with IOBC/WPRS IP - Guidelines for soft fruits. Differences in requirements for soil management, plant nutrition as well as crop protection and control procedures were noticed. Among the main problems of Integrated Production of soft fruits, the control measures for pests and diseases, a lack of resistant/tolerant cultivars, poor pesticide availability and unsatisfactory co-operation between institutions or organizations involved in soft fruit production were most important.

Key words: soft fruits, integrated production

Introduction

Soft fruit production constitutes an important branch of horticulture in many regions of Europe. According to a FAO database (faostat.fao.org), strawberries cover approximately an area of 133 thousand hectares and production in 2003 reached 1 million 15 thousand tons (table 1). The data classifies strawberry as the most important soft fruit in Europe. Poland is the European leader in strawberry production. However, Italy, France and Spain are also significant producers. Among soft fruit crops, currants, especially black currant are also important. Blackcurrant production amounted to nearly 700 thousand tons and was gained mainly from Poland, Russian Federation, Germany and the UK. Considering the area of cropping and production quantity, the next position is occupied by raspberry and other cane fruit crops. They are grown on a large scale in France, Serbia and Montenegro, as well as in Poland, Bulgaria or Hungary. Another important soft fruit crop, covering an area of 28 thousand hectares, is gooseberry. The biggest production of this fruit is in Poland, Germany and the Russian Federation. Examples of other soft fruit crops grown in Europe are elderberry, blueberry and kiwi. Among these, blueberry especially is being grown on an increasing scale.

Although soft fruits are a highly important branch of fruit production, development of their cultivation throughout Europe presents many difficulties, which are also directly connected with integrated production of these fruits.

Table 1. The area of production of the main soft fruit crops in Europe (FAO database, 2003)

Crop	Area (ha)	Production (ton)
Strawberry	133 234	1 152 724
Currants	122 124	679 074
Raspberry	68 932	315 060
Gooseberry	28 064	172 543
Blueberry	7 180	33 500

A state of integrated production of soft fruits in Europe

A first overview of the most striking pests and diseases, availability of control measures, and necessity of some research activities concerning soft fruit crops was given by Cravedi and Jörg in 1995 at Cedzyna (Poland) during the IOBC International Conference on Integrated Fruit Production. However some promising aspects were presented there. Special attention was paid to the many failings and gaps on this field. Several years later, a survey of progress in developing and implementation of IP for soft fruit in Europe was carried out by Gajek and Joerg (2003). In comparison with the previous survey mentioned above, only very limited development of integrated production was ascertained. With regard to particular soft fruit crops, the results of the survey showed the most advanced process of implementation of IP rules in strawberry growing, particularly in such countries as UK (APS – Assure Produce Scheme), Spain, Germany Sweden, Poland, Denmark and Italy. The extent of IP rules for other soft fruit crops was reported to be significantly smaller in comparison with strawberry. There were many reasons why the IP schemes for soft fruit crops had not been widely applied in Europe.

First of all, a big gap in the availability of plant protection products suitable for IP was noticed in many countries. In general, the number of products registered for soft fruit crop protection was especially low Germany, Denmark and Italy. There was a much better situation in Switzerland, Poland, Hungary and UK. However, some faults in these countries were recorded as well. The worst cases of this problem were noticed in the case of minor soft fruit crops such as blueberry, blackberry or elderberry. Many pests, diseases or weeds in these crops could not be controlled, because of a lack of registered products. Considering present activities in the frame of EU Act 91/414 (Plant Protection Products Directive), further restrictions of this field can be expected.

The use of plant protection products immediately involves thresholds or forecasting models, which should be common tools of IP practice. An investigation showed that many failings of this aspect have to be improved. A few monitoring or forecasting models, related mainly to strawberry growing, were elaborated and introduced into the practice. A possibility of reduction of chemical use through biological or biotechnical control measures seemed to be rather poor. The exploitation of predatory mites was the most frequently recorded example. Among other control measures, pheromones, sticky traps, entomopatogenic bacteria or *Tagetes* species were applied.

Another obstacle was the small range of soft fruit cultivars resistant or tolerant to pests/diseases. In case of soft fruit pests, the only examples were blackcurrant varieties resistant to the gall mite and raspberry varieties resistant to cane midges or aphids. Among fungal diseases, strawberry and raspberry cultivars resistant to *Phytophthora*, strawberry cultivars resistant to *Botrytis* and *Sphaerotheca* and also gooseberry and currants cultivars resistant to *Sphaerotheca mors - uvae* were found to be recommended.

In spite of problems related to plant protection, other obstacles was also recorded. Among them, the structure of farms producing soft fruits was mentioned. There are many regions of Europe where small or very small soft fruit plantations compose the main area of their growing. Therefore, adequate control and inspection procedures are hindered there.

The Guidelines for Integrated Production of Soft Fruits

The first guidelines for integrated production of soft fruits were elaborated and acted by different farmer' organizations or associations just at the beginning of nineties. An activation of other country/region IP rules for soft fruits have been and still are observed. Unfortunately, all of them more or less differ from each other. The most frequent differences related to IPM chemicals, soil management, plant nutrition or control procedures. Considering many aspects of diversity in the field of soft fruit production, an elaboration of European IP standards for these fruits seemed to be a particularly problematic challenge. On the other hand, performance of this task was the only chance to harmonize integrated production of soft fruits throughout Europe. An valuable experience as well as the basis for this work were IOBC principles of Integrated Production (Guidelines I and II), published in IOBC/WPRS Bulletin Vol. 22, 1999. From 1995, when the idea of the elaboration of such guidelines was born, up to 2000, when these guidelines were published (IOBC wprs Bulletin Vol. 23 (5) 2000), many steps and obstacles had to be overcome (Joerg & Cross 2000). One of the most important points discussed were the problem of genetically modified organisms (GMOs). The status of these organisms in agricultural production is questioned, because their direct or indirect influence on human health has not been completely exploited and accepted by the public so far. However, in some cases GMOs could reduce significantly the input of agrochemicals thanks to their modified metabolism or resistance mechanisms to some noxious agents. Considering this aspect, the IOBC - Council has taken a specific decision which permits to exploit GMOs in integrated production.

Another debatable problem concerned high-input systems such as heated glasshouses or non-soil systems, which are quite commonly applied for growing of some soft fruit crops. As integrated production is closely linked to sustainable agriculture, it was necessary to specify whether these systems fulfill requirements of sustainability. Taking into consideration all the pros and cons, the IOBC – Technical Guidelines III have been finally restricted only for soft fruit crops grown in the soil in the open or under non-heated protection. An assessment of different production systems of soft fruits under their compliance with IP principles have to be verified again in the near future.

Activities

The official beginning of the activities of the IOBC subgroup - Soft Fruits was in October 1997, when the "First Workshop on Problems in Integrated Production of Soft Fruits and on Guidelines for Integrated Production of Soft Fruits" was organized in Vienna (Austria). However, some discussions and initiatives were begun in 1995, at the International Conference on Integrated Fruit production in Cedzyna (Poland). An important event preceding the activation of this branch was also the meeting of a few experts, who met in Vienna in 1996 to discuss the main problems in IP of soft fruits.

After the workshop in Vienna (1997), three others were organized at two year intervals, successively in Warsaw (Poland) in 1999, in Dundee (Scotland) in 2001, and in Conthey (Switzerland) in 2003. All were recognized as successful meetings providing many valuable research achievements, useful for the development of IP soft fruits. Proceedings of particular workshops were published as IOBC/wprs Bulletins: Vol. 21(10) 1998, Vol. 23 (11) 2000, Vol. 26 (2) 2003, Vol. 27 (4) 2004.

Among the results of research presented, most were devoted to strawberry, cane fruits (raspberry and blackberry) and currants (table 2). Much less were concerned with other crops, like gooseberry, elderberry or blueberry. An explanation of this situation is certainly the importance and the growing area of particular soft fruit crops.

Table 2. Presentations on soft fruits crops given at the workshops of IOBC subgroup - Soft Fruits (1997-2003)

Workshop	Total no. of pre-tions (*)	Straw-berry	Cane fruits	Currants	Other crops	Other topics
Vienna (A) 1997	19	8	2	2	1	7
Warsaw (PL) 1999	33	11	2	9	1	10
Dundee (UK) 2001	34	11	14	4	1	7
Conthey (CH) 2003	31	13	5	4	4	5
Total	*117*	*43*	*23*	*19*	*7*	*31*

* The total number of presentations is not compatible with a sum of numbers mentioned in particular rows because some papers referred to more than one crop.

The main topics of presented papers, besides 'general overviews', referred mostly to general problems of pest or fungal disease control. It seemed especially promising that a similar share related to the biological and biotechnical control measures. This goal of the research focused primarily on the exploitation of predatory mites for biological control, however the problems of pheromone and sticky traps for pest monitoring, biological control of root nematodes by growing *Tagetes* species or the control of the soft fruit leafrollers (*Tortricidae*) by using entomopathogenic bacteria - *Bacillus thuringensis*, were also reported here. Some reports were also given on different problems of pesticide application or resistance breeding programs. Unfortunately, an highly important area of research on the thresholds has been missed.

Summarizing hitherto achievements of IOBC subgroup - Soft Fruits, the necessity of its further activities must be noticed. A development of integrated production of soft fruit in Europe can be possible only through extensive, international cooperation. This is a great challenge for both, scientists and extension workers for the future.

Table 3. The main topics of presentations given at the workshops of IOBC subgroup - Soft Fruits (1997-2003)

Topic	Vienna (A) 1997	Warsaw (PL) 1999	Dundee (UK) 2001	Conthey (CH) 2003	Total
▪ General overviews	12	6	4	1	23
▪ General problems of arthropod pest control	3	2	7	8	20
▪ General problems of fungal disease control	1	4	6	8	19
▪ Biological and biotechnical control measures	1	3	9	7	20
▪ Pesticides (availability, effectiveness, residues, application techniques)	2	7	2	2	13
▪ Resistance/tolerance of soft fruit cultivars	0	5	4	1	10
▪ Others	0	4	1	2	7

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Development of an integrated pest and disease management system for apples to produce fruit free from pesticide residues

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Abstract: In a large plot replicated orchard experiment, over three seasons, the pest and disease control achieved in managed plots, based on a zero pesticide residue management system was compared with that in plots sprayed conventionally or left untreated. The zero pesticide residue management system (MS /MR) is based on the use of conventional pesticides (excluding organophosphorus insecticides) up to petalfall and after harvest, but using biocontrol for dealing with pests and sulphur or cultural methods to control powdery mildew and storage rots between petalfall and harvest. Dormant season control of scab and powdery mildew is a key component of the system. Over the three seasons the control of scab, powdery mildew and storage rots achieved by the MS /MR system was as good as or better than that in the conventionally treated plots and at lower cost. Pest control in the system was satisfactory but more expensive than the conventional system because of the increased cost of selective insecticides compared to the broad spectrum products used in the conventional system. No residues of pesticides applied to the MS /MR plots were detected in harvested fruit.

Key words: Apple scab, powdery mildew, storage rots, rosy apple aphid, tortrix, codling moth, fungicide, insecticide.

Introduction

UK consumers expect perfect apples, but in recent years this requirement has extended to perfect apples that contain no pesticide residues. The main UK apple varieties Cox, Gala, Jonagold and Bramley are susceptible to all the major pests and diseases and the UK climate ensures that one or other of these problems are significant in most seasons. So to satisfy this consumer requirement is a very high expectation. Most apples in the UK are produced using integrated pest and disease management, and this ensures that pesticide use is targeted and therefore minimised so that residues, if present in the harvested crop, are well below the Maximum Residue Limit (MRL) permitted. However, consumers no longer trust science and to them the scientific facts of MRLs are irrelevant and the presence of any pesticide residue in apples is unacceptable. The challenge is therefore to develop crop protection systems that satisfy the consumer, but that are also profitable and sustainable for the grower. Other researchers have tackled the problem of the high pesticide input into apple crops (Wilcox et al, 1992; Jones et al, 1993). These studies concentrated on disease control only and their approach has been to focus on reduced fungicide inputs in the growing season. The approach tackled here is to develop production methods for pest and disease control where use of conventional pesticides is restricted to pre petalfall and after harvest to minimise overwintering inoculum. Cultural, biological and other non chemical methods could then be used to control pests and diseases during the apple development period in summer. Such an approach may be achievable since many important pests and diseases on apple such as scab or caterpillars could be controlled during the dormant season and/or pre bloom. There are however, certain pests and diseases such as powdery mildew, storage rots and rosy apple aphid that are mainly active post bloom and non chemical methods of control would need to be developed to effectively control these problems in such a residue free system. This project was initiated to determine whether this approach to residue-free production was feasible and to identify which pests and diseases were likely to be difficult to control.

Material and methods

Experimental plan

The trial was set up at East Malling where use could be made of existing established plots (planted in 1995) of disease susceptible apples (Cox, Gala, Fiesta) and scab resistant apples (Saturn, Ahra,). The variety Discovery was common to both sets of plots. Each plot consisted of 144 trees on M9 rootstock and was separated from adjacent plots by alder windbreaks. In these plots the pest and disease control achieved following a routine conventional pesticide programme was compared to that achieved following the zero pesticide residue management system (MS / MR). Untreated plots of disease susceptible and resistant varieties were included (Table 1). Each treatment was replicated twice in a randomised block design.

Treatments

Zero residue management system (MS, MR)

In the MS and MR system, pest control pre bloom was based on IPM monitoring and treatment with diflubenzuron, thiaclopyrid or fenoxycarb as necessary. Post bloom control of codling moth and other caterpillars was achieved using codling moth granulosis virus and *Bacillus thuringiensis* (Bt).

Scab control in MS plots pre bloom was based on a pre bud burst spray of copper oxychloride and use of conventional fungicides (dithianon, captan, myclobutanil) with ADEM key stage system (Berrie & Xu, 2003) to determine frequency of spraying. The final conventional fungicide spray was the key stage petalfall spray. In MR plots scab sprays were applied only at bud burst and petalfall. Post-bloom sulphur sprays were used for mildew control in both MS and MR plots. Post harvest (MS and MR plots), sprays of DMI fungicides and 5% urea were applied to limit the development of late scab and the overwintering scab sexual state and encourage leaf rotting.

Pre bloom, control of powdery mildew was based on removal of infected blossoms and shoots promptly at pink bud and petalfall and the use of DMI fungicides to suppress sporulation. Post bloom the mildew incidence was monitored weekly and the information used with ADEM to make decisions on sulphur use. Post harvest, DMI sprays were used to control mildew on late shoot growth. Any mildew overwintering in silvered shoots was removed during winter pruning.

Control of storage rots was achieved by cultural measures such as removal of cankers and fruit infected with *Monilinia fructigena* and mulching to limit exposure of fruit to soil. Before harvest an assessment of rot risk (Berrie, 2000; Cross & Berrie, 2001) was made to determine suitability for long term storage (Table 1). At harvest apples were picked selectively to avoid damaged or low hanging fruit being stored.

Conventionally treated system (CS, CR)

In the conventional system, pesticides were applied from bud burst to harvest as necessary, based on IPM monitoring. Chlorpyrifos and thiacloprid were used for insect control. Control of scab and mildew was based on a routine fungicide (dithianon, captan, myclobutanil, bupirimate) programme applied at ten day intervals from bud burst to harvest. In CR plots sprays for scab control were applied only at bud burst and petalfall. Captan, applied 28 and 14 days pre harvest was used for control of storage rots.

Table 1. Zero pesticide residues in apples: treatments, pesticide programmes and varieties

Treatment	Pesticide use	Varieties	IPDM programme
US	Untreated	Susceptible Cox, Gala, Fiesta, Discovery	None
CS	Conventional	Susceptible Cox, Gala, Fiesta, Discovery	Routine pesticides Captan (28 & 14 days pre harvest) for storage rot control
MS	Managed	Susceptible Cox, Gala, Discovery	Managed pesticides early and after harvest. Biocontrol during fruit development Rot risk, selective picking, inoculum removal for storage rot control
UR	Untreated	Scab-resistant Saturn, Ahra, Discovery	None
CR	Conventional	Scab-resistant Saturn, Ahra, Discovery	Routine insecticides and mildewicides. Reduced scab fungicide programme Captan (28 & 14 days pre harvest) for storage rot control
MR	Managed	Scab-resistant Saturn, Ahra, Discovery	Managed pesticides early and after harvest. Biocontrol during fruit development Rot risk, selective picking, inoculum removal for storage rot control

Untreated (US, UR)

No pest or disease controls were applied.

All treatments received the standard programme for nutrients (including calcium sprays) and for weed control.

Assessments

Pests and diseases were assessed using standard methods (Cross & Berrie, 1995) at standard IPM timings of green cluster/pink bud, petalfall and at monthly intervals to harvest in order to make decisions on pesticide use and to assess the success of the management systems. At harvest fruit yield and pest and disease incidence were assessed on the fruit from ten trees per variety per plot. Fruit quality (size and russet) were assessed on a random sample of 100 fruit per variety per plot. At harvest, fruit from each treatment (at least one bulk bin of fruit per variety per plot) was stored in a commercial controlled atmosphere (3.5°C; 1.25% O_2; < 1% CO_2) store and the incidence of rotting recorded in February or March. During the trial, records were kept of timings for cultural, monitoring and other management inputs such that an economic appraisal of the systems could be made.

Results and discussion

Disease control

The fungicide programme applied to plots in 2002 (Table 2) is given as an example. Fungicides applied in 2001 and 2003 were similar, apart from a pre bud burst spray of Cuprokylt (copper oxychloride) applied to managed plots in 2002 and 2003, but not in 2001.

Table 2. Fungicides applied to managed (MS, MR) and conventional (CS, CR) plots in 2002 Treatments were applied at the recommended dose unless stated.

Fungicide / Timing		Treatment / number of sprays (% dose)			
		CS	CR	MS	MR
Pre bud burst	copper	0	0	1	1
Bud Burst – Petal Fall	dithianon	2	1	2	1
	myclobutanil +captan	4	0	4	1
	myclobutanil	0	2	0	2
	captan	0	0	1(50)	0
	bupirimate	0	3	1(50)	3
Petal Fall - harvest	myclobutanil + captan	4	1	0	0
	myclobutanil	0	0	0	0
	bupirimate	4	7	0	0
	captan	4	2	0	0
	sulphur	0	0	9 (30-50)	9(30-50)
Post harvest	myclobutanil + captan	0	0	1	1
	urea	0	0	1	1
Cost	£/ha	384	304	262	217

This treatment was applied to control scab overwintering on the tree. Up to ten sprays of sulphur, none at the full dose, were applied to managed plots in all years. These treatments appeared to give adequate control of mildew, as the incidence of primary mildew in these plots the following season (2002, 2003) was negligible. Eventually it is hoped that alternative strategies can be developed for mildew control to replace or minimise the use of sulphur. The cost of fungicides in the managed programmes was 30 to 40% cheaper than in the conventional systems.

The incidence of scab and powdery mildew in 2001 and 2002 is presented in Table 3. In 2001, scab control in the MS plots was similar to that achieved in the routine sprayed plots (CS), with 1% or less of fruits scabbed at harvest compared to 20 to 72% on fruit from untreated plots. However in 2001 the scab risk was mainly in the early part of the season. The weather in 2002 was far more challenging for scab control, with a high scab risk at bud burst in March and from mid blossom onwards. Despite this, scab control in the MS plots was as good as or better than that achieved in the CS plots (Table 3). In 2003, in contrast to the previous two years, no scab periods were recorded until mid bloom (25 April) with most scab risk periods recorded after the conventional fungicide programme in MS plots had been completed. Nevertheless scab incidence was similar (zero or very low) in MS plots to that in CS plots, even on extension growth, compared to up to 80% scab recorded on fruit at harvest in untreated plots. No scab was recorded on the resistant varieties in the sprayed plots.
Mildew control in the MS / MR plots was also similar to that in the CS / CR plots, and did not exceed 20% of shoots mildewed. The primary mildew in managed plots at the start of 2002, 2003 and 2004 was negligible, indicating that the system was not resulting in an increase in overwintering mildew Sooty blotch (*Gloeodes pomigena)* and leaf / fruit spot (*Phoma* sp) were also recorded in the trial, mainly in unsprayed plots and mainly on the varieties Saturn and Fiesta.

Pest control
The insecticides applied to MS / MR and CS / CR plots in 2002 (Table 4) are given as an example. The insecticides applied in 2001 and 2003 were similar, except in 2001 no insecticide was applied at petalfall and in 2003 diflubenzuron was replaced with methoxyfenozide pre bloom with a second spray at petalfall.

The percentage of apples damaged by pests at harvest is given in Table5 (2001 and 2003 only). In 2001 the highest incidence of damage was recorded in untreated plots and the least in conventional plots. The incidence of rosy apple aphid at harvest in MS plots was greater than that in CS and CR plots indicating that a single pre blossom spray of thiacloprid was insufficient to give adequate control. The highest incidence of rosy apple aphid was recorded in untreated plots. The incidence of sawfly and tortrix damaged fruits was slightly higher in managed plots than in conventional plots, whereas early caterpillar damage was similar in both. The incidence of codling moth damage was low in all treatments. In 2002, thiacloprid was applied to MS / MR plots at pink bud and petal fall (Table 4) and this two spray combination gave adequate control of rosy apple aphid and sawfly (Table 5) compared to the CS / CR treatment, where chlorpyrifos was used pre bloom and thiacloprid post bloom.

In 2002, the incidence of tortrix damage to fruit at harvest was much higher than in 2001 with up to 12.7% of fruit damaged in untreated plots at harvest. Significant levels of damage were recorded in the MS / MR plots despite three sprays of *Bacillus thuringiensis* (Bt) during the summer. In 2003, methoxyfenozide was used pre bloom to reduce overwintering populations of tortrix. The incidence of fruit damage at harvest was lower and similar to that in CS / CR plots (Table 5). The incidence of codling moth recorded was again low in all treatments, but significant damage by *Rhynchites* weevils to fruit was recorded in some plots, particularly untreated plots of scab resistant varieties. The total pest damage to fruit at harvest was around 6% and similar in MS / MR and CS / CR plots compared to 30 to 40% damage in untreated plots.

Table 3. Incidence of powdery mildew and scab in managed (MS, MR), conventionally treated (CS, CR) and unsprayed (US / UR) plots in 2001 and 2002

Treatment / cultivar		2001				2002			
		Primary mildew May	% mildewed shoots June	% scab infected shoots July	% scab infected fruit harvest	Primary mildew May	% mildewed shoots June	% scab shoots July	% scab infected fruit harvest
US	Cox	2.3	60.0	65.0	20.0	0	65.0	97.5	43.2
	Gala	0	90.0	90.0	72.0	0	95.0	100.0	98.0
UR	Saturn	0	68.0	0	0	0	100.0	0	0
	Ahra	8.4	50.0	0	0	3.3	92.5	0	0.5
	Discovery	2.0	30.0	0	0.3	0	20.0	0	1.6
CS	Cox	0	5.0	0	0.1	0	7.5	2.5	1.4
	Gala	0	20.0	2.5	0.5	0	12.5	7.5	5.6
CR	Saturn	0	0	0	0	0	7.5	0	0
	Ahra	0.4	10.0	0	0	0.1	0	0	0
	Discovery	0	0	0	0	0	0	0	0
MS	Cox	0	13.0	0	0.3	0	0	0	0.4
	Gala	0	8.0	0	1.0	0	17.5	0	2.7
MR	Saturn	2.6	20.0	0	0	0	2.5	0	0
	Ahra	15.0	15.0	0	0	0.8	10.0	0	0
	Discovery	0	3.0	0	0	0	0	0	0.1

Table 4. Insecticides applied in 2002. Treatments were applied at recommended dose

Date	Growth stage	CS	CR	MS	MR
4 April	Green cluster	chlorpyrifos	chlorpyrifos	diflubenzuron	diflubenzuron
9 April	Pink bud	-	-	thiacloprid	thiacloprid
15 May	Petal fall	thiacloprid	thiacloprid	thiacloprid	thiacloprid
18 June	Fruitlet	chlorpyrifos	chlorpyrifos	Bt	Bt
4 July	Fruitlet	chlorpyrifos	chlorpyrifos	Bt	Bt
20 August	Pre harvest	Bt	Bt	Bt	Bt
Cost £/ha		111	111	236	236

The cost of the insecticide spray programme applied to MS / MR plots was 40 to 100% greater than the conventional programme (Table 4). Numbers of insecticides used were similar, but the cost of selective insecticides used in the managed programme was higher.

Table 5. Pest blemishes (% damaged fruit) recorded on fruit at harvest in 2001 and 2003 from Managed (MS, MR), Conventional (CS, CR) and Untreated (US, UR) plots

Pest	2001			2003		
	MS/MR	CS/CR	US/UR	MS/MR	CS/CR	US/UR
Rosy apple aphid						
Suscep cvs	5.6	0.3	6.8	0	0	1.2
Resist cvs	0.0	0.0	2.6	0	0	3.2
Sawfly						
Suscep cvs	1.6	1.0	2.2	0.4	0.2	5.4
Resist cvs	3.2	1.2	1.5	0.9	0.3	3.0
Tortrix						
Suscept cvs	1.8	0.6	4.2	1.4	1.3	13.9
Resist cvs	3.2	0.7	6.1	1.6	1.5	9.5
Early caterpillar						
Suscept cvs	1.0	0.6	1.6	0.2	0.3	1.9
Resist cvs	1.4	1.1	2.3	0.5	0.4	1.5
Codling						
Suscept cvs	0.3	0.0	0.4	0.3	0.5	3.3
Resist cvs	0.1	0.0	0.6	0.4	0.2	1.4
Earwig						
Suscept cvs	1.5	0.9	3.5	2.8	2.4	8.0
Resist cvs	1.5	2.0	3.6	1.9	2.8	5.7
Rhynchites						
Suscept cvs	-	-	-	0.4	0.8	11.0
Resist cvs	-	-	-	0.5	0.2	3.1
Total pest damage						
Suscept cvs	12.7	3.6	19.6	6.1	6.3	49.0
Resist cvs	9.7	5.0	17.4	6.9	6.2	32.1

Storage rots

In 2001, pre harvest rot risk assessment identified a risk of *Phytophthora* rot in the later harvested varieties (Gala and Saturn). This was effectively controlled by selective picking (only fruit > 0.5m above the ground picked for storage) in the MS / MR plots and a pre harvest captan spray in the CS / CR plots. Up to 4% rotting due to *Phytophthora* was recorded in Gala and Saturn from untreated plots. *Phytophthora* incidence was negligible in the earlier harvested varieties (Cox, Fiesta, Ahra), where no risk had been identified. This approach for control of storage rots was continued in subsequent seasons. The results from 2003 are shown in Table 6. Weather conditions pre harvest were dry and hence the risk of *Phytophthora* rot negligible. No *Phytophthora* rot was recorded in stored fruit (Table 6). Brown rot (*Monilinia fructigena*) was the main rot identified and this was adequately controlled by selective picking at harvest in the MS / MR plots.

Table 6. Mean % losses due to rots in apples cvs Cox, Gala, Fiesta and Saturn, harvested September 2003 from conventional (CS, CR), managed (MS, MR) and untreated plots (US, UR) and stored in CA (1.25% O_2 <1% CO_2) at 3.5°C until mid March 2004

Fungal Rot	Cox			Gala			Fiesta			Saturn		
	US	CS	MS	US	CS	MS	US	CS	MS	UR	CR	MR
Monilinia fructigena	16.0	2.7	1.7	12.6	0.2	0.6	15.4	3.5	2.5	0.6	0.4	0.7
Botrytis	0.3	0.1	0	1.1	0.02	0.06	0.3	0.1	0	0.2	0.3	0.3
Phytophthora	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium	1.8	0.4	0.2	1.1	0	0.05	1.7	0.7	2.4	0.6	2.2	0.3
Nectria	2.1	0.9	0.9	8.0	0.2	0.08	2.6	2.0	1.3	1.5	1.1	0.4
Gloeosporium	1.8	0.9	1.1	2.2	0.04	0.21	0.6	0.6	1.1	4.7	1.1	0.4
Other	3.7	1.4	0.9	1.3	0.3	0.3	1.6	3.3	2.0	6.2	19.6	7.2
Mean % loss	25.5	6.2	4.6	26.2	0.7	1.3	22.1	9.9	9.3	13.7	24.5	9.2

The disease control achieved by the zero pesticide residue management system (MS, MR) in this study has been as good as or better than that in the conventionally treated plots (CS, CR) and at lower cost. Dormant season disease control appears to be a key factor in the success of the system. Pest control in this system appears to be satisfactory but more expensive than the conventional system because of the increased cost of selective insecticides compared to the broad spectrum products used in the conventional system. No residues of pesticides applied to the MS and MR plots were detected in harvested fruit. Residues of captan, bupirimate and chlorpyrifos were frequently detected in apples from CS and CR plots, although all residues were below the MRL permitted.

The trial at East Malling will be continued for three further seasons to examine the impact of reduced pesticide programmes on the incidence of non target pests and diseases. The zero residue system will also be evaluated on commercial farms.

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Interaction between fruit tree canker and apple scab control in integrated apple orchards

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Abstract: Most important fungicides against fruit tree canker are benzimidazoles, copper and captan. The benzimidazole and copper fungicides applied to control fruit tree canker in autumn have a retarding effect on leaf degradation. And therefore jeopardise a low inoculum orchard for apple scab. Therefore, the objective was to measure the effect of calcium hydroxide as a new, environmentally-friendly method to control fruit tree canker on the control of apple scab.

A combination of a calcium hydroxide schedule against fruit tree canker, and sanitation practices to improve leaf degradation was compared to standard schedule of one application with a benzimidazole fungicide and a maximum of two applications of captan and no sanitation. Leaf degradation was significantly quicker in plots treated with calcium hydroxide against fruit tree canker combined with sanitation. Also, the control of apple scab tended to be better in these plots. On top of that, fruit tree canker control was at more or less the same level with calcium hydroxide as with the standard schedule. It is concluded that the control of apple scab can profit from the new, environmentally friendly method to control fruit tree canker in combination with sanitation methods.

Key words: *Nectria galligena, Venturia inaequalis*, calcium hydroxide, sanitation

Introduction

Fruit tree canker, caused by *Nectria galligena*, and apple scab, caused by *Venturia inaequalis* are major diseases in integrated apple growing in northern Europe. Several groups of fungicides have proven activity against those diseases. A most effective control of fruit tree canker is dependent of benzimidazole and copper fungicides. Also some broad spectrum preventive fungicides, like captan, have a reasonable good efficacy against fruit tree canker. However, the control of fruit tree canker becomes vulnerable, since copper fungicides are no longer permitted in Denmark and the Netherlands and benzimidazole fungicides, which have adverse effects on organisms living in water, might be restricted in future.

It has been demonstrated that a lower number of fungicide applications were needed to control scab in low inoculum orchards (MacHardy et al.,1993). Improvement of leaf degradation is, among others, an important measurement to achieve a low inoculum orchard for scab. Copper and benzimidazole fungicides applied to control fruit tree canker in autumn have a retarding effect on leaf degradation (Cook, 1999; Vink and van Straalen, 1999). And therefore jeopardise a low inoculum orchard for apple scab. At Applied Plant Research, a new strategy with calcium hydroxide schedules was developed to control fruit tree canker. Experiments have shown that this new strategy has no adverse effect on leaf degradation and a reasonable efficacy against fruit tree canker. Therefore, the objective was to measure the effect of calcium hydroxide as a new, environmentally friendly method to control fruit tree canker on the control of apple scab.

Material and methods

Experiments were conducted during two consecutive seasons, starting with autumn treatments in 2001 and ending with counting results in 2003 in a uniform apple orchard at Randwijk, the Netherlands. Two times three rows of apple cultivar Jonagold on M. 27 rootstock with a middle row of cultivar Discovery on M. 9 and outside rows of cultivar Alkmene on M. 9 were the experimental units of 946 trees (about 0.35 ha) each in a larger orchard. There were four replicates in the first season and three replicates in the second season. Trees were planted in a single row system of 3 x 1.25 m and pruned as slender spindles. There were two treatments: 1) the standard schedule for fruit tree canker and apple scab, denominated as S (standard) and 2) calcium hydroxide against fruit tree canker and sanitation against scab, denominated as N (new integrated). The standard schedule (S) was composed of one BCM treatment, carbendazim at 0.6 l/ha (as Luxan Carbendazim 500 FC; 500 g/l a.i.) and two captan treatments at 2.5 kg/ha (as Captosan spuitkorrel 80 WG; 80 % a.i.) targeted towards fruit tree canker applied during the leaf fall period. No sanitation measurements against apple scab were done in the standard schedule (S) during autumn and winter. The new integrated (N) schedule consisted of five applications of calcium hydroxide at 50 kg/ha (as Supercalco 95; 95 % a.i.) against fruit tree canker applied during the leaf fall period. Leaf shredding and one application of urea at 62.5 kg/ha to fallen leaves during winter was done to improve leaf degradation in the new integrated (N) schedule. Scab control was done with a weak schedule of only captan at 1.1 kg/ha (as Captosan spuitkorrel 80 WG; 80 % a.i.) or dithianon at 0.25 l/ha (as Delan flowable; 70 % a.i.) at weekly interval in spring till half May to magnify possible differences between treatments. A regular scab schedule followed from half May till harvest.

Leaf degradation was determined by the line intersect method on 7 January, 18 March and 17 April 2002 and on 6 February, 17 March and 29 April 2003. Symptoms of apple scab were counted on rosette leaves (15 May 2002 and 11 June 2003) as percentage of leaves with scab symptoms (incidence) and the number of lesions of scab (severity). New symptoms of fruit tree canker were counted 16 June 2003 and expressed as the percentage of trees with canker symptoms and the total number of cankers per 2 rows of trees (144 trees).

Results

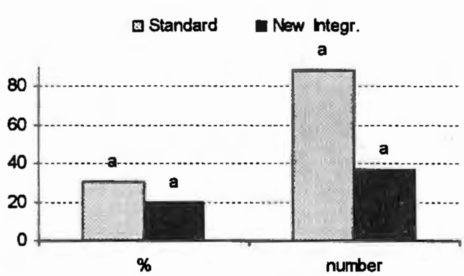

Figure 1. The percentage (%) of trees with new-formed cankers and the number of new-formed cankers per two rows in the standard (S) and the new integrated (N) strategy.

The average percentage of trees with newly formed cankers and the average total number of newly formed cankers per two rows of trees were similar (not significant different) for the standard schedule (S) and the calcium hydroxide schedule (N) (Figure 1). There were significant differences in the leaf degradation in early spring (Figure 2). Leaves decomposed faster in the new integrated strategy (N) compared to the standard schedule (S), especially in 2003.

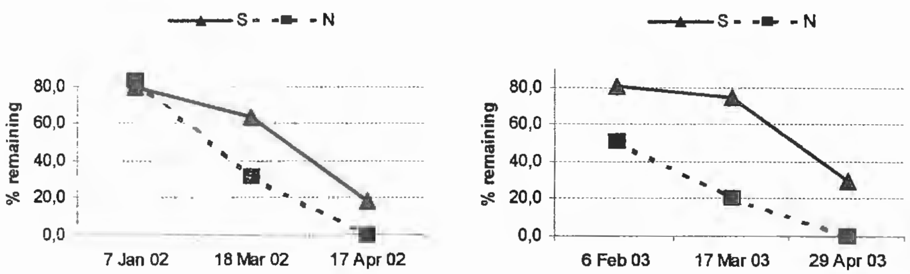

Figure 2. The average percentage remaining leaves in the standard (S) and the new integrated strategy (N).

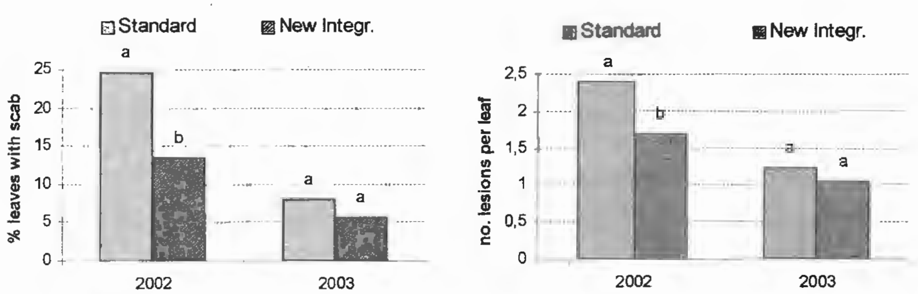

Figure 3. The average percentage leaves with scab and the average number of lesions of scab per leaf in the standard (S) and the new integrated strategy (N) for the rosette leaves.

The scab incidence, expressed as the average percentage of rosette leaves with lesions, was higher in the standard schedule (S) than in the new integrated strategy (N) in 2002. Similarly, the severity of scab on rosette leaves, expressed as the average number of lesions per leaf was higher in the standard schedule (S) than in the new integrated strategy (N) in 2002. The average infestation of scab was lower in 2003 than in 2002. There were no significant differences in scab incidence or in scab severity between treatments in 2003.

Discussion

There was a big variation in the number of newly formed cankers per tree and in total between plots. Therefore no significant differences could be demonstrated between the standard (S) and the new integrated (N) strategy. There seemed to be a tendency to a better efficacy of calcium hydroxide than the standard schedule from the presented data (Figure 1). This is opposite the results of earlier detailed studies in which calcium hydroxide was often less effective or, in the best case, as good as the standard schedule for the control of fruit tree canker (pers. comm. B. Heijne).

It is remarkable that leaf degradation was faster in 2003 than in 2002 in the new-integrated (N) strategy, while the scab infestation was lower in that year and not significantly different between the standard (S) and the new-integrated (N) strategy. A significant difference in scab infestations would have been expected. Most probably, this was the due to not applying a fungicide at the first scab infection period of the year in 2003. Missing this unforeseen severe infection period might have levelled out the low inoculum effect in the new-integrated (N) situation by the presence of lesions and conidial infections afterwards right at the beginning of the scab season.

In these experiments, combination treatments were compared. Therefore, it is not possible to attribute the results to separate elements of the combination treatments. However, the effect of sanitation practices, such as leaf shredding and the application of urea are well documented in literature, e.g. Sutton *et al* (2000). Also, the effect of retarding leaf degradation is known of BCM and copper fungicides (Vink and van Straalen, 1999). On the other hand, there was little effect of calcium hydroxide on leaf degradation compared to untreated leaves (pers. comm. B. Heijne). Reasoning from these literature based information, it seems logic that the new-integrated (N) strategy is retarding the scab epidemic in spring. It is concluded that the results of the combination treatments provide evidence for the statement that the use of calcium hydroxide against fruit tree canker in combination with sanitation practices against scab is conducive to a low inoculum orchard for apple scab.

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Preventive apple scab infection warnings: optimization of leaf wetness models and evaluation of regional weather forecast data.

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Abstract: Scab warning systems are currently based on a curative approach. Climatological and biological factors monitoring form the basis of estimating real scab risk. However, apart from the registration of the past climatological data, there are possibilities to obtain a more efficient control of fungal diseases using weather forecasts. 48 hours regional weather forecasts were evaluated for the use in preventive scab warning systems. The climate parameter leaf wetness, which is also needed for determining scab infection risks, had to be modeled. Two leaf wetness models, Sweb and Leafwet, were optimized for the apple orchard and tested. Accuracy of the weather forecast was evaluated positively, however the modeling of leaf wetness needs to be improved before incorporated in disease risk warnings. A preventive warning system based on a threshold of predicted rain hours is presented.

Key words: apple scab, leaf wetness, weather forecast, disease warnings, infection models

Introduction

Until now only curative warnings based on climatological risk calculated from measured meteorological data are available for scab control (Creemers, 2002). However a preventive warning system is demanded for the future. A preventive strategy offers several practical advantages: (1) better choice of time of application in function of spraying-weather conditions, (2) less selection pressure for resistance building versus curative fungicides, (3) better planning and organization of activities on the fruit farm, (4) reduction of the treatments based on preventive fungicides in organic farming. Recently the Royal Meteorological Institute of Belgium introduced new 48 hours regional weather forecasts based on the weather forecast model ALADIN (Termonia, 2001). However there is no forecast for the leaf wetness period, which is one of the most important factors involved in the epidemiology of fungal pathogens. In the case of scab, the infection degree can be determined by looking at the temperature and the duration of leaf wetness (Creemers, 2002). For the primary infection by ascospores in the beginning of the scab season, there is also the prerequisite of rain at the beginning of the leaf wetness period.

To make good estimates of forecast based climatological scab infection risks, two leaf wetness models were evaluated and optimized: Leafwet (Wittich, 1995) and Sweb (Magarey, 2004). The accuracy of the predicted leaf wetness depends on the accuracy of the leaf wetness model and accuracy of the regional weather forecast. Therefore also the weather forecast data were checked, especially rain because of its importance in primary scab risk.

Materials and Methods

Microclimatic measurements, weather forecast data and leaf wetness models

Hourly data on temperature, relative humidity, precipitation and leaf wetness were collected from 10 weather stations situated in apple orchards in Haspengouw, the most important fruit region in Belgium. Hourly data on radiation and wind were obtained from the Royal Meteorological Institute of Belgium. The regional weather forecast data, which are the outcome of the ALADIN-forecast model (Aire Limitée, Adaptation Dynamique, Développement International), comprise temperature, relative humidity, radiation, cloud cover, precipitation, wind direction and speed, downward directed radiation and atmospheric pressure (Termonia, 2001).

The Leafwet model (Wittich, 1995) predicts the surface wetness of the top leaf of an orchard. The Sweb model (Magarey, 2004) was designed for estimating leaf wetness duration in a grape canopy. Both models had several vegetation parameters, which allows adaptation for any given crop, in this case the apple orchard. To be able to modify the models and carry out a thorough evaluation, the models were transferred to the Mathlab environment. A comparison was made between modeled leaf wetness and recorded weather data of the foregoing years (2000-2003).

Field experimentation

To test the effectiveness of the different scab warnings systems, two trials were carried out in 2004 on the cultivars Jonagold and Golden delicious. The trial design is set up as a randomized block in 4 repetitions consisting out of 9 trees per repetition. In each block the different objects are divided in an aselect way. The warning systems under evaluation were Shorfprognosegrogramm, from H. Welte, Rimpro from M. Trapman (>100 and >300) and the warnings given by Research Station for Fruit Growing based on Mills a-3, plant development and ascospore release (Creemers, 2002). The curative fungicide Scala (pyrimethanil) was used in combination with delan (dithianon) to reduce the risk of resistance development. Disease assessment occurred on the rosette leaves and on shoot leaves. In each object four times hundred leaves were examined. The infection degree (TH3) and efficacy (Abbott value) were calculated according to the formula of Townsend-Heuberger and Abbott. Statistics were executed using Unistat software version 5.5 (Unistat Ltd., London, UK).

Results and Discussion

After a survey of the existent leaf wetness models, two models were selected for further evaluation, namely Sweb and Leafwet. Both models are based on the energy balance of evapotransporation and require the input of temperature, relative humidity, precipitation, radiation, wind and cloud cover. Both models also contain vegetation parameters that can be adjusted to the current situation, which is very important for a tree fruit like apple and associated diseases like scab. Primary scab infections occur after bud break during the early development of the leaves and flowers. In this period the leaf area index will change and the growing leaves will have a profound influence on the evaporation of the canopy. To reduce the complexity of the problem of modeling leaf wet in an apple orchard, these changes in evaporation due to growth were excluded in the analysis by selecting only the weather data at time points were no leaves were present on the tree or the tree was full-grown. Because the different vegetation parameters in the models interact with each other, all possible combinations were checked to achieve the best possible fit between measured and modeled leaf wetness. The evaluation was based on the comparison between measured and modeled leaf wetness period length (Table 1). Leafwet gives a good estimation for bare trees but not for full-grown trees. For Sweb, the difference is less and scores better for full-grown trees. However the overall accuracy and % wet well estimated are still too low for the use of these models in disease risk assessment. The next step of the research will include optimization of climatological parameters and the introduction of the measured leaf area index during growth.

Table 1. The maximum leaf wetness forecast accuracy of the two leaf wetness models obtained after optimization by changing the vegetation parameters of the models.

	Sweb/bare tree	Sweb/full grown tree	Leafwet/bare tree	Leafwet/full grown tree
forecast accuracy (%)	78.5	76.2	77.8	78.3
%dry well estimated	90.6	88.8	81.2	94.2
%wet well estimated	62.8	55.6	73.4	52.1

Table 2. Accuracy of the different weather variables from the regional weather forecast for the region Haspengouw in Belgium.

	T (°C)	RH (%)	Rain ($l/m^2/h$)	Windspeed (m/s)	Radiation ($joule/m^2$)
mean forcast error/h	-1.3±2.7	-1.6±2.2	-0.03±0.4	-0.7±1.3	-48±713
max forcast error/h	-3.2±4.1	-1.9±3.0	-0.2±1.5	-1.0±1.9	-378±2525

Table 3. Scab infestation on the leaves and rosette leaves and efficacy of different curative spraying schedules. (* warnings given by the Research Station for Fruit Growing, Belgium)

	number of treatments	golden		jonagold	
		rosette leaf	leaf	rosette leaf	leaf
untreated control	/	52.9%	47.0%	92.3%	54.8%
infection warnings*	6	99.3	99.5	99.5	99.5
infection model Rimpro>100	5	99	97.9	98.4	97.8
infection model Rimpro>300	3	99.3	98.9	98.5	98.5
infection model Welte	4	99.6	99.1	99.9	99.2

Table 4. Number of preventive treatments predicted after 24 and 48 hours at different rain hour thresholds and fungicide retention time and number of extra curative treatments necessary to cover all the climatological infection periods in the primary scab season in 2004.

predicted rainhours threshold	24 hours				48 hours			
	# preventive treatments		# extra curative treatments		# preventive treatments		# extra curative treatments	
	5 days	7 days	5 days	7 days	5 days	7 days	5 days	7 days
>4	9	7	0	0	9	7	0	0
>5	9	6	0	1	9	7	0	0
>6	8	5	1	1	9	6	0	1
>7	8	5	1	1	8	6	1	1
>8	8	5	1	1	8	6	1	1
>9	6	4	2	2	7	6	1	1
>10	5	4	2	2	6	6	1	2

The regional weather forecast was compared with the measured meteorological data of 10 weather stations of the agrimeteorological network situated in Haspengouw. Wind and radiation sensors are not present in the agrimeteorological network and data for these parameters were obtained from the Royal Meteorological Institute of Belgium. The evaluation was performed by calculating the forecast accuracy for each of the 48 forecast hours, which allows detecting variation of accuracy in function of forecast time. The mean and maximum deviation together with the variance, expressed by the standard deviation of the mean and the maximum, gives an idea on accuracy during the 48 hours and can point out repeated errors made by the forecasters (Table 2). Temperature, wind and relative humidity are predicted very accurately. In the case of temperature and wind, the variance shows a diurnal pattern (data not shown), which could be due to a regular forecast error or a difference between climatological readings in the orchard and forecasts for standard weather box. The diurnal patterns are also observed for radiation but here the variance is much bigger. A probable explanation for the variance observed, can be found in the facts that radiation is highly dependent on cloud cover and only one reference for measured data was available. Although 10 reference weather stations are available, precipitation is even more difficult to evaluate because of the different forms of precipitation and the geographically local aspect of rain showers. This is reflected in the high variance observed in the evaluation. However for the prediction of leaf wetness it is not the amount of rain that counts, but the number of rain hours during the day. An in-depth analysis revealed a relatively good prediction of the rain hours when there were at least 4 rain hours a day. As the primary scab infection is highly dependent on rain and rain can also prolong leaf wetness before and after dew, the predicted rain hours were evaluated for their use of predicting scab infection in the primary scab season.

In order to compare the effectiveness of preventive treatments based on predictive rain hours, the fungicide spray cover and retention were compared with those achieved with curative treatments in the primary scab season of 2004. Curative treatments based on different warning systems were performed in two trials on two different apple varieties. All spraying schedules had good efficacy towards scab (Table 3). No statistical differences could be observed between the different warning systems although the number of treatments differed. To achieve the same fungicide cover with the preventive warnings, fungicides had to be sprayed when at least 5 rain hours were predicted, however more applications were necessary (Table 4), the amount depending on the fungicide retention on the leaves. With a higher threshold, the number of preventive treatments declines, but curative treatments are becoming necessary to cover all the primary scab infections. When more data are available, a good threshold value can be chosen and this research can lead to a combined preventive/curative control of scab in the apple production, with preventive treatments on the basis of infection risks calculated on weather forecast, and with curative treatments if the registered data from the electronic weather stations differ from those of the forecast. However for the use of the more advanced warnings systems like Rimpro and Welte and also to use weather forecast data for other diseases that are less rain dependant, a good prediction of leaf wetness is necessary.

Acknowledgements

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Assessing the risk of reduced-risk apple pest control programs in New York State

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Abstract: A current on-farm project in NYS is concentrating on the use of IPM systems as an alternative to broad-spectrum insecticides to greatly reduce residues and worker exposure to organophosphate, carbamate, and pyrethroid insecticides and to evaluate, on a regional scale, reduced-risk tactics that will be effective, sustainable, economically viable, and compatible with biological control. This study addresses the effectiveness of tactics that include IGRs, botanically derived and selective pesticides, and pheromone disruption. Studies were conducted in 17 commercial orchards for the 2nd year to determine the effectiveness of reduced-risk tactics in managing key pests. Each site was a split-plot design in which the entire block received a program of selective insecticides, and a 2-ha portion of the block additionally received pheromones for mating disruption of internal worm pests. Results after 2 years of this 4-year study show that reduced-risk insecticides are effective against many secondary pests. However, fruit damage from internal lepidopterans and apple maggot in reduced-risk orchards tends to be slightly higher than that occurring in standard blocks. This project will help determine if selective insecticides, alone or integrated with mating disruption, can provide adequate control of direct pests of fruit for which there is no allowable market tolerance for damage. It will also help identify potential new pests, as well as natural enemies, that may occur in orchards treated with these new, selective tactics.

Key words: selective pesticides, pheromone disruption, predator mites, biological control

Introduction

Apples in New York State are a high value crop, with an estimated value of $100–140 million annually. Fresh and processed apple products constitute a major component in the diets of infants and children. Implementation of the Food Quality Protection Act of 1996 has begun and will continue to limit reliance on broad-spectrum insecticides, which have been the foundation of pest management programs in these crops for the past 40 years.

New York is a participant in a multi-state USDA-RAMP (Risk Avoidance and Mitigation Program) project that is examining pest control programs that use only selective reduced-risk pest control tactics on multiple farms throughout the state over a 4-year period. The goal of this project is to design pest management systems that will greatly reduce residues and worker exposure to organophosphate, carbamate, and pyrethroid insecticides and to evaluate, on a regional scale, reduced-risk tactics that previous research and experience indicate will be effective, sustainable, economically viable, and compatible with biological control. Among the approaches that are being used in these programs, which are designed for fresh market apple production, are selective (reduced-risk) insecticides, mating disruption, conservation of natural enemies, and cultural practices. These tactics are being integrated into specific pest management programs designed to be most appropriate for each major production region within the state. This article reports the results after the second (2003) year of the study.

Materials and methods

A total of 17 research sites were set up on commercial farms in all the major apple growing areas of New York: western NY, central NY, Hudson R. Valley, Capital District, and Champlain Valley. Each research site was a "split-plot design" in which the entire block (nominally 4 ha) received a program of reduced-risk insecticides, and a 2-ha portion of the block was additionally treated with pheromones for mating disruption of the later summer generations of codling moth (CM), *Cydia pomonella*; oriental fruit moth (OFM), *Grapholita molesta*; and lesser appleworm (LAW), *G. prunivora*. A grower standard comparison block, which had the same varieties and planting style, was also monitored at each site. These blocks all contained at least one fresh fruit variety such as 'Empire' or 'McIntosh' that might be selected for marketing in Europe or some other market outlet that could eventually require IPM protocols for market access.

Materials used in the blocks receiving a reduced-risk pesticide program included: clofentezine or horticultural mineral oil plus pyridaben or bifenazate (as needed in summer) for European red mite, *Panonychus ulmi*; thiamethoxam for early season pests (including rosy apple aphid, *Dysaphis plantaginea*; spotted tentiform leafminer, *Phyllonorycter blancardella*; plum curculio, *Conotrachelus nenuphar*; and tarnished plant bug, *Lygus lineolaris*), indoxacarb for post-petal fall pests such as plum curculio, internal Lepidoptera and apple maggot (*Rhagoletis pomonella*) plus tebufenozide and spinosad for obliquebanded leafroller (OBLR),*Choristoneura rosaceana*. All sprays were applied by the grower.

From 22 April–2 May, Trécé Pherocon IIB pheromone traps were hung in all three plots at each commercial orchard site as follows: one CM, OFM, and LAW trap group was placed at head height and arranged around the canopy of each of three trees in a middle row (one at each end and one in the center) of the Reduced-Risk Pesticides, Pheromone+Reduced-Risk Pesticides, and Grower Standard blocks at each site. All traps were checked and cleaned weekly until late August; CM lures were changed every 4 weeks, and OFM and LAW lures were changed during the middle two weeks of July. From 16 June–1 July, polyethylene pheromone tie dispensers were hung in the Pheromone+Reduced-Risk Pesticides plots at each site, using different products to disrupt two separate moth species: Isomate CTT ("twin-tube") at 500 ties/ha for codling moth, and Isomate M-100 at 250 ties/ha for oriental fruit moth and lesser appleworm. All OFM ties were hung at head height by hand; CM ties were hung in the upper 1/3 of the tree canopy by hand for dwarf trees, and using a pole+hoop applicator for trees taller than 7 ft. Average time requirements for deploying the pheromone ties were: Hand-applied, 3.18 hr/ha/person (or 0.32 ha/hr/person); 236 ties/hr/person; Pole+hoop: 3.10 hr/ha/person (or 0.32 ha/hr/person); 242 ties/hr/person.

All plots were sampled for representative arthropod pests throughout the season. Ten blossom terminals from each of 10 trees were inspected during the bloom-to-petal fall period for obliquebanded leafroller infestations; 20 fruits on each of 30 trees were examined for plum curculio damage after petal fall; apple aphid (*Aphis pomi*) infestations and predator incidence were assessed on 10 terminals per each of 10 trees several times during the summer months; and leafminer mines were counted on 10 terminal leaves from each of 10 trees in late summer. Mite populations were assessed 3–4 times during the summer by collecting four 25-leaf samples from each block and brushing them in the lab to count motile forms of phytophagous and predacious mites. Also, from 21–31July, fruit was examined for internal larval feeding damage in each plot by inspecting 20 random fruits on each of 30 trees along the edges and near hedgerows where pressure from immigrating moths was expected to be most severe. Shortly before the respective harvest date in each orchard, 20 fruits were picked from each of 35 trees in each plot: 6 trees grouped in the center of the plot, 12 trees from the mid-interior region (a few rows in from each of the four edges) and 12 trees from the outside edges + 5 extra along one edge designated as being at high risk for apple maggot injury (700 fruits total per plot). In cases where the Reduced-Risk Pesticides plot was separate from the Pheromones+Reduced-Risk Pesticides plot, a total of 16 trees along the "apple maggot edge" were sampled in each plot (860 fruits total per plot). All fruits were inspected for damage caused by diseases and insects, including the three internal Lepidoptera species.

Results

Pheromone trap catches from around the state revealed population patterns similar to those seen in the first year of this study for the different species. Codling moth levels were fairly low to moderate throughout the season in all the blocks, with catches rarely exceeding 10 moths per trap per week, and in many cases considerably fewer than 5 per trap. Abundance of the remaining two species was highly variable, depending on location. In the most western sites, lesser appleworm levels tended to be modest, but oriental fruit moth pressure was sometimes severe, with numbers exceeding 125 per trap per week in one instance. In the eastern orchards, the opposite trend was seen, with OFM scarcely present, particularly during the latter half of the season, and LAW at reasonably high levels (as much as 15--30 per trap per week) in most of these blocks, particularly towards the end of the season and beyond harvest. In all cases, the pheromone ties suppressed trap catches of not only the two target species (CM and OFM), but also LAW, at levels at or near zero. Interestingly, these low or zero-catch patterns were also seen in the pheromone-disrupted plots even during the first flight of these species; i.e., before the application of this season's pheromone tie dispensers. Because a normal number of moths were being caught in the adjacent non-disrupted plots, it must be assumed that either sufficient pheromone was still present from the previous year's pheromone dispensers to effect continued trap shutdown into the spring of this season, or else the previous year's pheromone treatment had had a locally suppressive effect on populations within the plot and few moths were migrating in from other plantings. The suppression of LAW is presumed to be due to the similarity of its pheromone blend (98:2 of Z:E-8 12-OAc) to that of OFM (92:8 of Z:E-8 12-OAc).

Data on European red mite and phytoseiid predators were analyzed by first determining the average density of each for the four times samples were collected from each plot. Densities were compared among the reduced-risk strategies and the standard strategy using analysis of variance. There were significantly more phytoseiids in the Reduced-Risk plots (0.15 per leaf) compared with the Standard plots (0.9 per leaf). There were no differences in European red mite densities between the two treatments. Most phytoseiids identified were *Typhlodromus pyri*; however, there was a difference in species composition between Reduced-Risk plots and the Standards, with T. vulgaris and *Amblyseius andersoni* found at different levels in the two treatments at later sample dates.

Fruit damage at harvest caused by fruit-feeding insects was uniformly low across all blocks and treatments (Table 1), with no statistically significant differences between the Reduced-Risk pesticide blocks, with or without pheromones, and the Grower Standards, similar to the 2002 results. Overall damage by internal-feeding Lepidoptera was somewhat reduced from that year, however, with only six farms exhibiting any internal worm damage in 2003, compared with eight farms in 2002. Some distinct differences did occur among the stratified samples taken within respective blocks, so that for instance, localized damage of up to 13–16% was noted along a specific orchard edge in one case.

In no instance were fruit damage readings statistically correlated with the pest management strategy used. However, for damage caused by internal Lepidoptera, which were responsible for an average of 0.24% fruit damage, the data suggest that this damage might be higher in the two reduced-risk programs. In these programs, the six highest incidences of fruit damaged occurred in either the Reduced-Risk or Pheromone+Reduced-Risk treatments. However, all of these six observations came from just three replicates (farms). Of the arthropod pests, the greatest experiment-wise incidence of fruit damage was due to the summer generations of OBLR (2.2%). The most consistent arthropod pests were plum curculio and tarnished plant bug, although the percent damaged fruit attributable to these pests was quite low. Location in the blocks influenced the proportion of fruit damaged by summer generations of OBLR, plum curculio, tarnished plant bug, and European apple sawfly, *Hoplocampa testudinea*. In all cases, the highest incidence of damage occurred in the exterior sections of the plots.

Table 1. RAMP plots 2003, summary of mean % fruit damage at harvest, across all sites.

Treatment	**Int. Lep stings**	**Int. Lep entries**	**Apple Maggot**	**Plum Curc**	**TarnPl. Bug**	**Rosy Apple Aphid**
Pheromones +Red.-Risk Pstcs	0.15	0.20	0.02	0.29	0.85	0.00
Reduced-risk Pstcs	0.35	0.40	0.13	0.33	0.81	0.00
Grower Std	0.19	0.11	0.06	0.33	0.94	0.02

Treatment	**Euro Apple Sawfly**	**Early OBLR**	**Late OBLR**	**San Jose Scale**	**Fruit Scab**	**Summer Diseases**	**% Clean***
Pheromones + Red.-Risk Pstcs	0.04	0.13	2.28	0.19	5.28	3.71	95.85
Reduced-risk Pstcs	0.00	0.06	2.20	0.05	5.97	2.89	95.67
Grower Std	0.03	0.08	2.18	0.08	5.81	4.45	95.98

* Results NSD (P = 0.05, Fisher's Protected lsd test; disease incidence not considered.)

Extensive evaluations of insect pest management programs that use organophosphate (OP) insecticides to control plum curculio, CM, OFM and apple maggot have shown their effectiveness. In addition, because some mite and aphid predators have become resistant to OP's, successful biocontrol of pest mites and aphids has been possible. However, because OP insecticides are toxic to other natural enemies in orchards, it has been difficult to obtain biocontrol of foliar pests such as leafhoppers and leafminers. Also, leafrollers, OFM and leafminers that were formerly of minor importance in orchards, have become resistant to OP's and now must be controlled with other classes of insecticides, many of which are toxic to mite predators. Results from small-plot evaluations of the new more selective, reduced-risk insecticides have shown that these compounds are effective against secondary pests such as aphids, leafhoppers, leafminers, and leafrollers. However, fruit damage from CM, OFM, and apple maggot in plots treated with reduced-risk materials has often been slightly higher than that occurring in plots treated with organophosphates.

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Development of a new paradigm for management of internal lepidoptera in western New York apple orchards

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Abstract: Apples in New York state are attacked by a complex of species of internal lepidoptera, the codling moth *Cydia pomonella* (Linnaeus), Oriental fruit moth *Grapholitha molesta* (Busck), and Lesser appleworm *Grapholitha pr.* In the past, NY apple gowers have obtained excellent control of these insects with organophosphate insecticide sprays for other key pests. During the last several years, damage from internal lepidoptera has gradually increased in the western NY apple production regions. Most of this damage has been caused by the oriental fruit moth. The objectives of this study were to test control programs for oriental fruit moths that can be used in developing more IPM compatible management systems for the complex of internal lepidoptera in western NY apple orchards: (1) Seasonal mating disruption, (2) A positive insecticide program using one spray/generation of OFM and (3) A monitoing program to determine the timing and need for control sprays based on pheromone trap thresholds and fruit damage monitoring. All three of these programs were tested in 10 grower orchareds that were classified as either "high risk" because of previous damage or "low risk". Trap catches were highly variable in research orchards and generally correlated with risk classifications of the orchards. Seasonal mating disruption provided good control of fruit damage except in one "high risk" orchard. Fruit damage from the first two generations of OFM was eliminated in the positive chemical and monitoring treatments except in one "high risk" orchard. These studies suggested that mating disruption may eliminate a need for special chemical control sprays against oriental fruit moth except in very "high risk" orchards. Also, pheromone monitoring traps can be useful in determining the need for control sprays for this pest, but additional work needs to be done to refine threshold levels. Finally, monitoring fruit on trees during the season can accurately detect low levels of infestation, but the technique has to be modified to be practical for growers and/or consultants to use.

Key Words: *Grapholitha molesta,* pheromone trap catch thresholds, mating disruption, integrated pest management

Introduction

Apples in New York state are attacked by a complex of species of internal lepidoptera, the codling moth *Cydia pomonella* (Linnaeus), Oriental fruit moth *Grapholitha molesta* (Busck), and Lesser by the oriental fruit moth. Traditionally, this complex of internal pests has not caused serious damage and New York growers have obtained good control using organophosphate sprays timed for other key pests. During the last several years, damage from internal lepidoptera has gradually increased in the western NY apple production regions. Most of this damage has been caused by oriental fruit moth, but a few orchards have also been infested by codling moth. In 2002, 113 loads of processing apples were rejected from 48 growers in western NY. In 2003, the growers with problem orchards were generally able to obtain control of internal lepidoptera and only 13 loads were rejected from 11 growers. However, many of these growers reverted to calendar spray applied at 14-day intervals, and used harsh materials such as synthetic pyrethroids, which are incompatible with current IPM programs. In 2003, studies showed that resistance ratios to azinphosmethyl of moths from severely infested orchards ranged from 2.1-2.7X, but chemical tests in commercial orchards showed that high rates of organophosphates generally still provided effective control. Studies were also begun to develop a new paradigm for managing oriental fruit moths in NY apple orchards with programs that are more compatible with current IPM systems (Fig. 1).

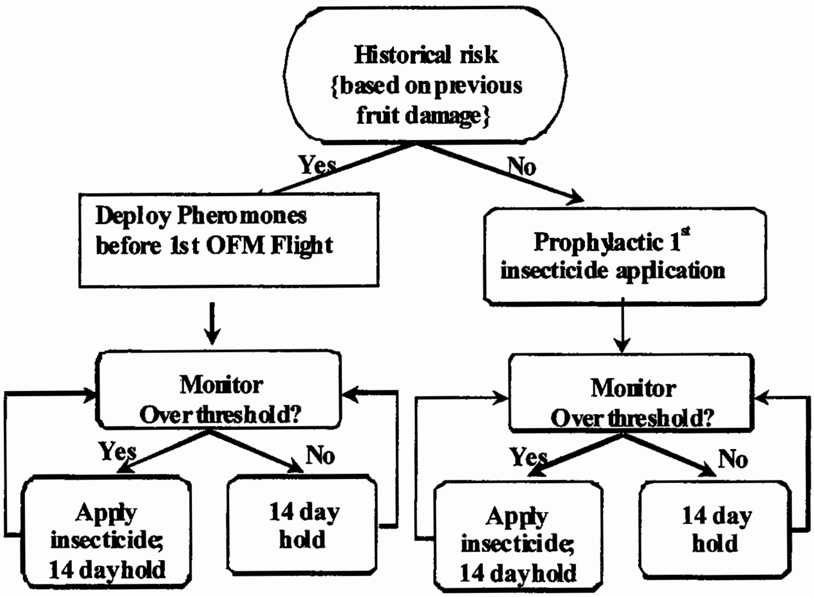

Fig. 1. A proposed new system for managing oriental fruit moth in apple orchards in western NY.

The specific objectives of research conducted in 2004 were to test some of the individual components of this management system in commercial apple orchards. The following three treatments were tested: (1) Seasonal mating disruption, (2) A positive insecticide program using one spray/generation of OFM and (3) A monitoring program to determine the timing and need for control sprays based on pheromone trap thresholds and fruit damage sampling during late summer.

Materials and Methods

Three management systems were compared in 10 commercial apple orchards in western NY. Plots were set up in both "high risk" orchards, which had previously suffered excessive damage from internal lepidoptera, primarily oriental fruit moths, and "low risk" orchards, which had not previously been infested by this complex of pests. Research plots were 5-10A and growers applied recommended insecticide treatments. Two pheromone traps for oriental fruit moths, codling moths and the lesser appleworm were set up in the center of each plot (two additional trap stations for oriental fruit moth were set up in the middle of each mating disruption plot). These traps were checked each week throughout the season until the first week in September. Fruit was sampled every other week (1000 apples/plot, 20 apples on 50 trees) on July 19, August 2, and August 17. Originally a DD model for oriental fruit moths developed at Pennsylvania State University was to be used to predict timing for a control spray at the estimated first egg hatch of each generation: first brood hatch=150DD, base temp=45°F after the initial biofix for the first flight, second brood hatch 1,150DD, and third brood hatch=2,350-2,400DD.

Isomate-M Rosso pheromone ties were deployed in the seasonal mating disruption treatment (200 ties/A) in April prior to the first flight of oriental fruit moths in 5A plots. Growers were advised not to apply special insecticide sprays for oriental fruit moths unless moths were captured in the pheromone monitoring traps deployed in the plots or fruit damage was observed during the summer sampling sessions. The growers were also advised to apply normal sprays against other key insect pests when necessary .

In the monitoring treatment a prophylactic control spray was applied at the pink bud stage to coincide with the predicted hatch of eggs from the first flight of oriental fruit moths. No other special control sprays were recommended for this pest unless moth catches averaged more than 10/trap/week or fruit damage was found in fruit sampling sessions. Other normal control sprays for other pests were recommended when necessary.

In the positive chemical control treatment, one special spray for oriental fruit moths was timed for the estimated first egg hatch of each generation 175-200DD, base temp=45°F) after the biofix of each of the three generations, which was estimated from weekly pheromone trap catches. This method was used because initial observations revealed that the PSU developmental model was not accurately predicting the seasonal activity of oriental fruit moths. The first spray was timed at pink during the middle of May. The second spray was recommended during the third week of July, and the final spray for the third generation was recommended during the last week in August.

The growers were not restricted in their choice of insecticides for the various control programs, and they used a wide variety of materials: chlorpyrifos (at pink only), phosmet, azinphosmethyl, fenpropathrin, lambda Cyhalaothrin, Indoxacarb,and Methoxyfenozide.

Results

The seasonal development of oriental fruit moths was later than normal during the 2004 growing season in NY, because of generally cool, rainy conditions. The first flight began on May 11, and ended on June 1. The second flight began on July 13, and ended on August 2, and the third flight began during the last week in August, and continued into September, when trap monitoring was discontinued (Fig. 2). The DD model developed at PSU did not accurately predict activity and predictions from this model lagged behind the observed flight patterns for each generation.

In the mating disruption plots, no moths were captured in the pheromone monitoring traps during the entire season in any of the research orchards. Codling moth catches were high in two of the orchards under mating disruption for oriental fruit moths.

No damaged fruit was observed in 8 out of 9 research orchards set up during the 2004 growing season in samples conducted in July and August. However, damage was observed in one high risk orchard in all treatments on all sampling dates. This damage was quite low in the mating disruption plot on the first two sampling dates (0.8%) and ranged from 0.5 to 1.1% in the monitoring plot. Damage in the positive chemical control plot ranged from 1.7-3.2% on the different sampling dates. Low damage (0.1%) was observed in another orchard in all treatments, but trap catches suggested that this damage was due to codling moth and not oriental fruit moth.

No oriental fruit moths were captured in any of the pheromone mating disruption plots during the entire season. No fruit damage was observed in this treatment, except in the one high risk block, which also had damage in the other treatments. A trace of damage was also observed in the mating disruption treatment in another orchard (0.1%), that also had identical damage levels in the other plots. Judging from the trap captures, in this orchard, this slight amount of damage was probably due to codling moth damage rather than an infestation of oriental fruit moths.

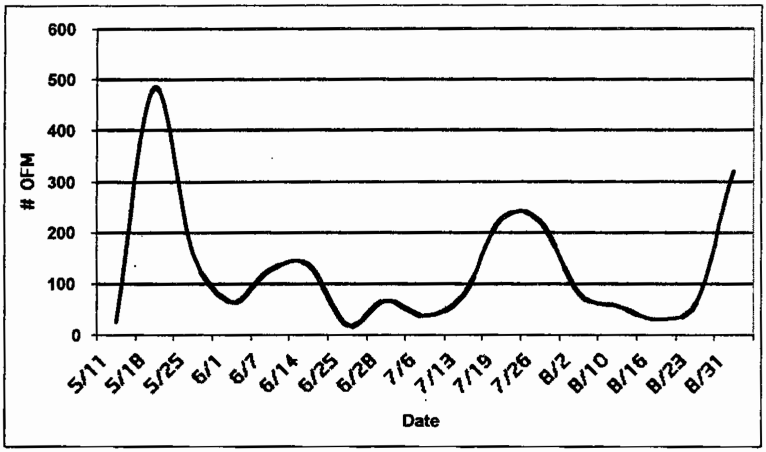

Fig. 2. Seasonal flight patterns of oriental fruit moths in NY apple orchards in 2004.

During the first flight of oriental fruit moth, trap catches exceeded the monitoring threshold of 10/ moths/trap in 5 of the orchards . Only 2 orchards exceeded the threshold level for the second generation, and only one orchard, which also had high catches during the previous generations, exceeded the threshold during the third generation. In general, the orchards in which trap catches exceeded threshold levels had been classified as "high risk" and catches in the "low risk" orchards usually remained below the threshold throughout the season.

Discussion

Final conclusions can not be made from this study until fruit harvest damage evaluations are completed and grower spray records are summarized. However, the preliminary results suggest that seasonal mating disruption can eliminate the need for special chemical control sprays against oriental fruit moths in NY apple orchards except in very high risk orchards. It appears that pheromone monitoring traps can be useful in determining the need for control sprays during the summer for oriental fruit moths, but additional work is needed to refine and test these threshold levels. Monitoring fruit on trees during the season can accurately detect low levels of fruit infestation, but this technique is currently too laborious to be used by growers on consultants. In the future, more rapid fruit sampling plans could be developed to detect defined fruit damage levels that will result in unacceptable risks for marketing fruit.

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Ecologically determined differences in the insect-host plant relations between various populations of codling moth

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Abstract: Host plants of insects may change due to agricultural activity of economy. As a result new agro-ecological conditions force phytophagous pests adapt to more narrow or enlarged assortment of host plants. The objective of this work is comparative study of fodder preferences of codling moth, i.e. the choice of plant to oviposit. Codling moth is a oligophagous insect. Its main host plant is apple-tree. In Armenia the pest damages also apricot, walnut, quince, pear and some other fruits. We have analyzed the oviposition behavior of codling moth females from apple and walnut monoculture orchards. Thus, our results show the existence of food preference mechanism in pest insect . Particularly it is expressed, , in the oviposition behavior of insect.

Key words: host plant, fodder, codling moth, monoculture orchards

Introduction

Host plants of insects may change due to agricultural activity of economy. Renovation of old orchards, introduction of new resistant to pests sorts of agricultural plants leads to alteration of insect habitats. As a result new agro-ecological conditions force phytophagous pests to adapt to more narrow or enlarged assortment of fodder plants (Becerra, 1997; Michaud, 1990, Azizyan, Ter-Hovhannesyan, 2000). Insects are known to possess ecological tolerance (Natale, *at al.*, 1999), but the change of plants influences on the number and areas of insect populations (Via Sara, 1990).

It is known that during the reproduction the instinct of progeny to food is expressed in insects (Thompson, Pellmur, 1991). Particularly, females lay eggs in sites where the future larvae will be supplied with food (Larsson, Strong, 1992; Singer, Moore, 1991).

The objective of this work is comparative study of fodder preferences of codling moth, i.e. the choice of plant to oviposit. Codling moth is an oligophagous insect. Its main host plant is apple-tree. In Armenia the pest damages also apricot, walnut, quince, pear and some other fruits. Earlier we have demonstrated that insects inhabiting various fruits have various fodder preferences (Ter-Hovhannesyan, Azizyan, 2003). This pest is able to synchronize its life cycle with the fruiting period of its host Here we have analyzed the oviposition behavior of codling moth females from apple and walnut monoculture orchards.

Material and methods

Two populations of codling moth from foothill zone of Armenia were studied: the one in apple orchard and the other in walnut. Insect inhabiting this zone has two generations a year. Diapausing larvae were collected with catching belts that were replaced every 15 days during all the vegetation season. The collected material was allowed to over winter in a cold room at 3-5^{0}C. In spring reactivated insects were used to test their oviposition behavior. For this moths were allowed to chose fodder (apple and walnut) or non-fodder (poplar and oak) plants (branches with leaves and fruits or extracts of these plants) to lay eggs. After 5 days the number of laid eggs was calculated. Breeding of codling moth, mating and oviposition were performed under condition 25-26^{0}C and 60% relative humidity.

Results

In the experiment where branches with leaves of host (apple) and non-host (poplar and oak) plants were exposed, the moths laid eggs mainly on the apple leaves (89%). The number of eggs on the oak leaves was as small as 8%, on poplar – only 3% (Fig.1). So, moths avoid non-fodder plants to lay eggs.

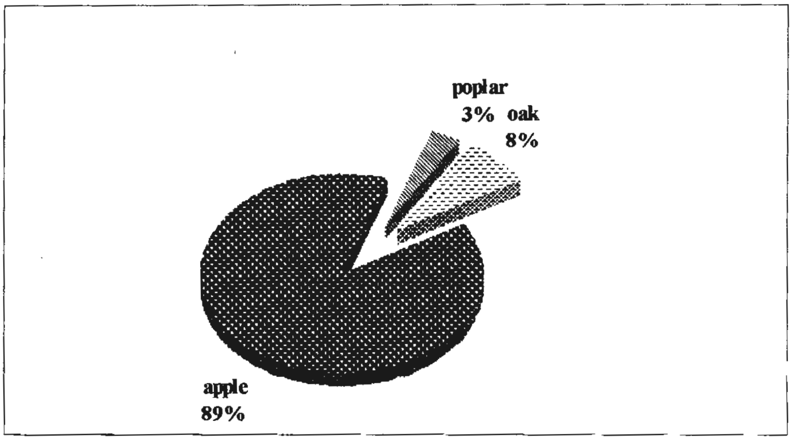

Figure 1. Distribution of eggs on the host and non-host plants laid by codling moth

In the next experiment moths from apple and walnut populations were allowed to choose leaves and fruits of both plants to lay eggs. The moths inhabiting apple orchard preferred apple (68,1%), and insects from walnut orchard – walnut (74,9%). The same pattern was observed when plants were substituted with extracts of leaves and fruits (Fig.2). So, the plant substances attracting insects are volatile, specific for every plant and provide the interaction "host plant-insect". It is obvious that these substances can induce chemotactic reactions of the insect.

The results obtained suggest that host plant forms fodder preferences of insects, probably, as a sequence of insect long-term adaptation reactions.

Codling moth larvae from apple and walnut populations were set on their own and other fruits. It was shown that change in fodder leads to the drop of insect viability and prolongs the terms of larva stage (Table1). So, the insect fodder preferences are important and essential for insect population survival.

Moths of the first and second generations were exposed to extracts of apple leaves and fruit to lay eggs. Moths of the first generations were attracted with both substrates (55,6 and 44,4 %, accordingly differences are not confidential). Moths of the summer generation were attracted with fruit in 70,1 %, i.e. the fruit extract was more preferable (Fig.3). So, the insect is able to recognize not only its own host plant but also different parts of plant and changes its fodder preferences dependending on plant phenology (Lombaria, Derridj, 2002). There are literature data on the alteration of attractability of plant parts for insects during vegetation season because of changes in biochemical composition (Bengtsson *at al.*, 2001, Stadler, Buser, 1984). It is a result of production of new secondary metabolites attractive for insects. Moreover, alterations in insect metabolism take place together with seasonal plant biochemical changes (Bernaus, 1996; Simson, Raubenheimer, 1993; Schoonhoven, 1968, 1972).

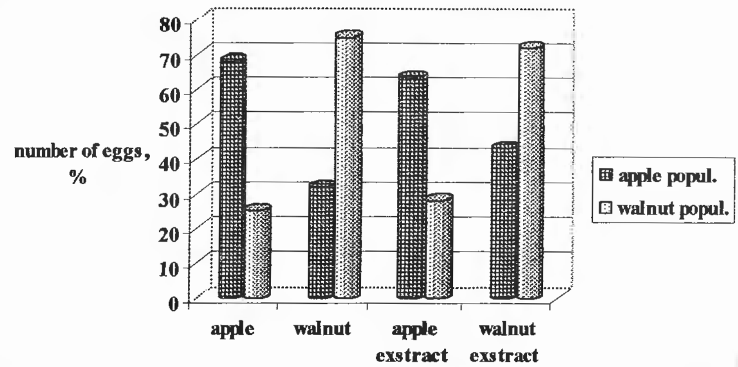

Figure 2. Distribution of eggs on the different fodder by apple and walnut populations of codling moth

Table 1.Development rate and viability of larvae fed with own and other fruits

Experimental variants	Number of larvae, n	Longevity of larvae stage, days	P	Number of imago, %
Larvae from apple populations –on walnut	130	21.3± 0.42	>0.01	36,1
Larvae from apple populations – on apple	98	19.1 ± 0.26	Control	69,4
Larvae from walnut populations- on apple	148	23.4 ± 0.09	>0.01	42,8
Larvae from walnut populations- on walnut	123	17.6± 0.13	Control	57,7

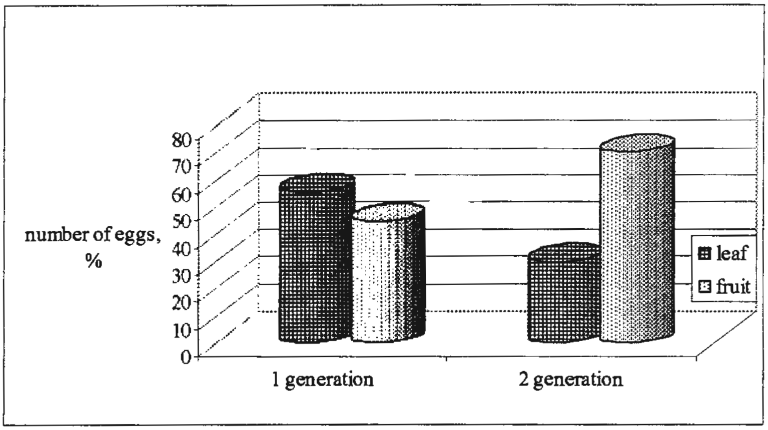

Figure 3. Distribution of eggs on plant parts laid by 1st and 2nd generations of codling moth

Thus, our results show the existence in pest insect the mechanism of food preference. It is expressed, particularly, in the oviposition behavior of insect. This insect feature is obviously adaptive and at the same time has vital importance for insect population survival. It is reasonable to note, that genetically oligophagous insect codling moth may express a trend to turn into monophagous organism under the limitation of host plant assortment. Illustrations of this trend may be seen codling moth populations from monoculture orchards. The ability of egg laying moth to recognize host plant attractive substances may be used in systems of integrated pest control.

Acknowledgements

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Spatial analysis of pheromone trap catches of *Cydia funebrana*, *Cydia pomonella*, *Cydia molesta* and *Anarsia lineatella*: contribution to the IPM in fruit crops

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Abstract: The study, realized in the Molise region (Central Italy), describes the temporal and spatial distribution of the pheromone trap catches of *Cydia funebrana* (Treit.), *Cydia pomonella* (L.), *Cydia molesta* (Busck) and *Anarsia lineatella* (Zell.). *Cydia funebrana* case - Spatial relationships between catches of male Plum Moth were studied (in 1999) at local scale inside and around a single plum orchard. Kriging procedures applied to trap catches showed that the number of males was lowest in the interior of a plum orchard and highest around a ravine, near irrigation channels and hedgerows in the orchard surroundings. *Cydia pomonella* case - Local distribution and dispersion patterns of Codling Moth were investigated (from 2000 to 2002) in two heterogeneous agro-ecosystems. Trap catches of male moths were clumped during almost all sampling weeks. Spatial characterizations obtained illustrate that the high trap capture foci were confined to the productive apple orchards. *Cydia molesta* and *Anarsia lineatella* case - Local distribution and dispersal patterns of Oriental Fruit Moth and Peach Twing Borer (2002 and 2003) were considered in a heterogeneous agro-ecosystem. Results showed that the main hot spot for both pests was in a peach orchard in the northern zone of the study area; other infested areas were in peach orchards and, in the case of *A. lineatella*, also in plum orchards. The river and the irrigation channels seem to act as barriers rather than ecological corridors.

Key words: orchards, geostatistics, spatial dynamics, heterogeneous agro-ecosystems.

Introduction

With the development of Integrated Pest Management (IPM) there has been an increase in the use of a variety of management tools to assist in the reduction of chemical control and to the development of more efficient monitoring strategies. These strategies depend on understanding specific insect behaviours and need precision targeting of interventions to maximize their efficacy. In this connection, geostatistical methods can be a powerful tool for the understanding of many spatially-related phenomena. The analysis can provide crucial information for improving sustainable pest control techniques in the context of precision IPM (Fleischer *et al.*, 1999; Sciarretta *et al.*, 2001; Trematerra *et al.*, 2004). In our paper the results obtained in spatial analysis of pheromone trap catches of *Cydia funebrana* (Treit.), *Cydia pomonella* (L.), *Cydia molesta* (Busck) and *Anarsia lineatella* (Zell.) are reported. The researches have been realized from 1999 to 2003 in the Molise region, Central Italy.

Material and methods

Study areas

C. funebrana monitoring was conducted in a plum orchard (Stanley cultivars), and in the surrounding area covering a zone of about 250 ha. The orchard is surrounded by cereal and sunflower fields; vegetables are grown between the orchard and a ravine with riparian vegetation including shrubs and blackthorn trees (Figure 1A). Sampling of *C. pomonella* was carried out in two heterogeneous agro-ecosystems (50 ha each), located 750-850 m above sea level, in hilly and mountainous landscapes. The agro-ecosystem I is characterized by the presence of two apple orchards, with different cultivars, about 350 m apart; the rest of the area comprises cereal and vegetable fields, and uncultivated fields and woodlots (Figure 2A). In agro-ecosystem II, there are two adjacent apple orchards, surrounded by cereal and vegetable fields, where abandoned apple, pear, service and walnut plants grow. In the case of *C. molesta* and *A. lineatella*, the experimental area, about 250 ha, consists of small plots of peach, plum, apple, pear and kiwi orchards, alternated with field and vegetable crops, irrigation channels, hedgerows and a river that divide the area into an eastern and a western sector (Figure 3A).

Data collection
The activity of adult males was monitored weekly using pheromone sticky traps (pagoda type for Codling moth, delta types for the other pests), baited with the specific synthetic sexual blends. The positioning of sampling points in the various cases was carried out as follows:

- *C. funebrana*: 15 traps were placed in 1999 inside and around the plum orchard.
- *C. pomonella*: in the agro-ecosystem I, 16, 20 and 26 pheromone traps were positioned during the years 2000, 2001 and 2002, respectively; in the agro-ecosystem II, 12 and 16 pheromone traps were placed during the 2001 and 2002.
- *C. molesta*: the activity of males was monitored using 26 traps, during 2002 and 2003. *A. lineatella*: 26 traps were positioned in 2003 at the same location of *C. molesta* traps.

Spatial analysis
Spatial analysis was carried out using Surfer Version 6 or 8 (Golden software, Golden, CO, USA) with x, y representing the coordinates and z the trap counts. The interpolation algorithm was kriging: exponential models were used for *C. pomonella*, linear models with zero nugget in the other cases. The interpolation grid obtained is represented graphically by a contour map, which shows the configuration of the surface by means of isolines representing equal z-values; a base map showing the experimental area, with the same coordinate system, was placed on top of the contour map. For a full explanation of methods, refer to Brenner *et al.* (1998).

Results and discussion

Cydia funebrana ***case***
High levels of *C. funebrana* trap catches inside the orchard occurred almost always near the borders, while lowest catches were obtained in the interior part of the field (Figure 1B). The zones outside the orchard with highest trap catches are located in and around the ravine with shrubs, including blackthorn, follow irrigation channels and hedgerows and arrive at the contact between irrigation channels and orchard; the lowest trap catches occurred on the opposite side of the orchard. Observed distribution of *C. funebrana* indicates high dispersal capability throughout the whole period flight. In particular, irrigation channels and hedgerows appear to serve as ecological corridors, where adults move from one zone to another. On the opposite side, arable fields act as a barrier to the movements of pest.

Cydia pomonella ***case***
Trap catches of male moths were clumped during almost all sampling weeks. Spatial characterizations obtained illustrate that the main trap capture foci were confined to the productive apple orchards for both agro-ecosystems (Figure 2B).

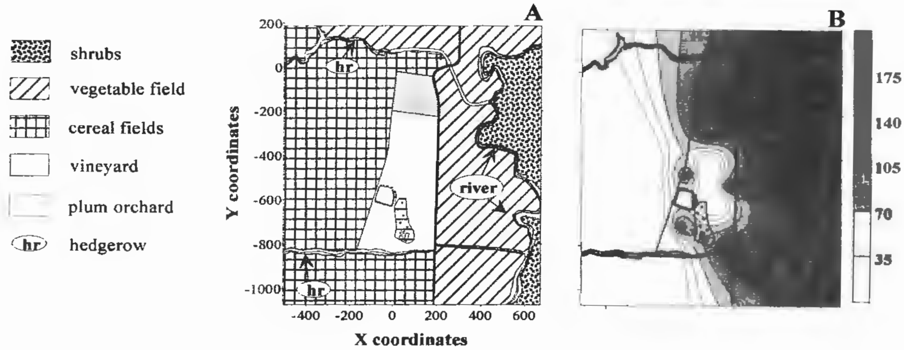

Figure 1A-B. *Cydia funebrana*: representation of the experimental area with important landscape elements (A); spatial distribution of male annual catches (year 1999) (B).

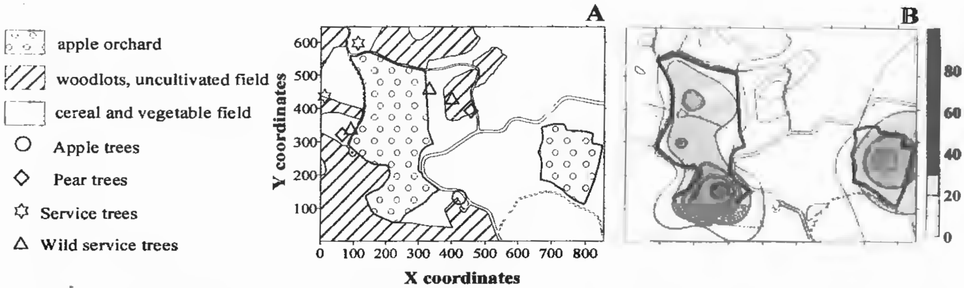

Figure 2A-B. *Cydia pomonella*: representation of the experimental area with important landscape elements (A); spatial distribution of male annual catches in agro-ecosystem I (year 2002) (B).

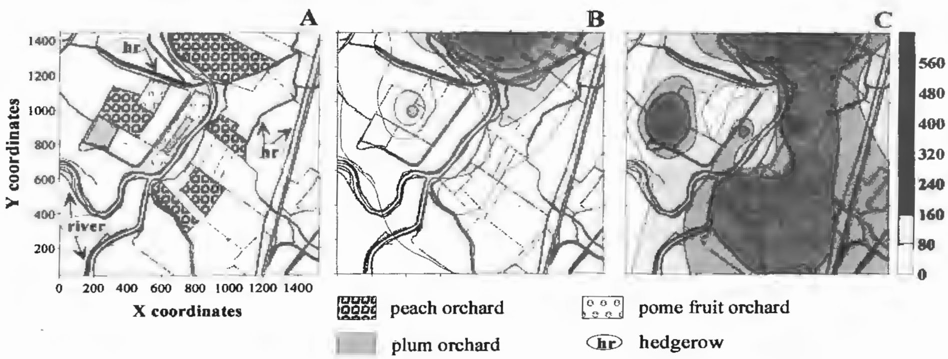

Figure 3A-C. *Cydia molesta* and *Anarsia lineatella*: representation of the experimental area with important landscape elements (A); spatial distribution of *C. molesta* male annual catches (year 2003) (B); spatial distribution of *A. lineatella* male annual catches (year 2003) (C).

High trap catch foci occurred also in the group of apple trees next to the orchards and, in the agro-ecosystem II in the zone with pear trees, service trees, and walnut trees. The highest pest density zones were isolated from each other, and only in few sporadic cases males were counted in traps located outside these zones. This distribution seems to indicate a low dispersal capability of codling moth males during the flight period.

Cydia molesta *and* Anarsia lineatella *case*

Results showed that the main hot spot for both pests was in a peach orchard in the northern zone of the study area (Figure 3B-C). *C. molesta* distribution extends southward in the other peach orchards, but with less intensity. Outside these zones, very few individuals were collected, mainly in pome fruit orchards during August and September (Figure 3B). Spatial distribution does not change between different flights and in the two years. *A. lineatella* distribution shows strong hot spots located in the stone fruit orchards, but infested zones involve also large areas on the western side of the river, mainly hedgerows with wild shrubs (Figure 3C). Spatial distribution of the three flights appears very different. The river and the irrigation channels seem to act as barriers rather than ecological corridors in both pests, but with different levels of efficacy, probably linked to the effective dispersion of the species in different patches.

The observed distribution patterns appeared very different in the four pests, *C. funebrana*, *C. pomonella*, *C. molesta* and *A. lineatella*, and seemed to be determined mainly by the location of the most important host plants and by the propensity of males to move in the environment, inside and outside the breeding and mating sites. Important landscape elements such as riparian vegetation and hedgerows can act as ecological barriers or corridors depending on the specific behaviour of a species.

On the basis of our results, adequate knowledge of both behavioural aspects of moths and landscape features must be achieved to improve the efficacy of precision IPM tactics. This is particularly important in heterogeneous landscapes, where potential sources of external infestation can invalidate some pest management methods employed in orchards (such as mating disruption or attract & kill methods). Indication obtained from spatial maps should be considered as an essential prerequisite for IPM precision targeting programs and incorporated in the development of monitoring and control strategies.

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The potential of integrating the Sterile Insect Technique as an environmentally friendly method for area-wide management of the codling moth (*Cydia pomonella*)

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Abstract: Over the last decades, numerous successful intervention programmes have validated the concept of managing economically important insect pests by manipulating their reproductive ability. A key pest of most pome fruit and walnut orchards in the temperate regions of the world is the codling moth (*Cydia pomonella* L.). In view of increased resistance of the codling moth to various broad-spectrum insecticides, the sterile insect technique (SIT) and inherited sterility (IS) could offer great potential as an additional component of area-wide integrated control campaigns. The operational releases of sterile codling moths in a suppression programme in Canada since 1994, has demonstrated the technical feasibility of the technology. This area-wide programme has resulted in a drastic decline in the retail and application of insecticides and in no detectable codling moth damage in more than 90% of the orchards. The SIT/IS has important advantages as compared to other control methods i.e. it is species specific, friendly to the environment, inversely density dependent and yields synergistic effects when combined with various other control techniques. Significant progress is being made with research supported by the FAO/IAEA on i.) the improvement of sterile codling moth quality control and management, ii.) mating compatibility of strains originating from geographically distinct areas and iii.) the development of genetic sexing strains. It is anticipated that the results of these research activities will enhance the efficiency of the SIT/IS for codling moth control.

Introduction

Many insect pests of agricultural importance continue to be controlled by the extensive use of broad-spectrum insecticides, resulting in major resistance problems, increased residues in food and contamination of water and the environment (Hendrichs, 2000). Consequently, there is a growing demand for more sustainable pest control approaches and methods, which are friendly to the environment and take into account the rising concerns about food safety, food quality and biodiversity. As a result, the concept of area-wide integrated pest management (AW-IPM), has gained considerably in acceptance.

The AW-IPM approach targets *entire* insect populations within a circumscribed area. Although this approach is area-bound and management intensive, and requires the involvement of grower organisations and of local or regional authorities, it results in more sustainable long-term pest control. Contrary to traditional localised pest management, in which individual growers address a pest problem on a field-by-field basis and do not deal with the pest insects in surrounding wild hosts, abandoned orchards and backyards, the AW-IPM approach is pro-active (action is taken before a damaging population develops) and protects large and small growers, enhancing agriculture in a whole area. The sterile insect technique (SIT) is one of the most effective tools for integration into such AW-IPM programmes.

Past control efforts against the codling moth (CM), *Cydia pomonella* (L.) epitomise the above-mentioned concerns. Increased resistance to broad-spectrum insecticides, the disruption of natural control of the secondary pest complex, the reduction of acceptable insecticide residue levels in major fruit importing countries and the withdrawal of a number of other essential insecticides have prompted and accelerated the search for viable alternative control methods. The application of synthetic insect growth regulators, the use of a granulosis virus and methods such as 'mating disruption' and 'attract and kill', have to a certain extent reduced the sole reliance on broad-spectrum insecticides for CM control. However, many of these control methods have limitations and are very often only effective when the CM population pressure is low.

The use of the SIT and inherited sterility (IS) could provide a practicable and cost-efficient control tool to supplement the already available suppression methods. Screening of the most important lepidopteran pests against factors such as global economic importance, rearing feasibility, radiation biology, migration behaviour, host range etc. the CM was considered a good candidate for the application of the SIT as part of an area-wide integrated intervention approach. (FAO/IAEA, 2000).

The use of SIT against economically important insect pests including Lepidoptera

The concept of suppressing, excluding or eliminating economically important insect pests by affecting their reproductive behaviour was conceived by E. Knipling in the 1930's (Knipling, 1955). The SIT relies on the production of large numbers of the target insect in specialised production centres, the sterilisation of one or both of the sexes, and the sustained dispersal in the natural habitat in numbers large enough to allow sterile males to out-compete wild males for matings with wild females. The concept was first demonstrated on the island of Curaçao in 1954, where the New World Screwworm Fly (*Cochliomyia hominivorax*) was driven to extinction through the sustained, sequential release of sufficient numbers of sterile males (Baumhover et al., 1955). These encouraging results ultimately culminated in the largest and most successful SIT-based eradication campaign ever undertaken against a major insect pest i.e. the elimination of screwworm from the Southern US, Mexico and Central America (1957 - 2002) (Wyss, 2000). The SIT has likewise been used successfully for suppressing, excluding or eliminating major crop pests such as the Mediterranean fruit fly (*Ceratitis capitata*), the melon fly (*Bactrocera cucurbitae*), the Queensland fruit fly (*Bactrocera tryoni*), the Mexican fruit fly (*Anastrepha ludens*) and the West Indian fruit fly (*Anastrepha obliqua*) (Cayol et al., 2002).

With respect to genetic pest control, Lepidoptera represent a special case in view of their high resistance to radiation (LaChance et al., 1967), which has negative consequences for their competitiveness in the field. In the IS (F_1 sterility) approach, the moths are subjected to lower, sub-sterilising doses of ionising radiation, which reduces the negative effects of the radio-resistance and results in more competitive insects. After the mating of released sub-sterile male moths with wild females, the F_1 male generation will be completely sterile and inherit the radiation induced deleterious effects to the next generation. Moreover, as female moths are in general more radiosensitive than males of the same species, a careful selection of the sub-sterilising dose will allow the release of completely sterile females and partially sterile males.

The area-wide application of the SIT against a major lepidopteran pest, the pink bollworm (*Pectinophora gossypiella*), has since 1968 successfully prevented the establishment of the pest in the San Joaquin Valley, California protecting a cotton crop with an annual value of US$ 1 billion (Bloem et al., in press). The AW-IPM programme against the CM in British Columbia, Canada is another fine example of the successful suppression of a major lepidopteran pest through the release of sterile insects. A pilot programme, conducted from 1976 to 1978 in the Similkameen valley of BC, demonstrated that CM populations could be eliminated by a combination of suppressive action with chemicals and the release of sterile moths (Proverbs et al., 1982). The SIT programme in the Okanangan Valley of BC was initiated in 1992, with the construction of a mass rearing facility and releases of sterile moths starting in 1994. The trap catches of the wild CM population have decreased in the first intervention zone from an average of 13 and 2.5 moths/trap/week in 1995 for the first and second generation, respectively to an average of 0.08 moths/traps/week in 2000 (Bloem et al., in press). The proportion of orchards in the first zone with no detectable CM damage had increased from 42% in 1995 to 91% in 2000. In addition, the retail of organophosphate insecticides has declined from 18,903 kg in 1991 to 3,403 kg in 2001 (Bloem et al., in press).

Advantages and limitations of the SIT and economic considerations

Like any other control tactic, the SIT has certain advantages and limitations. Especially its non-polluting character and species specificity makes the technology particularly friendly to the environment. There is no risk of the development of resistance (provided adequate quality assurance is practiced in the production process), and, unlike other biological control methods, the released insects cannot become established in the target area and attack non-target species. The sterile insects are usually released from the air making intervention possible even in difficult to access areas such as forests, mountains or in areas of civil unrest. Arguably the most important benefit of the technique is its inverse density-dependency i.e. the SIT becomes more efficient as the natural population declines and increasing sterile to wild ratios are obtained.

Most insect pest populations occur in high densities, however, and suppression is required before the SIT can be applied. The release of sterile insects will not result in a rapid 'kill of' of the native pest insects and as a result there is a 'delayed effect' on the number of insects present. The technique is susceptible to the immigration of already mated females from the surroundings, and thus needs to be applied on an area wide basis (above mentioned limitations also apply for methods such as mating disruption). In addition, the technology requires a comprehensive knowledge of the ecology and mating behaviour of the insect, the colonisation and mass production of the target insect must be feasible at a reasonable cost, the competitiveness of the sterilised insects should be as close as possible to that of the native insects and the sperm of sterile males should be as competitive as the sperm of the native males.

The integration of SIT in AW-IPM prevention, eradication and suppression strategies, have resulted in enormous economic benefits. Benefit to cost ratios between 146 and 400 have been calculated for fruit fly prevention and eradication programmes in California, Mexico-Guatemala and Chile. In the SIT fruit fly suppression programme in South Africa, the substitution of insecticides with the release of sterile males has resulted in annual direct benefits of US$ 370,000 or a cost to benefit ratio of 1.8 to 1 for the 2001/2002 season (Enkerlin, in press). Data on the potential benefits of SIT-based AW-IPM approaches against CM are scarce, but one economic study revealed that the elimination of the CM from Syria over a period of 15 years, would result in a benefit-cost ratio of 3.98, an internal rate of return of 42% and a return on equity of 5.9 (including both direct and indirect benefits) (Walther Enkerlin, unpublished IAEA report).

The potential of integrating the SIT/IS with other pest management strategies for CM control

No singular strategy can be expected to manage a pest problem in an optimal way and IPM specifically aims at defining strategies, which can be combined without having deleterious effects, and which preferably have an additive or even synergistic outcome. The compatibility of the SIT/IS with control methods such as synthetic pheromones, natural enemies, host plant resistance, entomo-pathogens and insecticides has been demonstrated in the laboratory or in the field for several lepidopteran pests (Carpenter et al., in press). The compatibility emanates from the similar deleterious action of the methods used against the irradiated and the wild insects. As such, the use of insecticides and of the host plant defences during a continuous release of irradiated insects would benefit the intervention programme, as the killing agent would both destroy the irradiated and native insects in the same proportion. Continued releases of irradiated insects would each time increase the sterile to native insect ratio, making the programme more effective.

The integration of inundative releases of parasitoids with sterile insects will likewise result in additive and synergistic effects (Knipling, 1979), provided that the parasitoids strategies do not negatively impact irradiated insects and their progeny more than that of the wild population (Carpenter et al., in press). The effectiveness of SIT would increase the ratio of adult parasitoids to adult hosts, whereas the effectiveness of parasitoids increases the ratio of sterile to fertile insects. The combination of these 2 techniques could be 10,000 more efficient that each technique used alone (Knipling, 1992). Even greater suppression can be anticipated when the use of IS can be combined with the release of parasitoids i.e. the IS produces sterile F_1 larvae or eggs which can be used as hosts for the released parasitoids.

Although these examples are impressive, caution should be exercised in extrapolating these results to species such as the CM. Little data are available for CM, although combining 'mating disruption' with IS proved to be compatible in Washington State, US (Bloem et al., 2001) and releasing sterile CM with parasitoids in large field cages resulted in less fruit damage than either method used alone (Bloem et al., 1998). More research and pilot trials will be needed to assess which combinations will provide the best synergistic effects with CM.

Recent developments to enhance the SIT for suppression of Codling Moth

The Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, in Vienna, Austria, has the mandate to assist Member States with the development of the SIT for integration in AW-IPM programmes against major livestock and crop insect pests. This mandate is accomplished through a series of normative functions (training courses, meetings, publications etc.), field application through Technical Cooperation Projects and Research and Development. As part of the latter, an FAO/IAEA Coordinated Research Project entitled 'Improvement of CM SIT to facilitate expansion of field application' was initiated in 2002 with the goal to coordinate focussed research over a 5-year period to improve CM SIT for application in orchard and urban areas internationally. Since its inception, significant progress is being made in the area of CM rearing, quality management and genetics and some of the results to date can be summarised as follows:

One of the problems of current CM SIT is the reduced quality of non-diapausing sterile moths in the first generation of releases. The combination of the use of diapause and radiation treatments with sub-sterilising doses showed significant improvements with respect to recapture days after release. Dispersal studies with CM males treated with a radiation dose of

250 – 350 Gy also indicated higher catches in pheromone traps at different distances from the central release point with the lower doses.

The economical agar-free larval diet used in the CM-SIT programme in BC, Canada allows for the collection of adult moths with minimal labour input. However, the collection of pupae is difficult, precluding the shipment to potential customers in distant locations. A combination of water sprays and desilking agents was used to extract successfully CM cocoons from the sawdust-based diet. Emerging adults appeared normal.

CM populations consist of long and short distance flyers and these flight characteristics are inheritable. Studies showed that mobile and sedentary strains can be selected and significant correlations were found between mobility and longevity, fertility, female size and the intrinsic rate of increase (r_m), i.e. characteristics important for the selection of desirable CM strains for SIT programs.

CM is wide spread in the temperate regions of the world, but no information is available on the mating compatibility of the populations originating from different geographical regions. Small cage and field studies using moths originating from Canada and South Africa indicated the complete absence of mating barriers. Studies among other populations are in progress.

Initial data of field studies carried out with the CM egg parasitoids, *Trichogramma nerudai* and *T. cacoeciae* in experimental apple orchards in Argentina showed that both parasitoids were capable of infesting sterile and partially sterile CM eggs, suggesting that there is potential for the integration of biological control methods with SIT against the CM.

Based on the experience with the Mediterranean Fruit Fly it is assumed that the efficiency of SIT against the CM could be significantly improved by releasing male moths only. This would require the development of a genetic sexing strain and the approach of using dominant conditional lethal mutations (DCLM), linked to the female-determining chromosome (W), would offer the best potential (Marec et al. submitted). Basic genetic research has shown that the karyotype of the CM consists of 2n=56 chromosomes, with a sex chromosome mechanism of the WZ type and with females being the heterogametic sex. The W and Z sex chromosomes are well differentiated making CM suitable for sex chromosome-based genetic sexing. In addition, transgenesis of CM has been achieved through the use of the *piggyBac* transposon and lines with a green fluorescent protein have been maintained in the laboratory for over 37 generations, indicating the stability of the transgene in these lines. Preliminary assessment in transgenic CM of temperature sensitivity of a truncated form of the *Notch* gene, N^{60G11}, indicated that this temperature sensitive DCLM could be useful in development of genetic sexing lines. The use of this approach would have the advantage that following the insertion of the conditional lethal gene into the W chromosome, all females would die following the application of the restrictive condition, but the males used for sterilization and release would not contain the transgene.

Future prospects

The increased intensification of cropping combined with the growth of monocultures will continue to create future imbalances of the agricultural environment. More attention is therefore warranted to the preservation of biodiversity, the promotion of control methods friendly to the environment and the reduction of noxious and toxic chemicals. The SIT programme in British Columbia has demonstrated the technical feasibility of integrating the release of sterile CM in an AW-IPM programme. The success of this programme is, however, not a warranty that the SIT technology for CM is 'perfect' or 'failure proof'. The FAO/IAEA Coordinated Research Project clearly aims to address some of the shortcomings related to the rearing, radiation biology, genetic sexing, management of the quality of the released insects, monitoring etc. that will require further improvement and refinement to enhance the efficiency of the technique and to reduce its costs.

The increased resistance of the CM to broad-spectrum insecticides, rising public pressure to decrease insecticide applications and to implement more environment-friendly alternatives, and the limited effectiveness of available alternative methods (*e.g.* mating disruption remains expensive and seems only effective at low population densities and resistance problems with the insect growth regulators) has resulted in an increased awareness of the potential of CM SIT in many parts of the world. In that respect, the Governments of South Africa, Argentina, Brazil and Chile have all expressed serious interest in the further development and application of the SIT for CM suppression and various programmes are being initiated and are in different stages of development. Unfortunately, the adaptation of the area-wide concept of pest control in general and the use of the SIT in particular, has never really germinated in Europe, despite the availability of considerable expertise e.g. in the rearing of CM for the production of the granulosis virus. In that respect, increasing the awareness in the private industry of the enormous potential of the SIT (e.g. which would create jobs in the rearing facilities) for CM control would certainly contribute to help turn the tide.

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Components of an ecologically and economically sustainable orchard

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Abstract: We are developing a sustainable system for eastern North American orchards beginning with redesigning the concept of what constitutes an orchard production system. Specific components of the system include the use of compost mulch, companion plantings, interplanting fruit trees with extrafloral nectaries, and a novel planting design to maximize interaction among the components. Inclusion of compost mulch, companion plantings and interplanting of fruit trees producing extrafloral nectar will maximize the functional biodiversity of the orchard and biological control. The novel orchard design is based on the concepts of ecological sustainability but is also based on economical feasibility so that it will be both environmentally sound and practical to implement.

Kew words: compost, companion plants, interplanting, orchard design, functional biodiversity

Introduction

Our goal is to develop a sustainable orchard management program for eastern North America based on biological control. A key aspect of such a management program is maintaining a high degree of functional biodiversity. Integrated Fruit Production with an emphasis on supporting high functional biodiversity will lead to an orchard production system that will maximize the ability of the system to exert biological control of orchard pests (Boller, 2001; Brown, 2001a). Because of high variability in environmental conditions and the large number of potential orchard pests, effective biological control must be through a diverse community of natural enemies. By applying principles of conservation biological control to orchard management we can encourage the development of a stable, diverse guild of predators and parasitoids. Not only is ecological sustainability needed for the continued production of fruit, but economic sustainability is also critical. Only if fruit growers can earn a profit from applying new methods of production will they adopt the practice, whether it is ecologically sustainable or not.

Components

Compost Mulch

Compost addition to the soil is well known to enhance soil structure and to be a tool for sustainable soil nutrition management. Application of a compost mulch to an apple orchard also has been found to enhance the biodiversity of arthropod predators in the detritus-based food chain (Mathews *et al.*, 2002). Brown & Tworkoski (2004) also found an increase in natural enemy diversity with a corresponding decrease in abundance of *Phyllornorycter blancardella* and *Eriosoma lanigerum* by the addition of compost mulch. Brown &

Tworkoski (2004) also found that a compost mulch provided weed control equal to herbicides for up to a year and, in the laboratory, reduced the growth of *Monolia fructicola* mycelium.

Companion Plants

Companion planting of annual flowers in the orchard increases the diversity of insect natural enemies (Jenser *et al.*, 1999) and can replace many traditional insecticide inputs without reducing fruit quality (Brown & Glenn, 1999; Bostanian *et al.*, 2004). In most of the studies using companion plants there has been a reduction in yield either by root competition with the companion plants (Brown & Glenn, 1999) or by reducing the number of trees per ha (Bostanian *et al.*, 2004). For the use of companion plants to be accepted in an orchard management system they must be added to the orchard in a way so as not to significantly reduce yield.

Interplanting

Extrafloral nectaries on peach trees increase the abundance of natural enemies on peach and have been shown to increase the biological control of peach pest insects (Mathews, 2004). When interplanted with apple trees, peach extrafloral nectar also increased the diversity of arthropod predators and parasitoids on adjacent apple trees (Brown & Schmitt, 2001). Interplanting of peach trees has also been shown to provide enough control of most arthropod pests of apple to be comparable to chemical control (Brown, 2001b).

Other Components

Disease resistant cultivars provide the best option for sustainable disease management in apple. The economic sustainability of disease resistant cultivars has been shown by Baicu *et al.* (1997) through reducing the required number of fungicide sprays by more than 50%. Behavior based pest management, being discussed in many presentations at this meeting, also provides very useful tools for sustainable pest management. The key aspect of these approaches is that they allow management of one pest species without disrupting the dynamics of other species in the system. Selective plant protection chemicals that have a narrow spectrum of activity and cause little disruption of the ecosystem are also needed to control those pests that are not adequately controlled by the above components.

Orchard Redesign

Creation of a sustainable orchard that incorporates some, or all, of the components described above will not be an easy task. To maximize the positive effects of these components on orchard functional biodiversity the whole orchard agroecosystem must be redesigned in the sense of Hill *et al.* (1999). Orchards have been designed by horticulturists to maximize production. We need to create a new idea of what an orchard should look like, keeping in mind the horticultural goal of high production but also with input from pest management specialists to maximize the sustainability of the ecosystem. Horticulturists, pest managers, soil scientists, hydrologists, ecologists, economists and others all have to contribute to optimize all the inputs and develop an orchard system that is both environmentally and economically sustainable.

As a starting point for such a redesigned orchard that could incorporate the sustainable pest management components discussed, we suggest a system such as depicted in Fig. 1. This orchard has a traditional width alley between every other tree row to allow tractor traffic to perform the required pest management and horticultural activities. The more narrow alleys between tree rows are for companion plants. Weed control activities, compost application, and other management could be performed to both tree rows from the traditional width allies.

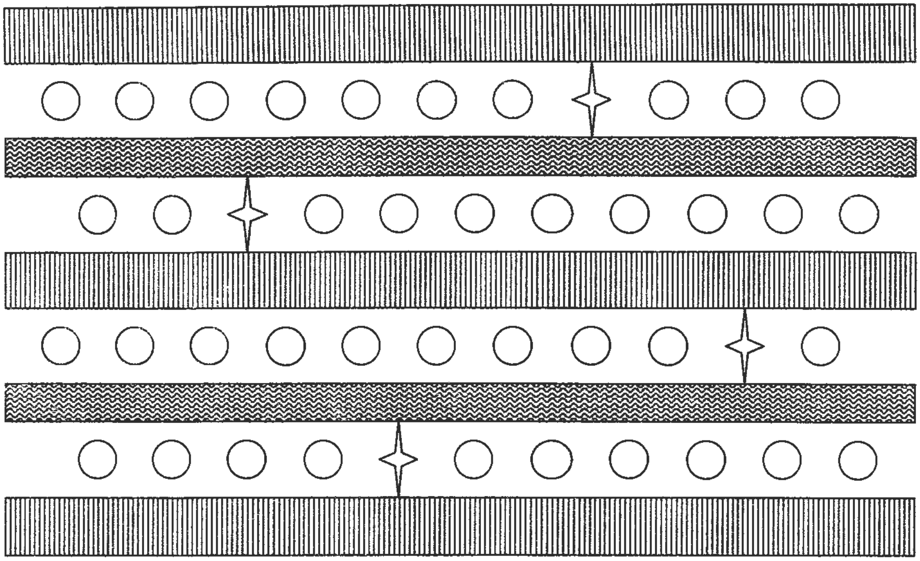

Figure 1. Proposed orchard redesign plan with standard width grass alley (vertical hatching), narrow strip of companion flowering plants (wavy line hatching), interplanted peach trees supplying extrafloral nectar (stars), and apple trees (circles), offset to allow adequate spray coverage on both sides of the trees by spraying from the grass alleys.

The offset planting of the trees within rows would allow for spray treatments to effectively cover both sides of each tree row. Trees within a row would be spaced slightly farther apart to enhance spray coverage between rows but the overall tree density would be the same because there would be more rows per hectare due to every other alley being narrower than a conventional orchard. A few peach trees would replace apple trees to provide a nectar source all season. Research currently underway will provide information to decide the needed density of peach trees to affect biological control, but within this design we suggest 20 to 50 per hectare.

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Role of leaf miners' parasitoids in an IPM sour cherry plantations

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Abstract: Based on results of fundamental and applied research, authors have worked out ingtegrated production techniques of sour cherry which takes the population regulating ability of leaf miners' natural enemies into consideration. The trials were carried out in provocative, conventional and IPM sour cherry plantations using pyrethroids, organo phosphates and selective insecticides, respectively. Among the leaf miners, pear leaf blister moth populations (*Leucoptera malifoliella* Costa) were important, therefore their presence was considered in the management programme. This pest's parasitoids occurred in all the three plantations. The 37,6 % of parasitism observed in the IPM plantation indicated that no particular control of pear leaf blister moth was necessary in the third year of the programme because the population regulation by the parasitoids was shown up. The dominating parasitoids detected in *L. malifoliella*: *Chrysocharis pentheus* Walker, *Minotetrastichus frontalis* Walker, *Pnigalio pectinicornis* Linnaeus, *Closterocerus trifasciatus* Westwood, *Sympiesis sericeicornis* Nees, *Bariscapus nigroviolaceus* Nees.

Key words: IPM, sour cherry, *Leucoptera malifoliella*, parasitoid

Introduction

Sour cherry is the second most important fruit produced in Hungary. The use of safe, successful and environmentally-friendly plant protection is the basis for the economical production of high quality fruit.

In compliance with the guidelines for integrated fruit production, the integrated production of sour cherry has been worked out by giving priorities to pest management methods which minimize the use of agrochemicals in the plantations and consider the role of main natural enemies of the pests. Several results (Balázs, 1992, 1996, 1997, Cross *et al.*, 1999, Mey 1993) obtained in apple plantations confirm that the parasitoids of different leaf miners may well reduce the number of their host animal. The increase in their abundance and number of species can be reached within the plantation by using control techniques safe for the parasitoids and, in exchange, they may well regulate the population (Balázs *et al.*, 1996, Balázs & Jenser, 1999, Jenser *et al.*, 1997).

Furthermore, we wanted to know how the use of various plant protection products influences the populations of pests and their related natural enemies. Therefore, the trials were carried out in provocative, conventional and IPM sour cherry plantations using pyrethroids, organo phosphates and selective insecticides, respectively.

Material and methods

The trials were carried out in the sour cherry plantations of the Research and Extension Centre for Fruit Growing in Újfehértó. Between 1999 and 2003, the presence and parasitism of leaf rollers and leaf miners were regularly monitored in the experimental areas. We also studied the population dynamics in the areas.

Surveys were made every second week during the growing season. In all parts of the plantations, at each survey, 1000 leaves were evaluated and the infested plant material was grown for the determination of the quantity and parasitism of the occurring species, as well as the number of species and abundance of the parasitoids.

Results and discussion

It was found with the plant surveys that only leaf miners – pear leaf blister moth (*Leucoptera malifoliella), Phyllonorycter corylifoliella, Ph. blancardella, Ph. cerasicolella* and *Lyonetia clerkella* – occurred in sour cherry plantations.

Studying the infestation values (figure 1), it is noted that the dominating species was *L. malifoliella* in all areas, therefore this leaf miner was only considered during the control programme. Occurrence of all other species was below the damage threshold in every year, however they were important because they could hold the polyphagous parasitoids in the area.

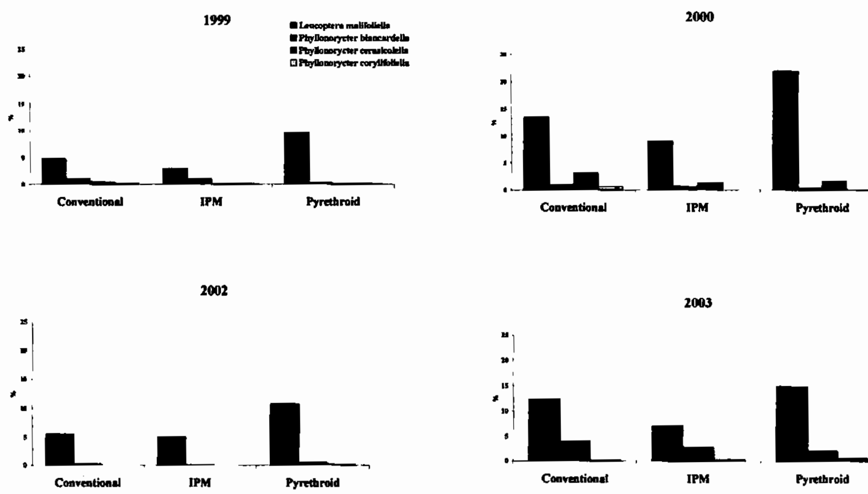

Figure 1. Procent of infestation by leaf miners

The difference in infestation level between the IPM and the provocative plantations was significant already in the very first year.

In the first year of the programme, data of parasitism indicate well that the integrated management encourages the increase of abundance of parasitoid which, in turn, plays an important role in reducing the population of miners in the sour cherry plantations (table 1).

Table 1. Procent of parasitism of leaf miners (sour cherry, Újfehértó)

	1999	2000	2001	2002
Conventional	2,2	5,3	17,3	9,3
Pyrethroid application	2,6	4,0	8,4	4,1
IPM	**14,8**	**13,7**	**37,6**	**28,5**

In the third year, an average parasitism of 37,6 % in the IPM plantation indicates that the parasitoids could reduce the number of hosts to such an extent that no control was necessary. This statement was confirmed by the data of parasitism in 2002.
In the population reducing activity of *Leucoptera malifoliella*, the role of 10 Chalcidoidea species was identified in the following order of importance: *Chrysocharis pentheus* Walker, *Minotetrastichus frontalis* Walker, *Pnigalio pectinicornis* Linnaeus, *Closterocerus trifasciatus* Westwood, *Sympiesis sericeicornis* Nees, *Bariscapus nigroviolaceus* Nees, *Pediobius pyrgo* Walker, *Pediobius saulius* Walker, *Cirrospilus viticola* Rondani, *Cirrospilus pictus* Nees. Of the Braconidae species, occurrence of *Pholetesor bicolor* Nees was observed, but only in the fifth year of the trials.

As far as population dynamics is concerned, it is important to note that the increase was not only observed in the abundance of parasitoids but also in the number of species in the IPM areas during the years. The presence of 3, 4, 9 and 5 parasitoid species was identified from *Leucoptera malifoliella* in 1999, 2000, 2001 and 2002, resp (figure 2).

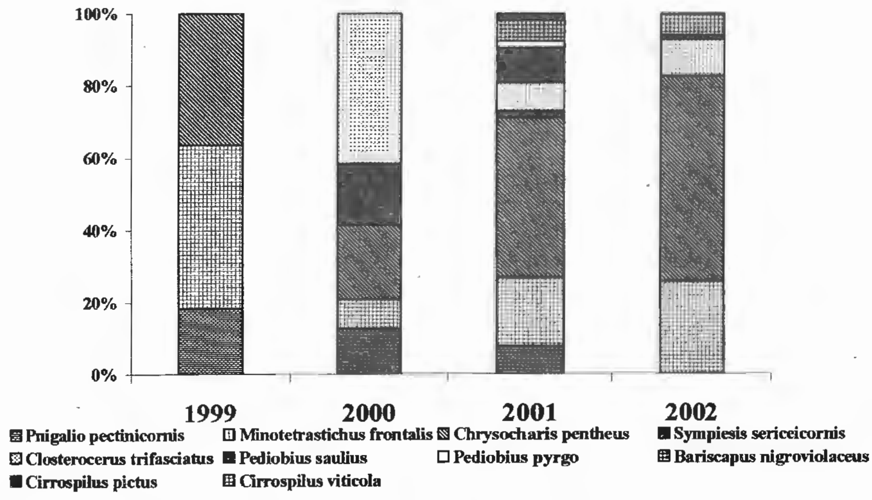

Figure 2. Change of the *L. malifoliella* parasitoids in IPM sour cherry plantation

Thus, the environmentally-friendly management has improved biodiversity. As a good effect, the abundance of the various Chalcidoid species has increased so much that the number of their hosts has greatly decreased. In this relation, it was also noted that the foliar infestation and the parasitism of leaf miners are inversely proportional. The more beneficial Chalcidoid

wasp species are in the plantation, the lower foliar infestation occurs, with other words the lower is the level of damages.
Integrated pest management means a safe treatment for the parasitoids. How can this be put in practice? If, on the basis of the catches of pheromone traps, it is necessary to make treatments in the plantations, sprayings shall be made when we are sure to enhance safeguards to the natural enemies (at least the dominant species). The simplest way is to consider, and make use of, the characteristics of the way of life of both the pest and its parasitoids and the difference between the flight periods due to their development. Parasitoids follow the flight of their host animals with some days (7-16 days, depending on the species)(figure 3).

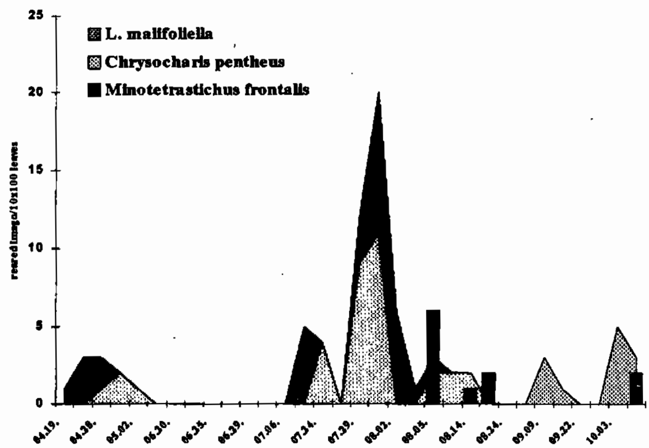

Figure 3. Flight activity of *L. malifoliella* and two parasitoids (sour cherry, IPM, Újfehértó)

Thus, we have always time to protect the most frequent and most important Chalcidoid species, without injuring them during the control of the pests. This statement is true, of course, for the control of other pests – e.g. cherry fruit fly *(Rhagoletis cerasi)*, fall webworm *(Hyphantria cunea)*, and *Melolontha melolontha* -, too.

Acknowledgements

The authors thank Jenő Papp for the determination of the Braconidae species.
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Reduction of insecticide sprayings by using alternative methods in commercial apple orchards.

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Abstract: An experiment in a Girona (Catalonia, Spain) fruit-producing area based on alternative methods to chemical insecticides was carried out in 2002 and 2003, from post-bloom to harvest, to control the codling moth (*Cydia pomonella* L.), the leopard moth *(Zeuzera pyrina* L.*)* and the Mediterranean fruit fly *(Ceratitis capitata* Wied.) in commercial apple orchards (33 ha in 2002 and 56 ha in 2003). Mating disruption was used to control the codling moth using Isomate-C® (Shin-Etsu) pheromone dispensers. In young orchards, mating disruption with Isonet-Z® (Shin-Etsu) dispensers was also used to control the leopard moth (*Zeuzera pyrina* L). A mass trapping method was used to prevent damage from the Mediterranean fruit fly by means of Tephry® and Probodelt® traps provided with Trypack® (Kenogard), and Pherolab® attractants. Target pests were well controlled and no important outbreaks were observed. There was a significant reduction of the total amount of insecticides sprayed in summer (65 %) and fruits were of higher quality because they had been sprayed with less insecticides than fruits from the standard Integrated Production orchards.

Key words: apple, Integrated Fruit Production, alternative methods, mating disruption, mass trapping.

Introduction

Integrated Pest Management (IPM) in fruit orchards was implemented in Girona (Catalonia, Spain) in the 80's. Once the Integrated Fruit Production (IFP) rules for apples were approved by the Department of Agriculture of the Government of Catalonia *(Generalitat de Catalunya)* (1995), fruit growers' cooperatives of Girona (70% of the production) promoted their members to produce according to the IFP methodology (Batllori *et al.* 2003).

Since then the surface area devoted to IFP has continuously increased. During the first years growers had indirect help with the financing costs of the required analysis (soil, leaves, water and pesticides) and, in recent years, grants to produce fruit using alternatives to chemicals or complementary methods, by minimizing the use of inputs (e.g., pheromone traps for pest monitoring) and caring for the environment. Nowadays, most of the fruit in Girona is produced according to the IFP methodology. IFP has allowed the Girona cooperatives to know and also offer traceability of their own production to dealers and, as a result, to be able to sell in the European markets which are more exigent in matters of food safety.

Fruit growers must attend IFP training courses and make a great effort to become IFP producers. Private companies, on the other hand, certify the fruit production by visiting the IFP orchards, taking samples for pesticide analysis and revising the field book (which contains all orchard inputs which must be based on soil analysis, water needs, pests and diseases thresholds, etc).

Related to specific plant protection, several field trials have been carried out in Girona over the years to reduce insecticide sprayings. Since 1987 different kinds of mating disruption materials have been checked to control the codling moth (*Cydia pomonella* L.) (=CM), the leaf roller (*Pandemis heparana* Denis & Schiff.), the apple clearwing (*Synanthedon myopaeformis* Borkhausen) and the leopard moth (*Zeuzera pyrina* L.), and mass trapping methods have been used for the Mediterranean fruit fly (*Ceratitis capitata* Wied) (=MFF), the apple clearwing and the leopard moth (Batllori *et al.* 2003). Since 2001 specific new plant protection efforts have been made to reduce the total amount of insecticide sprayings by implementing a project entitled 'Pilot Area of Insecticide Spraying Reduction' (APRI in Catalan) which consists of using alternative methods, rather than chemicals, to control the most important summer apple pests.

Materials and methods.

The APRI project was carried out in 2002 and 2003 in a flat apple fruit growing area of Girona (Ullà, Baix Empordà) with a total surface area of 33 ha (2002) and 56 ha (2003), where Golden Smoothee was the main cultivar and where CM pressure had not been high during recent years.

The methods used were mating disruption for the CM and the leopard moth (young orchards) and mass trapping for the MFF. Isomate-C and Gynko (Shin-Etsu) (respectively, 1000 and 500 dispensers/ha) were used to control the CM, and Isonet Z (Shin-Etsu) (300 dispensers/ha) for mating disruption of the leopard moth, while mass trapping for the MFF (50 traps/ha) was done with Tri-pack (Kenogard) (whose active ingredients are Ammonia acetate, Putrescine, Trimetilamine) and Ferag CC D TM (SDEC) (Ammonia acetate, Diaminalcane, Trimetilamine) attractants.

In all mating disruption orchards codling moth pheromone dispensers were installed in April, and in young orchards leopard moth dispensers were installed in May. In all APRI areas pheromone traps were installed for the CM, the leaf roller *(Pandemis heparana* Denis & Schiff.*)* and the leopard moth, and MFF attractants were installed for monitoring purposes (Fig. 2). In orchards where the MFF was above the threshold, mass trapping methods were used in 2.2 ha (2002) and 12.83 ha (2003).

Fig. 1 - Isomate C ® dispensers (left) and Probodelt® traps (right) with Tri-pack attractants were the materials used, respectively, for CM mating disruption and for MFF mass trapping in the APRI area.

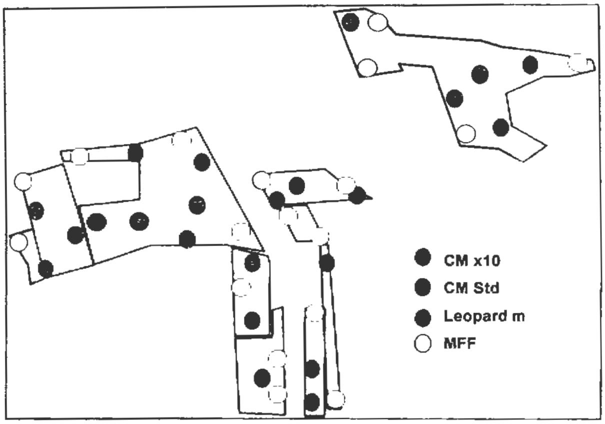

Fig. 2- Location of the monitoring traps in the APRI area (2002).

Evaluation of the applied methods was done by monitoring traps weekly (codling moth standard lures and 10x concentrate (Biolure CM 10X (Consep)) and with field evaluations of the pest damage to fruits, at the end of the first two codling moth generations and at preharvest time, by looking carefully at 10 fruits per tree in approximately 100 trees per hectare (75 in the inner part and 25 along the perimeter line).

Results

Data from the APRI project are shown in the following terms: CM and MFF trap catches (Table 1 and Figure 4), observed CM and MFF fruit damage in field evaluations (Fig. 3 and 5), sprayed insecticides and miticides (Fig. 6) and common effects of the techniques used on the occurrence of other pests.

Table 1 - Codling moth catches (May-October) in monitoring traps in the APRI area compared with standard IFP apple orchards.

AREA	*YEAR*	*USED LURES*	*Nº TRAPS*	*AVERAGE CATCHES/TRAP*	
APRI	**2002**	***Monitoring (standard)***	**17**	**0.47 c**	
	2003		**22**		**0.04 c**
APRI	**2002**	***Monitoring* X 10**	**5**	**2.40 b**	
	2003		**6**		**0.33 b**
STANDARD ORCHARDS	**2002**	***Monitoring (standard)***	**6**	**10.2 a**	
	2003		**6**		**13.70 a**

Values followed by the same letter (in the same column) are not statistically different (Tukey; α=0.05).

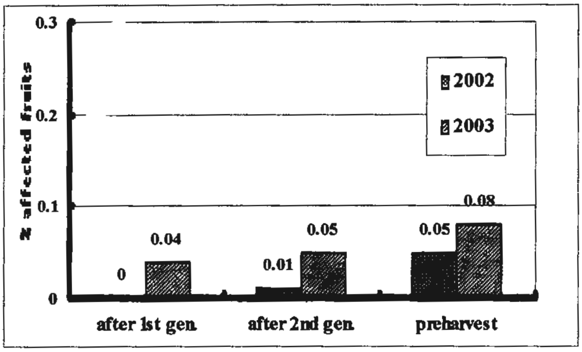

Fig. 3 - Codling moth damage to fruit after each CM generation in the APRI area during the 2002 and 2003 seasons.

Inhibition of the CM catches in the APRI area was important due to the few observed catches compared with standard IFP orchards, particularly in the second year. Catches in the 10x concentrated lure traps reveal that the CM pressure was low.

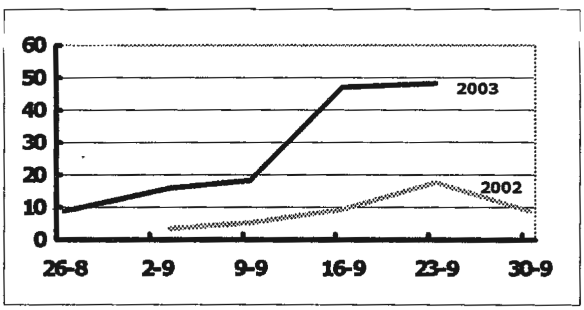

Fig. 4 - Average catches of MFF per trap and week in apple orchards where mass trapping was used.

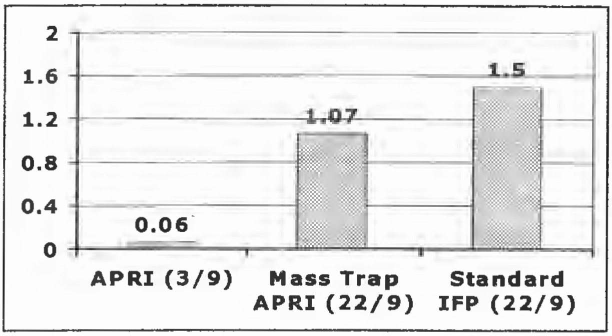

Fig. 5 - Percentage of MFF damage to fruits in mass trapping APRI orchards compared with IFP standard orchards (2003).

The MFF pressure on fruit orchards depended on the year. On pome fruits it may cause important damage later than mid August if no direct control measures are adopted. Damage to fruits caused by MFF until the first days of September were very low, but in the following weeks they increased quickly. Where the mass trapping method was used, the percentage of affected fruits at harvest was similar to that of the standard IFP area.

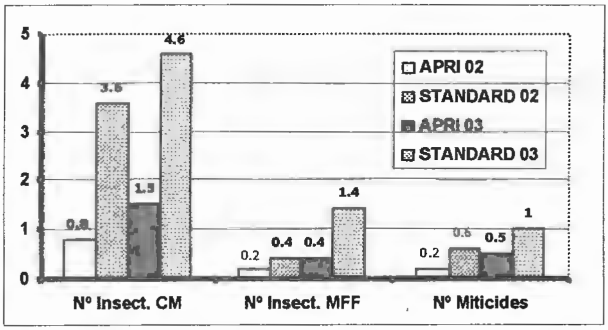

Fig. 6 - Average number of sprayed insecticides against CM and MFF and average sprayed miticides in the APRI area compared with standard IFP orchards (2002-03) from June to harvest.

Throughout 2002 and 2003, an average of 3 insecticides and 0.5 miticides less per season were sprayed on the APRI orchards. In 2003 (MFF pressure considered to be medium) it was necessary to spray an average of one less insecticide in the APRI area. Most of the APRI orchards did not receive insecticides from bloom to harvest.

In young orchards where mating disruption for the leopard moth was done, 0.5% of the trees were bored, while in untreated orchards (for this pest) 1% of the trees were affected the following winter (2003). There was not important damage by other pests - only leafroller

(Pandemis heparana) and San José scale *(Quadraspidiotus perniciosus)* outbreaks in orchards in which both pests had already shown some activity.

Conclusions

1. Target pests were well controlled by using alternative methods to chemicals.
2. Low occurrence of other pests; specific insecticides had to be sprayed in only a few orchards against the leafrollers and the San José scale.
3. Finally, the APRI project allowed an important reduction in the use of summer insecticides (a total reduction in most of the orchards) so the production obtained met the dealers' interests better and offered an additional positive aspect to its commercialisation.

Acknowledgements

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The Tortricidae population in Croatian apple orchards and a possibility for mating disruption techniques

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Abstract: The mating disruption technique against key pests in apple orchards such as *Cydia pomonella* and *Cydia molesta* is a very common method in many European countries. This ecological safety technique has been applied in Croatia only experimentally. Investigation was focused on reasons for bad results in apple protection against codling moths with the mating disruption technique in Croatia. In the course of two years (2002 and 2003) the tortricids fauna research was carried out in apple orchards in the northwestern region of Croatia.

In addition to *Cydia pomonella*, Croatian apple orchards also had a considerable population of *Cydia molesta, Archips podana, Pandemis heparana, Spilonota ocellana* and *Adoxophies orana*.

Key words: apple, TORTRICIDAE, mating disruption technique.

Introduction

The mating disruption technique against key pests such as *Cydia pomonella* and *Cydia molesta* in combination, or *Cydia pomonella* with another species of *Tortricidae*, has a long tradition in many European countries as an ecological safety tool in plant protection. In Italy, France and Switzerland this is a regular method in integrated fruit protection (Rama et all. 2001; Charmillot, Pasquier 2003). The Guidelines for Integrated Production of Pome Fruits in Europe (2002) recommend this measure against the codling moth and tortricids.

In Croatia the first experiments with a mating disruption technique against codling moth started in 1999. RAK 3 + 4 was used in 1999 and 2000 (Ciglar and all, 2000). Two years later the attract-and-kill technique with Apeall was used in the same orchard. In 2003 the mating disruption technique was used again with Isomate C. During five years the damage resulting from attacked fruits in that orchard varied from 8% to 12%, except in 2003 when apple fruits were attacked in the range from 8% to 35%. In addition to a high percentage of the fruits attacked by codling moths, there were a considerable percentage of fruits damaged by other tortricides. The question was raised about the cause of bad results of mating disruption technique in Croatian orchards, as well as about the profitability of this method under conditions of Croatian orchards.

The aim of our work was to carry out research on the population of some Tortricidae species in Croatian apple orchards as the pest on the apple fruit.

Material and methods

Monitoring of five species of Tortricidae was carried out in apple orchards where the mating disruption technique was used for three years, while the attract-and-kill technique was used for two years. *Csalomon* pheromone traps were used for monitoring. *Cydia pomonella* was monitored in the commercial orchard. The number of caught codling moths was evaluated daily, and pheromone traps for the other tortricids were expected once per week.

The temperature data was measured at the meteorological station with Celsius thermometers for minimum and maximum temperatures.

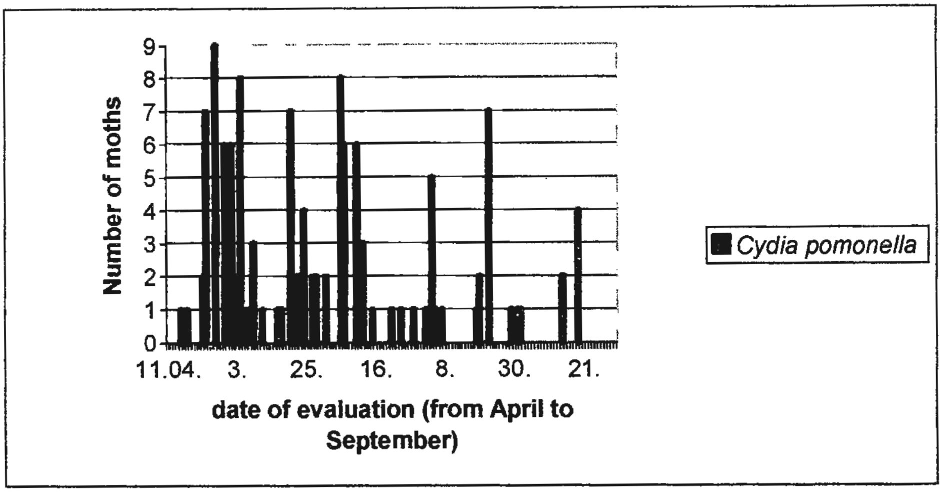

Figure 1. Number of codling moths caught per trap in 2002

Results and discussion

Two-year monitoring of the Tortricidae population showed a high population of *Cydia pomonella* and proved it to be a key pest in Croatian apple orchards. In addition, monitoring showed a serious increase of population of other species. *Cydia molesta* was caught during both years of our investigation in high numbers per trap. A population of *Adoxophies orana* is increasing in many countries, but in Croatian orchards its population was low. The appearing of moths varied in the first and second year of monitoring. In 2002 the first generation of *Adoxophies orana* moths was strong and the second generation was absent. In 2003 results were the opposite in case of *Adoxophies orana* moths catches. The second generation was stronger than the first, and moth's flight persisted until early September.

Monitoring of *Archips podana* showed a stronger population in the first year. The first moths were caught in late June and their flight stopped in early September. In 2003 there were two captures, the first from mid May to mid July, and the second from late July to late August. In both cases a number of captured moths was small.

Pandemis heparana is a very common pest in Croatian orchards. Occasionally there is a change in the intensity of its population. Usually there are two flight periods per year, the first from May to mid June and the second from late July to late August.

Spilonota ocellana was observed only in 2003. The first records showed the presence of this pest during a whole vegetation period.

These two-year results showed a presence of five monitored species of Tortricidae except for *Cydia pomonella*. A period of their attack on fruits is similar and persists during a whole vegetation period.

A mating disruption technique is a very expensive method protecting the orchard against one or two pests. Other pests in the same orchard raise a question about profitability.

In addition to profitability another issue is the efficacy of the pheromone dispensers in high temperature conditions, and of the release rate dispensers. Croatia has a continental climate with very high summer temperatures. The temperature data analysis showed that many summer days had a maximum temperature of 35°C. Countries that apply a mating disruption technique as a common measure against key pests have small oscillation in air temperatures.

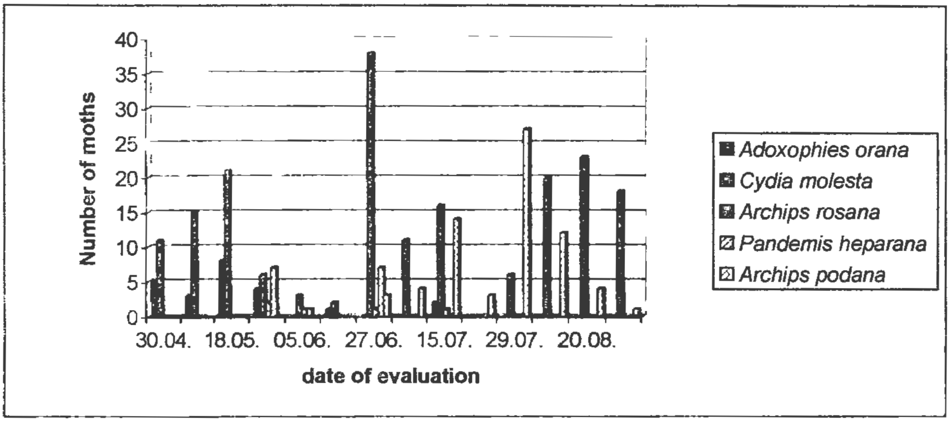

Figure 2. Number of Tortricidae moths caught per trap in 2002

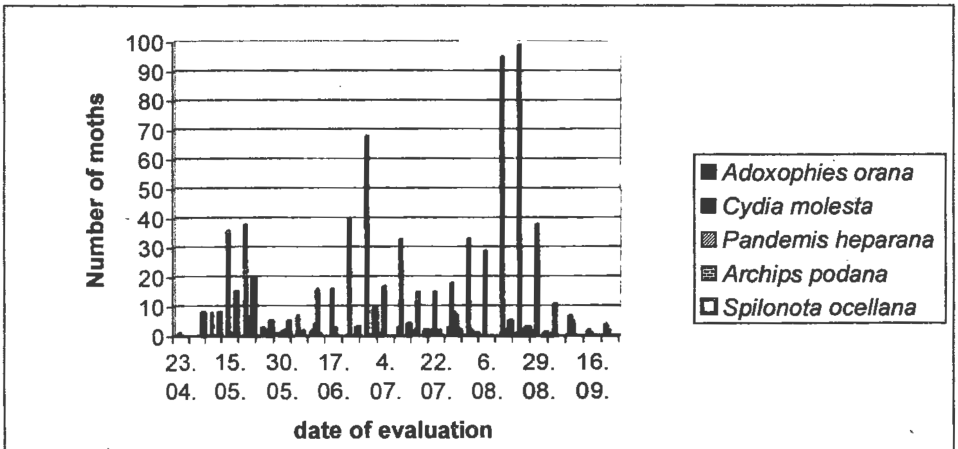

Figure 3. Number of Tortricidae moths caught per trap in 2003

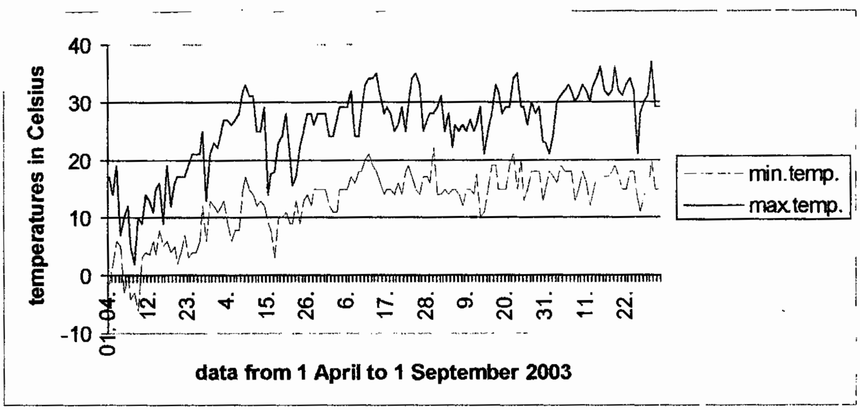

Figure 4. The temperature records for 2003, northwestern Croatia

Conclusions

Five species of Tortricidae were monitored in the course of two years (2002 and 2003). To conclude, all monitored species *(Cydia molesta, Archips podana, Spilonota ocellana, Pandemis heparana* and *Adoxophies orana*) are present in Croatian apple orchards. Due to their biology they are damaging the apple during a whole vegetation period. A time of their appearance is similar to *Cydia pomonella.*

High summer temperatures may have negative influence on dispensers' release rate in the mating disruption technique. Considering conditions in Croatian apple orchards and abundant presence of the tortricidae species, a mating disruption technique proved to be neither efficient nor profitable.

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Development of IPM programs in apple orchards by autosterilization of codling moth wild populations

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Abstract: The purpose of this work is development and testing of integrated pest management (IPM) and an apple production system suitable for Armenian farmers who have limited acreages and financial resources. This IPM should be applicable to various geographical zones of the country to increase production quality. The integrated system of measures includes such main elements as meteorological monitoring of pest populations and management of pest control. The objective of our study is to reveal the applicability of the pest autosterilization technique to wild codling moth populations to protect fruit yield in various regions of Armenia. For the purpose sterilizing traps were placed in apple orchards. The inner surface of the traps was treated with 4% solution of TIOTEPA. The drop of reproductive potential of codling moth population as a result of sterilizing agent action was revealed. The result should the efficacy of codling moth control method via sterilization of moths immediately in pheromone traps.

Keywords: autosterilization, codling moth, trap, integrated pest management

Introduction

Codling moth (*Cydia pomonella* L) (*Lepidoptera: Tortricidae*) is a cosmopolitan insect of deciduous fruits in Armenia. Apple is the preferred host of codling moth. That is why the codling moth occurs where apple-tree grows (plain, foothill and mountain zones of the country). Besides the apple, codling moth can develop on pome fruits such as pear and quince, on stone fruits such as apricot, plum, peaches, walnuts, *etc*. Codling moth possesses a remarkable ability to adapt to a wide range of climatic conditions. This pest is able to synchronize its life cycle with the fruiting period of host plant (Ter-Hovhannesyan, Azizyan, 2003).

At present the control of this pest in Armenia mainly performed by from two to six insecticide applications (depending on the number of generations of the area). Application of insecticides resulted from the inside effects such as destruction of useful natural pest enemies and development of resistance to insecticides (Kilpatrick, Quarterman, 1952; Charmillot *et al*, 2002; Sauphanor, Bouvier, 1998,a, Georghiou, 1972; Knight, *at al*., 1994)

Single tactic systems are promising for codling moth control. The most promising tactics include mating disruption, insect growth regulations, a granulosis virus, insect autosterization, sterile insect release, and cultural practices, most likely used in various combinations (Ascher, 1964; Charmillot, *at al*., 1991; Cross, 1994). The entomo-phenological forecasting is very important for the construction of population growth models, the establishment of time periods and place to carry out quantitative stock-taking, as well as for the the time periods for pest control (Azizyan *et al*., 2001; Riedl, 1983).

As it was mentioned above, the purpose of this work is development and testing of IPM and an apple production system for Armenian farmers having limited acreages and financial resources. This IPM should be applicable to various geographical zones of the country to increase in the production quality.

The objective of our study was to reveal the applicability of the pest autosterilization technique to wild codling moth populations to protect the fruit yield at various regions of Armenia.

Material and methods

The experiments were carried out in apple orchards located in three various geographic zones of Armenia (plain zone-- 850 m , foothill zone - 1300 m, and mountain zone - 1600 m).

A chemosterilizing agent TIOTEPA (3-ethylen-imid-thiophosphoric acid) was used.

To determine the sterilizing TIOTEPA dose codling moths were exposed to various doses of TIOTEPA. Male moths were plated into Petri dishes. Dish inner surface was previously treated with TIOTEPA (3, 4, 5 and 6% solution) mixed with thick soup. In 5 minutes they were allowed to mate with intact females. After oviposition females were tested for spermataphore existence (i.e. for male sexual activity), the number of laid eggs and hatched larvae was calculated.

Attractive sterilizing traps (AST) were placed in three mentioned orchards (30 traps per 1 ha). The trap inner surface was treated with 4% Tiothepha solution. In early spring the sterilizing traps with pheromone capsules were hung on trees of experimental orchards. The sterilizing agent and pheromone capsules were renewed every 10-15 days. Additionally five control glue catching traps were placed in the same orchards to observe the moth flight dynamics. Around the experimental orchards eight glue catching traps were placed to prevent emigration of males from the outside. To evaluate fruit damage, the damaged fruits were counted in the yield of 10 trees in each orchard.

Phenology of codling moth was studied in field investigations using pheromone traps and trap bands. Every 10-15 days the trap bands were replaced with new ones and removed bands were placed separately in netted nurseries for further observations. Moths were paired up and mated in order to determine the sexual activity and fertility.

At the end of the season number of infected apples and the productivity of the experimental orchards were calculated by the equation:

$$BE = \frac{K-O}{K} x100\%,$$

where:
BE - biological efficacy, %
K- number of fruit damaged in the control
O - number of fruit damaged in the experiment.

Results

Methods of sexual sterilization may be effective if sterilized males retain their sexual activity and normal sexual behavior that in the nature allows them to find females and mate. So, we have tried to determine TIOTEPA doses that will sterilize mail and, at the same time, do not decrease their sexual activity.

According to literature data (Ascher, 1964), the effective sterilizing dose of chemosteriliant resulting 98-100% egg death is 0.4-1.0 % at 2-4 h exposition. It is reasonable to note that under field condition the term of codling moth stay in pheromone trap is as short as 5-10 minutes. That is why we have determined the TIOTEPA sterilizing dose at 5-min.

exposition. Results (Table 1) show that the optimal TIOTEPA dose is 4%. This dose leads to decrease in offspring number to 1,6 % in comparison with control and, at the same time, it only slightly suppresses male sexual activity. Moreover, it doesn't influence the male life span (11-14 days). This dose was applied in field investigations.

Table 1. Sterilizing effect of TIOTEPA various doses at 5 min exposition (experimental conditions)

Tiotepha concentrations, %	Mating number, n	Number of females with spermataphores, %	Numer of eggs per a female, n	Larva hatching, %	Offspring number, % of control	Longevity of male life span, days
0	20	85,0	80,1	79,2	100	14,6
3	19	73,6	70,9±3.1	11,8±2.4	4,8	15,3
4	21	71.4	43,1±4.5	1,2±0.06	1,6	13,0
5	16	37,5	47,2±3.8	0	0	14,2
6	15	6,6	0	0	0	11,7

To determine the efficacy of autosterilization, larvae of the first and second generations were collected in plain zone at cocoon forming stage. Moths were mated to reveal the lethal load (Fig.1) it was shown that in treated orchards the decrease in the number of larvae trapped with belts takes place from the first generation to the following. On the trees under observation the relation of trapped larvae/tree was 6.1 ±1.5 for the first generation, for the second this value was only 2.6± 0.3. It decreased in total pest number in the orchard as a result of their autosterilization.

The autosterilization was shown to influence on both the flight dynamics and moth number all over the vegetative season. In Ararat valley where codling moth has three generations we have observed the constant drop in the insect populations of the 2nd and 3rd generations (Fig.2). This trend seems is a result of accumulation of the lethal load from one generation to another as the sterilizing agent is exposed for all the season.

Larvae of the 1st generation were collected from treated orchard and hatched males were allowed to match with intact females. The progeny was shown to bear high lethal load (42.1 % ± 11.6 of the offspring death). This lethal load is accumulated in the moths of the second generation (56.4 % ± 19,2) (Fig.3).

Determination of fruit damage (Table 2) showed that the yield from experimental orchards is higher than in intact orchard and the same is in chemically treated one. It is true for all three investigated climatic zones.

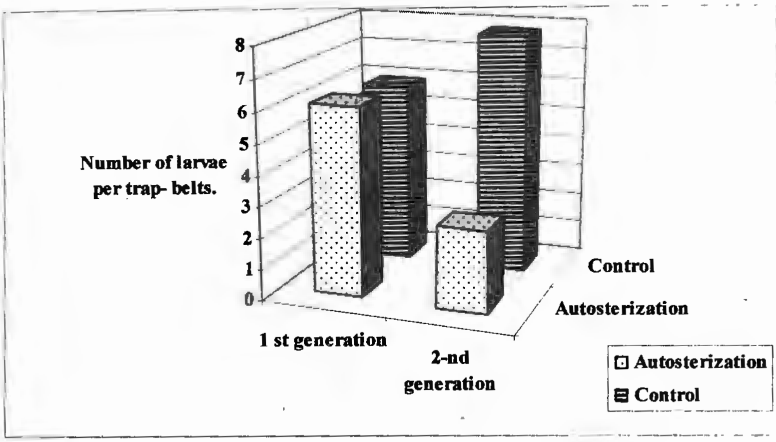

Figure 1. Trapped larvae/tree number in the experimental and control orchards

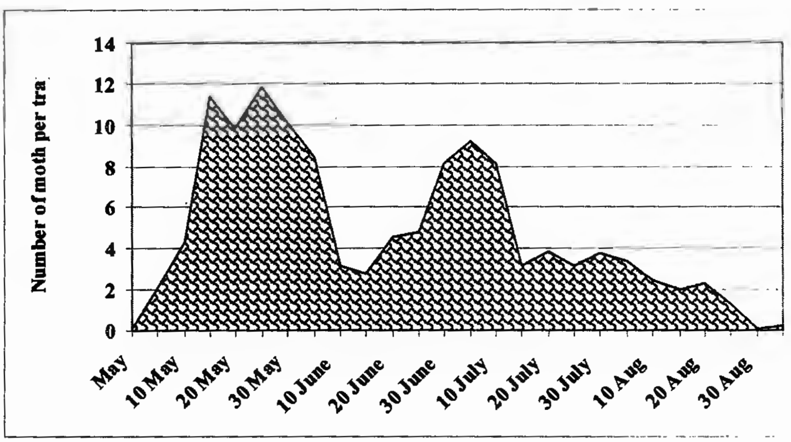

Figure 2. Dynamics of codling moth fly in Ararat valley orchards after autosterilization

Thus, the 4% solution of TIOTEPA as a tool for pest autosterilization is effective and decreases the yield damage. The application of this technique solves the problem of availability of pest for treatment. It is known that a part of wild pest population is not available at insecticide application. In our case sterilized males retain their sexual activity and find themselves females to mate. The lethal load expresses a trend to accumulate in pest progeny.

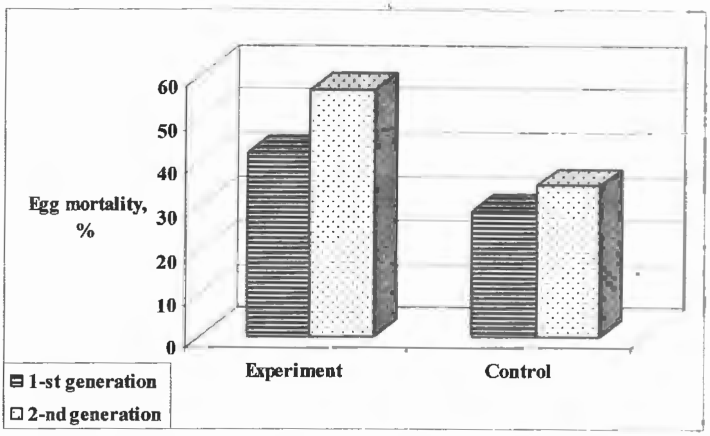

Figure 3. Mortality of codling moth eggs in experimental and control orchards of Ararat valley

Table 2. The efficacy of autosterilizing method of codling moth control

Climatic zones	Variants of experiment	Fruit damage, %	Biological efficacy, %	Yield Productivity, Metric centner/ha
Plain	Experimental (0.5 ha)	1.9 ±0.43	97.8	359
	Chemical control (1.0 ha)	0.9 ±0.25	98.9	371
	Intact control (0.2 ha)	87.3 ±13.2	-	59
Foothill	Experimental (1.0 ha)	2.1 ±0.62	96.2	250
	Chemical control (1 ha)	1.4 ± 0.32	97.5	268
	Intact control (0.2 ha)	56.2 ±13.4	-	118
Mountain	Experimental (0.5 ha)	0.9 ±0.31	97.7	232
	Chemical control (1.5 ha)	1.2 ±0.41	96.9	249
	Intact control (0.3 ha)	39.4 ±9.8	-	140

Acknowledgements

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Pilot project for sustained management of codling moth, *Cydia pomonella* L., with minimal SIT, in British Columbia, 2001– 2003

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Abstract: A pilot project of area-wide management for codling moth, *Cydia pomonella*, was conducted in 131ha of apple orchards and 12ha of pears set in 895 ha of the Similkameen Valley, 2001-2003. Moths were monitored each season, with codlemone-baited or pear ester lures (DA kairomone) in traps, using cardboard tree bands, by visual inspection of fruit and surveys when moths or damage were found, and were mapped using a GIS. *C. pomonella* was managed by release of low numbers of sterile moths weekly or every two weeks in the entire area, or weekly for two weeks in a targeted area following capture of unmarked moths. Mating disruptant (Isomate-C+) was applied to one orchard (10ha) in 2003. In total, 27 unmarked codling moths were detected and very light fruit damage occurred near a few traps but never exceeded 1-2 boxes (ca. 12 kg) in any year. No evidence of a resident *C. pomonella* population was found and it was not the target of any insecticide from 2001-2003. Insecticide use for other purposes was limited in 71-73% (by surface area) of orchards to treatment with *B. thuringiensis*, or Spinosad in a few sites, or carbaryl as a fruit thinner. Where codling moth populations are very low, area-wide programs can use successfully a strategy of monitoring and targeted response with SIT. Movement of diapausing moths in or on bins presented the most serious reinfestation risk to apparently moth-free areas. Experimental use of moths treated at 100-150Gy complicated the interpretation of traps and may have led to the "sting" damage that was observed and to unnecessary treatments.

Key words: codling moth, area-wide management, sterile insect technique, monitoring

Introduction

An area-wide management program for codling moth, *Cydia pomonella,* has been operating in British Columbia, in the key fruit-growing areas, and in neighbouring urban, native, or public lands, since 1994. It is the largest horticultural project in Canada, operating in >5,000ha of pome fruits and in >29,000 non-commercial sites such as parks and private gardens. By 2005 *C. pomonella* should be at non-economic levels in commercial orchards, and should be maintained thereafter in a cost-effective fashion. We are investigating biological factors affecting efficacy at very low population levels, often poorly understood (cf. Proverbs, 1974), and developing or testing approaches for long-term monitoring and management of the codling moth (Thistlewood & Judd, 2002; Thistlewood et al. 2003).

As part of this work, we conducted a pilot study using sterile insect technique (SIT) in an area of 895 ha of the Similkameen Valley, 2001-2003, with 131ha of apples, 12ha of pears, in 39 properties of 25 growers, and containing 27 private gardens with fruit trees.

Materials and Methods

Area-wide management of *C. pomonella* in B.C. occurs in orchards (land with >20 trees) and in urban or rural gardens (<20 trees). Moth populations were monitored daily or weekly each season with codlemone-baited 1mg lures) in 185-250 pheromone traps (min. 1/ha in orchards, 1 /urban site), pear ester lures (DA kairomone) in 25-175 traps, or ca. 3,000-5,000 cardboard tree bands (Judd et al., 1997) per year spaced regularly (2001), in orchards with unmarked moths or damage or placed at random (2001-3), by inspections of ca. 25,000-50,500 fruit seasonally and at harvest, by grower and homeowner surveys when moths or damage occurred, and were mapped using a geographic information system (ArcInfo, ESRI Corp.).

Releases of internally and externally marked and sterile (irradiated at 100-250Gy) *C. pomonella* moths occurred weekly or every two weeks in the entire area (May 2001; 2003 season) or weekly for two weeks in a targeted area following capture of unmarked moths (remainder of 2001; 2002 season), or for experimental purposes. Mating disruptant (Isomate-C+ 500 dispensers/ha) was applied to one orchard (10 ha) in 2003.

When unmarked moths were found, we notified the grower, centred sterile moth releases in and around the orchard containing the trap, searched for possible sources of wild codling moth (host trees, bins, woodpiles), searched for damage in the area, asked growers and residents for explanations, and banded trees, so as to detect any possible local populations. Each winter, growers were contacted for any observations, and records of pesticide use.

Results and Discussion

Detection of C. pomonella *and damage*

In 2001-2003, 27 unmarked codling moths were detected in 20 traps and very light fruit damage occurred near a few traps. Insect damage never exceeded 1-2 boxes (ca. 12kg) of apples in any year and for *C. pomonella* was from "stings" only (partial entry of larvae).

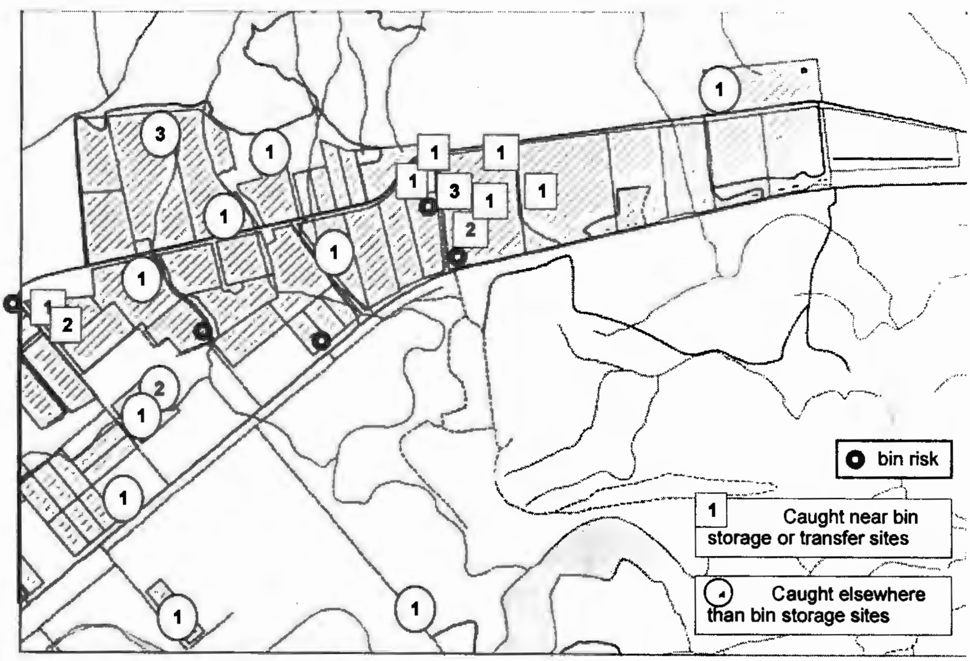

Fig 1. Unmarked codling moths captured in Cawston study area, 2001 to 2003, by location

No evidence of a resident and fertile *C. pomonella* population was discovered in traps, fruit damage, or cardboard bands. Many moth captures (13/27) were located near bin storage areas, cold storage facilities, or bin pieces imported from outside the area (Fig 1) (we were unable to monitor all bin movements within the area at every harvest). Six other moths were caught on relatively high ground in an area where high numbers of codling moths have been found in traps since the 1970s, arising from topography and wind exposure.

***Management of* C. pomonella**

No insecticides were targeted at codling moth from 2001-2003. The area-wide program staff only permitted us to begin work in June 2001, and they released ca. 1.4 million moths in May. Subsequently in 2001, we released 163,000 sterile moths all season at an overall rate of 52 moths/ha/week. In 2002, we released during the season 304,000 sterile moths at an overall rate of 85 moths/ha/week. In 2003, we released approximately 404,000 sterile moths at an overall rate of 280 moths/ha/week or 0.125 Petri dishes/acre/week. This rate is half of that used by the area-wide Program in 2003, but is also 3-5 times the low rate that we employed successfully in the summer of 2001 and throughout 2002. In 2003, one grower was concerned by captures of unmarked moths in the area of a packing shed and we deployed nearby Isomate C+ mating disruptant at 500 dispensers/ha, in10 ha.

Limited insecticide use for other insects

Insecticide use for all other purposes was limited in 71-73% (by surface area) of the orchards to treatment with *B. thuringiensis* once or twice per season, or Spinosad once/year in 2002 and 2003 in a few sites, or carbaryl as a fruit thinner. The remaining 27-29% of the area, managed by a few growers, received some organophosphate insecticide for other insects.

Estimated costs

Using figures from Dendy et al. (2001) and known costs, we estimate roughly the monitoring and management costs in the study area (Table 1), including some research and unnecessary use of sterile moths in May 2001 and July-Sept. 2003, as requested by program staff.

Table 1. Estimated costs in the project area, by year, activity, and on a per ha basis.

	2001	2002	2003
Trap monitoring	$7,980	$7,900	$7,901
Tree Banding	$1,084	$6,085	$5,626
Urban Program	$525	$506	$486
Release costs	$5,346	$5,384	$5,384
Moth costs	$11,862	$2,313	$5,649
Mating disruptant			$1,082
TOTAL	$26,797	$22,189	$26,128
per ha	$188.71	$155.17	$182.72

Conclusions

By 2001, the population density of *C. pomonella* in one region was very difficult to detect or absent following seven years of area-wide management. Where populations of *C. pomonella* are that low, area-wide Sterile Insect Technique programs can employ successfully a strategy of monitoring and targeted release. Our costs in the pilot area, including some research and unnecessary applications, were similar to or less than current programs using insecticides ($196-249/ha), or pheromone-based mating disruption of multiple species ($271-540/ha).

As suggested earlier by Proverbs & Newton (1975), our results show that the movement of diapausing moths on bins [Fig. 1] was the most likely method of reinfesting previously moth-free areas, rather than from undetected local populations. Our pilot project also prevented the very significant reinfestation that appeared elsewhere in the area-wide program during 2002 and 2003 (11.4% of traps in 2003 vs. ca. 35% in Zone 1).

The experimental use of moths irradiated at only 100-150Gy complicated the interpretation of presence of unmarked moths in traps because in theory some unmarked (but sterile) males may be caught in traps as a result of a few sterile-wild matings (cf. Henneberry 1992), and may also have led to the "sting" damage that was observed (from late embryonic lethality of sterile larvae) and so to unnecessary treatments. In choosing a suitable dose (100-300Gy), issues of sterility, potential damage, competitiveness, and trap interpretation must be balanced but are at present poorly understood. (Thistlewood et al. 2003).

This and other related research is leading to a better understanding of an appropriate mix of technologies that could provide minimal inputs or maximal cost-effectiveness, e.g. combinations of insecticides, mating disruption, SIT, *Cp* granulosis virus, attract-and-kill, etc.

Acknowledgements

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The egg stage as the target for the control of the Oriental Fruit Moth (*Cydia molesta* (Busck); Lepidoptera: Tortricidae)

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Abstract: *Cydia molesta* (Busck) is a serious pest in many peach and pome fruit orchards in Italy. The increasing attention to the environment has greatly reduced the use of many traditional neurotoxic insecticides to control infestations of this pest during the larval stage. Active ingredients acting on insect specific metabolic targets like chitin synthesis inhibitor insecticides are now greatly preferred in several Italian integrated pest management guidelines. To investigate the activity of these insecticides against the eggs of the Oriental Fruit Moth, a series of bioassays have been performed. Results of these bioassays are presented and discussed in relationship to integrated pest management strategies.

Key words: *Cydia molesta*, IGR, bioassay

Introduction:

The importance of *Cydia molesta* (Busck) in peach and pome fruit orchards in Italy has increased for the last years. Traditionally the control of this key pest was based on the application of neurotoxic insecticides, mainly organophosphates like azimphos-methyl, against the larval stage. Now the increasing attention towards environment and farmers' safety has greatly reduced the use of many traditional neurotoxic insecticides to control the infestations of this pest (Civolani *et al.*, 1998; Forti *et al.*, 2003). Active ingredients acting on insect specific metabolic targets like chitin synthesis inhibitors insecticides are now greatly preferred in several Italian integrated pest management guidelines. Moreover, new active ingredients acting on the nervous system of Lepidoptera and also some traditional products but with a different formulation are now available (Schreiber & Rovetto, 2003). In literature in general many data are available on the mode of action and efficacy of these products against the codling moth but only a few and incomplete information are available on *C. molesta* (Broadbent & Pree, 1984; Rigo & Goio, 1996; Charmillot *et al.*, 2001; Forti *et al.*, 2003). To evaluate the efficacy of these new strategies, a series of bioassays have been performed to investigate the activity of some insecticides acting as insect growth regulators against the eggs of the Oriental Fruit Moth.

Material and methods

Insects: a laboratory colony of *C. molesta* with no selection pressure by insecticides for 10 years was used in the tests.

Two types of bioassays were used to test the efficacy of a series of insecticides: a) application of the insecticide solution on eggs of known age (post-oviposition test) b) application of the insecticide solution on the substrate where eggs had to be oviposited (pre-oviposition test). In the first case adults were allowed to oviposit on a plastic (PET) substrate for 24 hours. A given number of days after oviposition the eggs on the substrate were dipped into the insecticides. Eggs used as control were dipped in water. The efficacy was evaluated scoring the number of hatched eggs two weeks after the application.

In the second case a small amount of the insecticide (500 µl) was dispersed inside a 500 ml plastic bottle. The insecticide was allowed to dry and then 15 adults were introduced inside of the bottle. After 24 hours adults were removed and transferred to a new, untreated bottle and maintained here till death with food supply and allowing them to oviposit on the untreated surface. The efficacy was evaluated scoring the number of hatched eggs laid during the 24 hours period on the treated surface and then the on the untreated one.

The list of insecticide used is reported in table 1. Commercial formulations of the insecticides were used. For each test 4 concentrations were prepared and the following coefficients (compared to the label field rate) were adopted to get the range of concentrations: 1/100; 1/10; 1; 2.

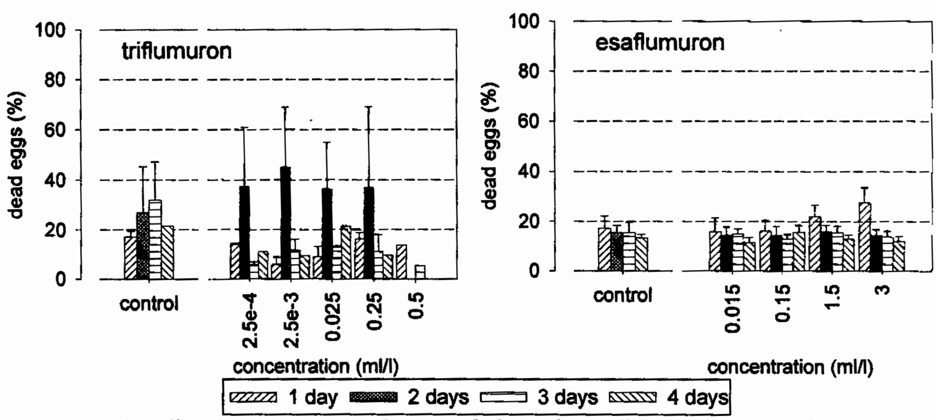
Table 1 - Insecticide used in the tests. Field rate is the dose registered against *C. molesta* in Italy. The filed rate was used as reference point in the efficacy tests.

insecticide (a.i.)	field rate (ml/hl)
alsystin (triflumuron)	25
dream (esaflumuron)	150
match (lufenuron)	100
nomolt (teflubenzuron)	50

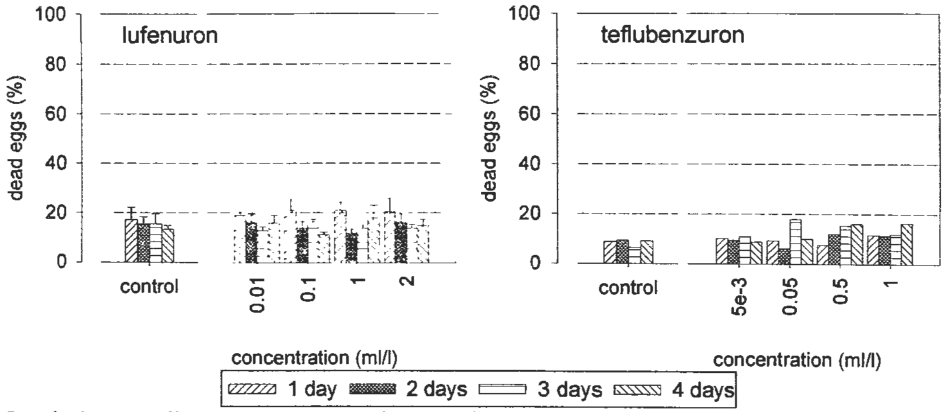
Results

Post-oviposition tests

Each insecticide was tested on eggs of 4 different ages of the eggs (1 to 4 day old) and was replicated at least 3 times. Test with "nomolt" were replicated only once. Results (mortality of the eggs) are reported in graphs 1 and 2. In general mortality is quite low. Only in the case of "alsystin" an increase of mortality, compared to the control, was observed when the product was applied against 2 day old eggs.



Graph 1. Mortality (mean ± s.e.) of eggs of *C. molesta* dipped 1, 2, 3 and 4 days after deposition, in various concentrations of triflumuron (left) and esaflumuron (right).



Graph 2. Mortality (mean ± s.e.) of eggs of *C. molesta* dipped 1, 2, 3 and 4 days after deposition, in various concentrations of lufenuron (left) and teflubenzuron (right).

Pre-oviposition tests

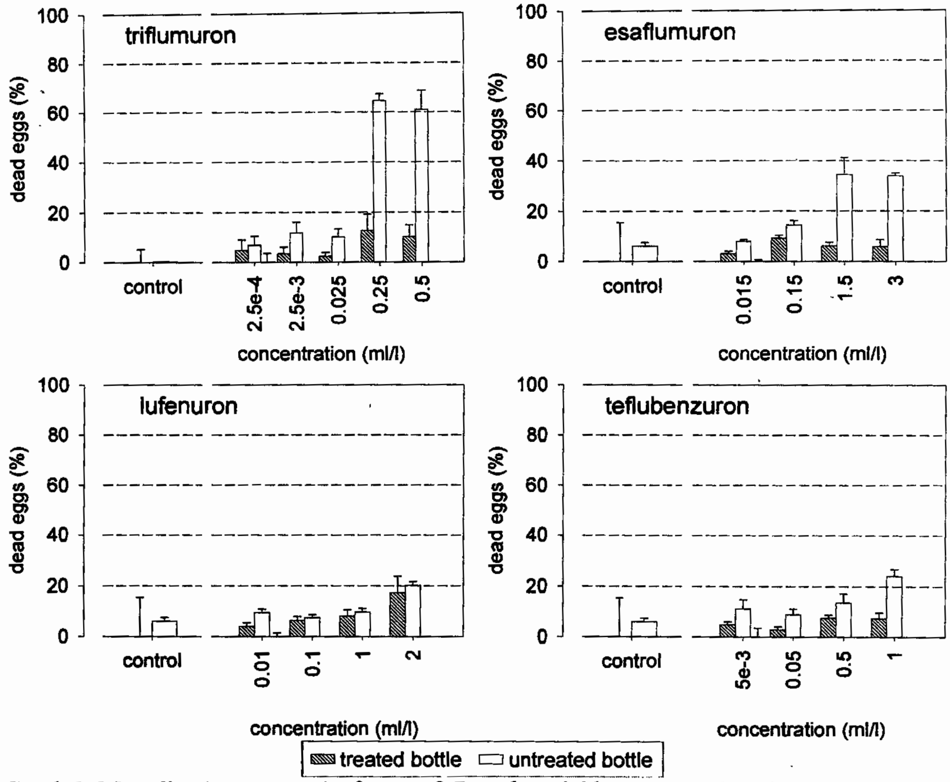
The aim of these tests was to assess the efficacy of insecticide residues against eggs laid on a treated substrate. Each test was replicated 3 times. All the products show a minimal increase of mortality, at least at the highest rate (1X and 2X) (Graphs 3 and 4). This is more evident in "alsistin" and "match". However mortality of eggs laid on treated surface is clearly lower than the mortality observed in eggs laid later by the same adults after being transferred on a untreated surface. This is extremely evident in the case of "alsistin" (up to 60% of dead eggs) but, even if at a lower grade, it can be observed also with the other insecticides.

Conclusions

According to laboratory data collected in the present study post-oviposition treatments against the eggs of *C. molesta* using IGR insecticides have not a great efficacy.

Variability, above all in the case of "alsystin", is quite big but in any case it is rather difficult to show clearly any "dose - response" relationship in these data. Moreover no difference in sensitivity of different age eggs emerges, with the exception of 2 days old eggs treated with "alsistin".

Also the activity of these insecticides on eggs laid on treated surface seems to be quite low but nevertheless mortality of eggs laid by "treated" females can be interesting (at least at the higher doses). This confirms a similar behaviour already reported in *Cydia pomonella* in which a "transovaric" effect was observed (Badowska-Czubik *et al.*, 1991).



Graph 3. Mortality (mean ± s.e.) of eggs of *C. molesta* laid on a treated surface compared with the mortality of eggs laid later by the same adults on an untreated surface.

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Seasonal fluctuation of Oriental Fruit Moth *Grapholita molesta* (Busck) with sexual pheromone trap in peach orchards in Bento Gonçalves, RS, Brazil

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Abstract: Seasonal fluctuation of *Grapholita molesta* (Busck, 1916) (Lepidoptera: Tortricidae) males was evaluated in two commercial peach orchards cv Chiripá in Bento Gonçalves, RS, during June of 2000 to July of 2002. The insects were evaluated weekly using Delta traps (two/orchard) baited with commercial synthetic sex pheromone (E-8-dodecenyl-acetate + Z-8-dodecenyl-acetate + Z-8-dodecenol). Four peaks were observed during peach production period: last week of August, first of November and December and second of January. Medium temperature seven days before evaluation was positively correlated with catches of *G. molesta* males in the traps. Information about seasonal fluctuation can be used for growers as a tool to guide insecticide treatments for pest control in peach orchards.

Key words: Oriental Fruit Moth, seasonal fluctuation, peach, Integrated Fruit Production.

Introduction

Peaches, which represent one of the most important temperate fruit in the country, are grown on nearly 20.000 ha in Brazil. The production is concentrated in Rio Grande do Sul (RS), São Paulo (SP), Santa Catarina (SC), Paraná (PR) and Minas Gerais (MG) states (Marondin & Sartori, 2000; Sidra, 2002). The main producer state is Rio Grande do Sul (12.000 ha) and the fruits are for the internal market, being 50% for fresh consumption and 50% for the canning industry.

Since 1950, *Grapholita molesta* (Busck, 1916) (= *Çydia m.*) (Lepidoptera: Tortricidae) has been an important peach pest in Brazil (Lepage, 1943). Presently, insect control is based on broad-spectrum insecticide treatments (organophosphates, carbamates and pyrethroids), but growers usually do not monitor pest infestation and do not follow threshold levels, established as 20 adults per pheromone trap per week (Fachinello *et al.*, 2003). As a result of this pest management, many negative effects such as the possibility of residues in fruits, toxic effects for the growers and environmental contamination are acute concerns for growers, technicians and consumers (Botton *et al.*, 2001 and 2002). For these reasons, safer and more rational control methods than conventional chemical insecticide use need to be developed.

The development of a sex pheromone trap for OFM led to increased efforts for monitoring insect population, so that a better timing of spraying could be achieved (Roelofs *et al.*, 1969; Rice *et al.*, 1982). The following report presents two years of seasonal adult fluctuation of *G. molesta* males in Bento Gonçalves, RS, Brazil, located in the major table peach production region. The study also shows a relation between climate and OFM biology offering data for farmers to take decisions on the management of this pest.

Material and Methods

The study was conducted during 2000 and 2001 seasons in two commercial peach orchards (cv.`Chiripá). Peach trees, ca.10 years old and 3.5-4 m tall, were planted on a 4 x 6 m (tree x row) spacing. The OFM adult male population was monitored by using Delta traps (baited with commercial *G. molesta* synthetic sex pheromone dispenser with 1 mg of E-8-dodecenyl-acetate (95%) + Z-8-dodecenyl-acetate (5%) + Z-8-dodecenol (1%) (Isca Tecnologias Ltda, Rio Grande do Sul, Brazil). The traps were placed in the orchards in July 2000 and maintained in the field for the whole duration of the monitoring period. At each orchard two traps were placed on 30 m spacing, 1.7 m from the top of the peach orchard canopy. The traps were serviced weekly and the captured males were removed and recorded. The pheromone dispensers were changed at 6-week intervals while the traps were replaced when necessary.

Weekly catches of *G. molesta* males in the traps were correlated with average temperature (°C) and rainfall (mm) recorded during the preceding seven days. Climatic data were recorded daily on a standard hygrothermograph station located nearly 1.500 m far from the orchards. The spray program for pest control was performed according to the farmers' decision.

Results and discussion

Four distinct peaks were recorded in the flight pattern of *G. molesta* each year during the vegetative period (August-January). In both years, no catch was recorded in June, only sporadic catches in July and a major presence in mid August (Figs. 1 and 2).

In the region, Biofix can be set in the first week of August. This parameter indicates the starting of stable presence of the insect, that is the first catch followed by catches in the next sampling. This is the basis for the application of temperature-based forecasting models.

The first generation adults fly in October and early November, the other peaks occur in early December and mid January. The latter was the highest in both years with an increasing overlapping of generations (Figs. 1 and 2).

The level of catches was positively correlated with temperature data of the preceding week, but not with rainfall.

Information gathered in the Bento Gonçalves region has been used to give advice to the growers of area, pointing out the four major periods of the adults' presence. These results make it advisable to carry out monitoring in every orchard or to set up a monitoring net. It is also important to define thresholds for specific situations.

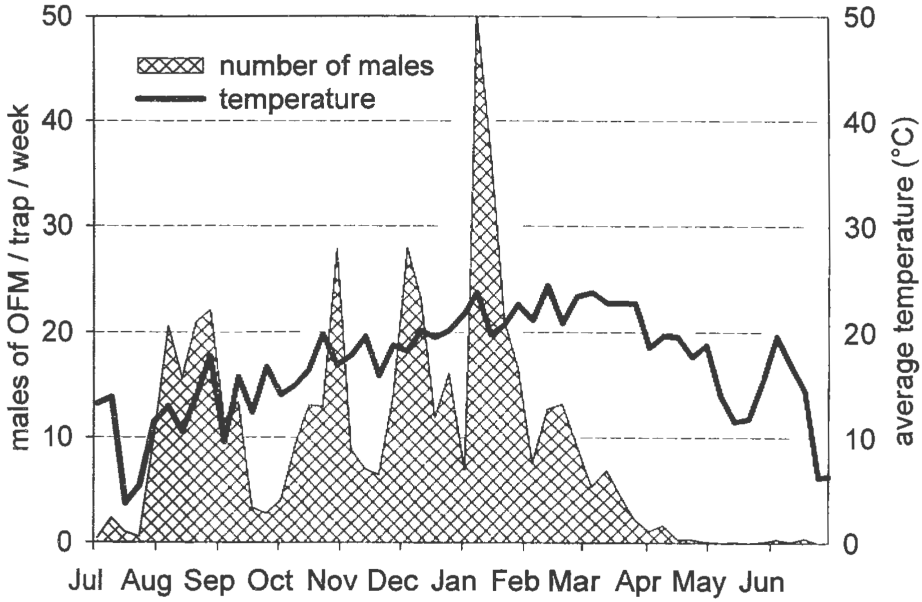

Figure 1. Seasonal fluctuation of oriental fruit moths male caught per pheromone sexual traps in 2000/2001 season.

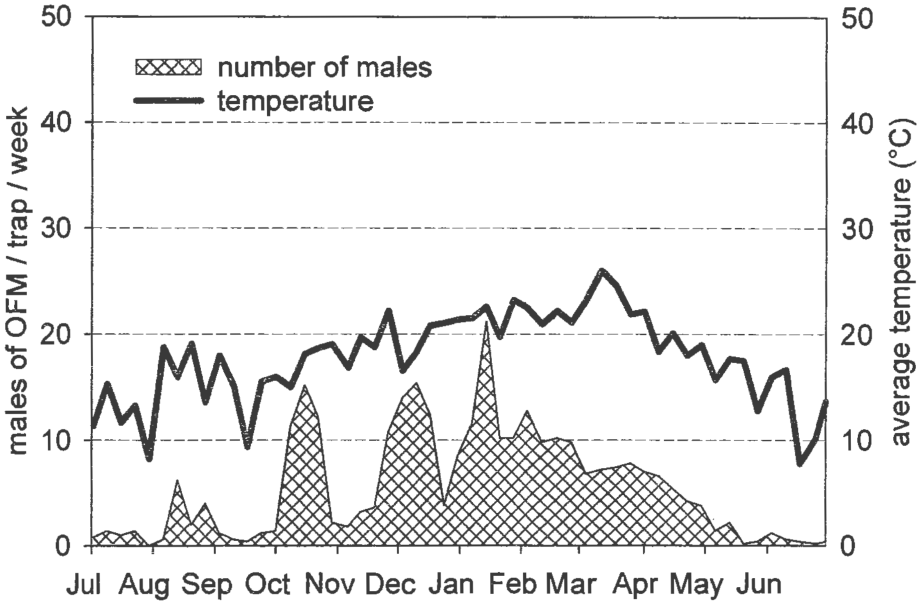

Figure 2. Seasonal fluctuation of oriental fruit moths male caught per pheromone sexual traps in 2001/2002 season.

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A forecasting model for the peach twig borer, *Anarsia lineatella* Zeller

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Abstract: Research has been done since 2000 in order to set up a simulation model of the phenology of Peach Twig Borer (PTB), *Anarsia lineatella* Zeller. The model can give, based on the temperature of a specific area, a figure of the percentage of the different stages of the pest.
Developmental data are collected in the laboratory in order to fit the model parameters to the specific pest. Larvae have been reared on artificial diet as well as on different natural food sources, that could induce differences in the duration of the larval stage.
After positive validation, the model will be used by technicians in Emilia Romagna in IFP to give indication on the right moment to apply control measures.

Key words: *Anarsia lineatella,* forecasting model, Italy.

Introduction

Research has been done since 2000 in order to set up a simulation model of the phenology of Peach Twig Borer (PTB), *Anarsia lineatella* Zeller (Lepidoptera: Gelechiidae).

This model can describe the age-class distribution and genetic variability of poikilothermal organism population. To set up the model, the thermal response of each stage must be defined. Developmental data of eggs, larvae, pupae and adults are collected in controlled-evironment cells at constant temperature, relative humidity and photoperiod. To simulate the development of early stages, the function of Logan *et al.* (1976) is used and a linear function for adult's longevity. Another parameter to be defined is the mean fecundity of female as function of age. We fit a modified Bieri's function (Bieri *et al.*, 1983) to the daily fecundities observed in females reared at the optimal temperature. To simulate the flux of individuals of the same population through the different stages (stochastic process) we use a time-distributed delay model (Manetsch, 1976). This model lets the individuals flow through a series of substages. The number of substages has to be experimentally determined considering the variability of each stage.

When all the parameters have been defined the model can simulate the development of the insect population on the basis of temperature input. The cumulating percentages of egg-laying, egg-hatching, pupation and adult emergence are given day by day.

Material and methods

The parameters needed for the model are being collected in the laboratory of the Istituto di Entomologia e Patologia vegetale in Piacenza University, recording the developmental data. Complete data are already available for egg incubation, larval and pupal stage duration of individuals reared on artificial diet, while adult life span and fecundity are still under investigation.

Development on fruits

Larvae have been reared on different natural food sources, in order to assess whether differences in the duration of the larval and pupal stage, as well as in the adult lifespan and fecundity are induced. For this work, still in progress, larvae are reared on fruits of peach, apricot and plum at constant temperature (15 °C, 18°C, 20 °C, 23 °C, 26 °C, 29 °C and 32 °C) and 70% R.H.

Newly hatched larvae are put on fruits, previously acclimatized at the test temperature, using a small brush.

When necessary, due to fruit deterioration, larvae are transferred on new fruits to complete the development

Adult life span and fecundity

Batches of adults of both sexes are put in plastic bottles for mating and oviposition. Egg laying and mortality are checked daily and dead individuals sexed. Mortality is recorded at 20 °C, 23 °C and 26 °C; egg laying is checked at 23 °C.

Results

Rearing on fruits showed a series of problems, mainly due to molds or phisiological decay of the fruits, particularly on apricots; larvae must be frequently trasferred on new fruits and larval mortality is quite high.

A slower development on plums have been recorded.

At the temperatures tested till now the time required for larval + pupal development ranged from 24 days (apricots, 26 °C) to about 50 days (plums, 20 °C)(Tab. 1).

The lifespan of adults obtained by larvae reared on fruits is highly variable, ranging from 7 to 80 days and, like in the case of artificial diet, females live much longer than males, (Tab. 2).

Apricot and peach fruits induce a faster development than artificial diet.

Table 1. Duration (days) of larval and pupal stage of insect reared on different natural food sources at different temperature.

Temperature	*20 °C*		*23 °C*		*26° C*	
Stage	Larvae	Pupae	Larvae	Pupae	Larvae	Pupae
Peaches	25.4±0.16	13.8±0.29	19.0±0.25	10.0±0.20	16.0±0.24	8.0±0.32
Plums	30.4±1.39	19.2±0.59	20.9±0.53	10.7±0.18	19.0±0.39	8.0±0.18
Apricots	24.4±0.48	14.6±0.42	19.0±0.40	10.0±0.42	17.0±0.38	7.0±0.14

At present, functions of Logan have been defined for egg incubation, larvae and pupae from artificial diet; table 3 shows the duration of the egg stage at different constant temperatures and fig. 1 the very good fitting of experimental data to a Logan function.

Table 2. Adult's lifespan of insect reared on different natural food sources at different temperature.

Temperature	*20 °C*		*23 °C*		*26° C*	
Adults	Male	Female	Male	Female	Male	Female
Peaches	14.5±2.45	13.8±3.88	16.0±1.94	30.0±4.07	19.0±1.70	16.0±3.78
Plums	21.0±2.56	25.3±0.97	30.9±3.43	32.5±3.48	11.0±0.72	14.0±0.76
Apricots	7.0±1.73	21.7±1.31	20.0±3.74	31.0±5.39	9.0±0.82	15.0±1.04

Table 3. Incubation of PTB eggs under different temperatures (°C) (RH 70%)

Temperature	*18°*	*23°*	*26°*	*29°*	*32°*
Incubation days	11.38±0.062	7.00±0.063	5.28±0.045	3.95±0.040	3.87±0.044

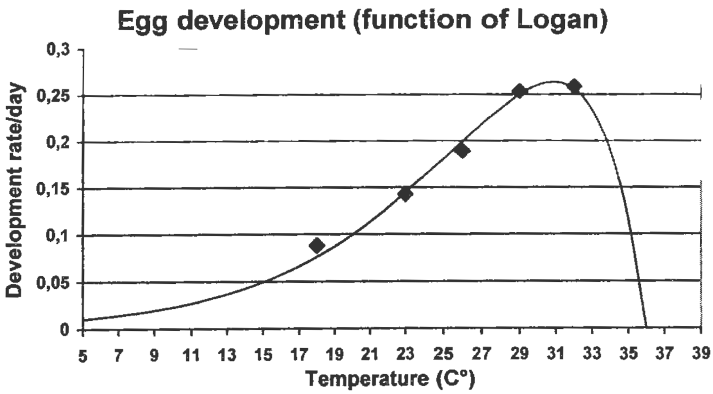

Fig. 1. Function of Logan for developmental rate of the eggs of PTB at different temperatures.

After positive validation, the model will be used by technicians in Emilia Romagna in IFP to give indication on the right moment to apply control measures.

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Occurrence of visual symptoms of pest and disease in 15 sweet cherry varieties under organic cultivation

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Abstract: In a cherry orchard located the in the South of Italy, with 15 sweet cherry varieties producing fruit suitable for processing, observations are carried out with regard to the occurrence of the visual symptoms of some biotic and abiotic adversities. Among the former, *Archips rosanus, Cylindrosporium padi, Monosteira unicostata,Operophthera brumata , Tetranicus* spp.and summer phylloptosis are considered, whilst the main abiotic adversity monitored is fruit cracking. After 3 years of observations, it is concluded that the incidence of damage seems to be influenced by genotype and year. No variety is fully resistant, although fruit obtained under organic cultivation remains suitable for processing.

Key words : cherries for processing, biotic and abiotic adversities.

Introduction

Among the many requirements expressed by the processing industry (Roversi,1997 and 2001) as regards the product characteristics of cherries, over the last few years it seems that the type of growing method, i.e. fruit from organic or integrated production, has been added to the list. Organic production of cherries in Italy is very limited (Caruso, 2004), and indeed nil for the processing industry. However, as of this year a research programme financed by MiPAF (Ortofrubio), which includes cherries for processing, has begun. After three years of research carried out under integrated production (Roversi & Monteforte, 2001), it was decided to continue research in the same cherry orchard, which had switched to organic production, with the aim of seeking confirmation of the varying susceptibility to some biotic and abiotic adversities of 15 varieties for processing.

Material and methods

The research involved a 16-year-old cherry orchard established with 15 cultivars for the processing industry previously described (Roversi, 1995; Roversi & Monteforte, l.c.) and for which in the last 3 years treatments were carried out in accordance with regional regulations for organic production. For *Cylindrosporium padi* no specific treatments was carried out.

For each cultivar, 10 representative trees were chosen, on each of which 2 fruit-bearing branches were labelled; over the three-year period 2002 – 2204 these were used for gathering data regarding the incidence of some adversities using an empirical scale of severity increasing from 0 (= absent) to 5. For each of the 3 years considered, all the data, always for the same plants and the same branches, was gathered independently by the same group of 3 technicians (valuers) and covered:

- as regards biotic adversity, *Archips rosanus, Cylindrosporium padi, Monosteira unicostata, Operophthera brumata , Tetranycus* spp. In addition, the degree of summer phyllopthosis was also measured, with the aim of confirming (Roversi & Monteforte,l.c.) its possible cause by certain of these adversities.

- as regards abiotic adversity, only the occurrence of fruit cracking was recorded.

The data was elaborated statistically with non-parametric tests, i.e. Mann-Whitney's U test for 'drawing out' the genotype, year and valuers effects, and Spearman's Rho coefficient to test qualitatively the relationship between biotic adversities and phyllopthosis .

Results and discussion

The data gathered over the 3 year period and its statistical analysis have led to the highlighting of the following.

Archips rosanus

The occurrence of this adversity varied much more between the cultivars (Tab.1) than from year to year (Tab.2). Indeed, the difference between years is not at all significant , while the effect of genotype is highly significant (Tab.3).Of the cultivars, the one least affected by this insect was Haciomer Karasi (with only 1.5 points); Roccamonfina (Tabellaria) was almost as resistent, with only a slightly higher score. Karobodur (3.78) was particularly susceptible, followed by Denisenova (3.73) and Ferrovia (3.67).

Cylindrosporium padi

The degree of visual symptoms of infection by *Cylindrosporium* was highly significant (Tab. 3) for both genotype and year. Of the genotypes studied, the least susceptible (Tab.1) cultivar was Cherie, with a score of 1.22. Stark Glorious Gold (1.63) and Karabodur (1.67) were also relatively resistant to this fungus. In contrast, Montagnola (3.69), Colafemmina (3.53) and Pagliarsa (3.44) were particularly damaged. Comparing years (Tab.2), the highest incidence (2.91) was in 2004 and the lowest (2.14) in 2003. It should be noted that the year with the highest incidence was also the one with frequent, heavy, spring rainfalls.

Monosteira unicostata

The leaf symptoms of the presence of this small bug were similar (just over 2 points) for all the cultivars studied, as shown in Tab. 1 (see also Tab. 3). The only exception was Haciomer Karasi, which for *Monosteira* reached 2.83 points.

However, the difference between years was highly significant (Tab. 3), with a maximum of 2.94 and a minimum of 1.11 in 2002 and 2004 respectively (see Tab.2). The lower level recorded in 2004 is perhaps be due to frequent and heavy summer showers, which may have limited populations.

Operophthera brumata

The damages caused by this lepidopter were highly significant, both for year and genotype, as shown by Tab. 3. More specifically, the incidence of damages of this adversity increased (Tab.2) from 2002 to 2004 , from an average of 0.84 to 1.38. The least susceptible genotypes (Tab.1) were Ferrovia and Stark Glorious Gold, both with 0.33 points. In contrast, Pagliarsa (2.78) and Montagnola (2.72) were much more affected. Data here not reported reveal this adversity was entirely absent (= 0 points) for some years and for some cultivars, e.g. in 2002 for Cherie, Droganova, Ferrovia, Karabodur and Stark Gloriuos Gold, in 2003 Marrasquino and Roccamonfina tardiva. On the contrary, the highest level (3.33) was recorded for Pagliarsa in 2004.

Tetranycus *spp.*

Damages by mites (see Tab. 2) decreased continuously from the beginning (2.38) to the end (0.82) of the3 year period under consideration, and these differences are highly significant (Tab. 3). The differences noted between cultivars, however, do not appear significant, being generally just above 1 and constantly lower than 2, except for Pagliarsa which reached 2.44.

Summer phyllopthosis

This phenomenon varied (Tab.1) widely among cultivars, with a very high significance (Tab. 3). Over the years, the scores increased (Tab.2) from the first (1.97) to the last (2.71) year of study, but statistical significance was not reached. The cultivars with the higher summer leaf drop were (Tab.1) Pagliarsa with a score of 3.56 and Colafemmina with 2.33; whilst the least affected cultivar was Roccamonfina della norma with a score of just 1.11.

Tab. 1 Comparative susceptibility of the genotypes studied to biotic and abiotic adversities

Varieties	*Archips* a	*Cylindr.* b	*Monost.* c	*Operopt.* d	*Tetranyc.* e	Σ a - e	Phyllopt.	Cracking
Cherie	2.44	1.22	2.00	0.67	1.11	7.44	1.67	0.22
Colafemmina	2.36	3.53	2.28	1.25	1.78	11.20	3.33	1.22
Denisenova	3.73	1.89	2.27	0.49	1.64	10.02	1.89	1.04
Droganova	3.44	1.89	2.11	0.56	1.93	9.93	1.33	1.19
Ferrovia	3.67	1.89	2.11	0.33	1.44	9.44	1.44	2.33
Haciomer Karasi	1.50	2.67	2.83	1.50	1.39	9.89	1.33	1.61
Karabodur	3.78	1.67	2.33	0.67	1.00	9.45	1.89	1.50
Marrasquino	2.78	2.67	2.00	0.67	1.11	9.23	1.78	3.22
Montagnola	2.19	3.69	2.58	2.72	1.97	13.15	2.83	0.56
Pagliarsa	2.11	3.44	2.00	2.78	2.44	12.77	3.56	1.11
Roccamonfina della norma	2.22	2.33	2.33	1.78	1.67	10.33	1.11	0.56
Roccamonfina (Tabellaria)	1.78	2.33	2.00	0.78	1.56	8.45	2.33	0.22
Roccamonfina tardiva	2.22	2.00	2.11	0.67	1.44	8.44	1.67	0.78
Stark Glorious Gold	3.22	1.63	2.33	0.33	1.37	8.88	1.93	0.78
Stella	2.74	2.67	2.33	0.89	1.67	10.30	2.96	1.11

The calculation of Spearman's Rho for the 3 year data revealed a highly significant and positive correlation between summer phyllopthosis and occurrences of both *Cylindrosporium* (.416 **) and *Operophthera* (.221**). This confirms the findings of a similar previous study (Roversi & Monteforte, l.c.).

Table. 2 Susceptibility trend over the years of observations

Adversities	2002	2003	2004	Σ
Archips	2.88	2.73	2.84	8.45
Cylindrosporium	2.35	2.14	2.91	7.40
Monosteira	2.94	2.93	1.11	6.98
Opheropthera	0.84	1.02	1.38	3.24
Tetranychus	2.38	1.68	0.82	4.88
Phylloptosis	1.97	2.03	2.71	6.71
Fruit cracking	0.98	0.84	1.47	3.29

Fruit cracking

As is known, the occurrence of this abiotic adversity, genotypes being equal, depends directly on rainfall during the period from the changing of skin colour to harvest. Indeed, the year (see Tab. 3) is highly significant. In any case, Tab. 1 shows that the cultivar most prone to cracking is Marrasquino with a score of 3.22. In contrast, Cherie and Roccamonfina della norma suffer far less, both with a score of just 0.22.

At the risk of stating the obvious, it should be borne in mind that this adversity, in contrast with biotic adversities, does not depend in any way on whether or not phytoiatric treatments are carried out.

Valuers concordance

The empirical scale adopted for evaluating the degree of adversities under study (Roversi & Monteforte, l.c.) has proved itself a good working instrument being that the scores given by the 3 different valuers did not vary significantly (Tab. 3).

Table. 3 Chi^2 values for the considered adversities as related to varieties, years and valuers.

Adversities	Varieties	Years	Valuers
Archips	127.34 **(.000)**	1.18 **(.553)**	.64 **(.967)**
Cylindrosporium	149.03 **(.000)**	16.58 **(.000)**	2.23 **(.328)**
Monosteira	11.79 **(.623)**	169.77 **(.000)**	1.49 **(.474)**
Operophthera	129.07 **(.000)**	16.98 **(.000)**	.48 **(.976)**
Tetranycus	23.56 **(.052)**	126.47 **(.000)**	6.02 **(.059)**
Phylloptosis	91.95 **(.000)**	5.60 **(.061)**	.32 **(.851)**
Cracking	76.64 **(.000)**	13.33 **(.001)**	2.40 **(.301)**

After 3 years of observations, in a cherry organic cultivation it is noted that :

- the lack of phytoiatric treatments permitted under traditional cultivation, or the use of only those permitted by organic production protocol, is unable to effectively control the adversities considered, especially *Archips rosanus* and *Cylindrosporium padi;*

- the susceptibility of 15 cultivars for fruit processing, to fungus, insects and mites, depends directly on the genotype. Indeed, even without specific treatment, some cultivars have proved to be constantly less attractive to insects and mites and less vulnerable to *Cylindrosporium.*- taking as a whole the occurrence of biotic adversities (Tab. 1), it appears that the least susceptible cultivar is Cherie (Σ=7.44); whilst the most vulnerable is Montagnola (Σ=13.15);

- as was to be expected, the year and therefore the climatic conditions, and the development, or lack of development of a biologic 'equilibrium', had an important role in the occurrence of the adversities considered. In this regard, for example, over the years, the occurrence of *Operophthera brumata* increased noticeably and significantly, while *Monosteira* and *Tetranychus* decreased in a similarly significant manner;
- summer phylloptosis, related to the occurrence of *Cylindrosporium* and *Operophthera,* while increasing constantly over the 3 year period studied, so far does not seem to have compromi ,ed the normal productivity of the cherry orchard.

To conclude, this study has shown how organic cultivation of a cherry orchard can co-exist with the adversities considered, which do not seem to prejudice the quantity or the quality of the fruits suitable for processing.

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Preliminary study of *Forficula* sp. (Dermaptera: Forficulidae) as predator of *Cacopsylla pyri*, (Homoptera: Psyllidae), under field conditions in Lleida (Spain).

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Abstract: The predatory effect of earwigs (*Forficula* sp.) on *Cacopsylla pyri* (L.) under field conditions was studied in Lleida (NE Spain). Pear cultivars monitored were Blanquilla and Conference. Pear trees were grouped in three treatments according to the earwigs manipulation done: Treatment 1, no manipulation of earwigs population; Treatment 2, artificial decrease of earwigs population and Treatment 3, artificial increase of earwigs population. In Treatment 2, earwigs were captured by shelter traps weekly from March to October in 2002 and from March to December in 2003 and then released onto the ground of trees of the Treatment 3. The number of immature stages of *C. pyri* were monitored on the five apical leaves of three young shoots per tree and the number of adults with beating trays. The predatory effect of earwigs was manifested in both pear cultivars and led to a significant decrease in the number of *C. pyri* eggs. Nymphs and adults of *C. pyri* were also lower in earwig-enhanced plots but differences were not statistically significant. In accordance with these results and those reported for other areas, the role of earwigs should be considered when IPM programmes in pear orchards are proposed.

Key words: earwigs, pear psylla, predators, *Forficula*, *Cacopsylla pyri*.

Introduction

Pear psylla (*Cacopsylla pyri* (L.)), is one of the most damaging pest of pear trees in the area of Lleida (NE Iberian Peninsula). The increase of pear psylla populations has been mainly due to their resistance to the insecticides commonly used and to the decrease of its natural enemies. In Lleida, earwigs (Dermaptera: Forficulidae) are always present in pear orchards. Some authors have reported that earwigs are effective predators of pear psyllids (Lenfant *et al.* 1994; Jaworska *et al.* 2002; Sauphanor *et al.* 1994; Solomon, *et al.*, 2000). This study aims to determine the predatory capacity of *Forficula* sp. on *C. pyri* populations in field conditions.

Material and methods

The experiment was carried out in Gimenells (Lleida) in a pear orchard (0.5 ha) that had never received any insecticide nor acaricide treatments. The trees belonged to the cv. Conference (5 rows; 90 trees per row) and Blanquilla (4 rows; 78 trees per row) and they were 10 years old. To asses the effect of *Forficula* sp. on *C. pyri* populations, pear trees were grouped into three groups and each group randomly assigned to one of the three treatments applied according to the population manipulation done. Treatment 1 was used as a control and no manipulation of the earwig population was done. In Treatment 2, earwig populations were decreased by placing corrugated cardboard shelter traps in the trunk, near the base of pear trees, and removing weekly the earwigs caught in the traps from March to October in 2002 and from March to December in 2003; after that the number of earwigs caught were recorded. In

Treatment 3, earwig populations were increased by releasing the earwigs caught in Treatment 2 onto the ground of the five central trees. Corrugated cardboards were wrapped around the base of tree trunks in Treatment 3 to provide artificial refuges to the earwig individuals. A total number of 8,920 earwigs were transferred from plots of the treatment 2 in the year 2002; the corresponding number transferred in 2003 were 2,147. Four replicates per treatment and cultivar were used (13-15 trees/replicate).
Pear psylla populations were sampled on the central trees of each replicate. The different immature stages were monitored by scouting on the five apical leaves of three young shoots per tree and the adults were monitored with beating trays.

Statistical analyses
To determine the influence of the treatment on psylla populations, a split-plot like model was used in which the year and cultivar were the main plots and the treatment the subplot. All factors, excepted blocks, were considered fixed. The least significant test, LSD, was used to compare means when needed.

Results and discussion

The analysis of the results show that independently of the pear cultivar and year, there were significant differences between treatments in the number of *C. pyri* eggs, but not in the number of nymphs or adults (Table 1).

Table 1. Values of F (probabilities) of the factors Year, Cultivar and Treatment in the ANOVA of *C. pyri* eggs, nymphs and adults. Double interactions were not significant. Super indexes show the degrees of freedom.

Psylla stadia	Year^{1,9}	Cultivar^{1,9}	Treatment
Eggs	F=77.02 (*P*<0.001)	F=10.24 (*P*=0.08)	F=15.82^{2,18} (*P*=0.01)
Nymphs	F=94.52 (*P*<0.001)	F=15.58 (*P*<0.001)	F=0.20^{2,18} (*P*=0.82)
Adults	F=91.20 (*P*<0.001)	F=9.13 (*P*=0.01)	F=1.99^{2,23} (*P*=0.16)

Figure 1 shows that the number of psylla eggs were higher in pear trees where the earwig population was decreased. The results show that *Forficula sp.* is, at least partially, responsible of *C. pyri* egg reduction. Consistently with that found here, Lenfant *et al.* (1994) and Sauphanor *et al.* (1994) reported the high capacity of third instars earwigs to consume pear psyllid eggs (1000 per day in laboratory conditions).

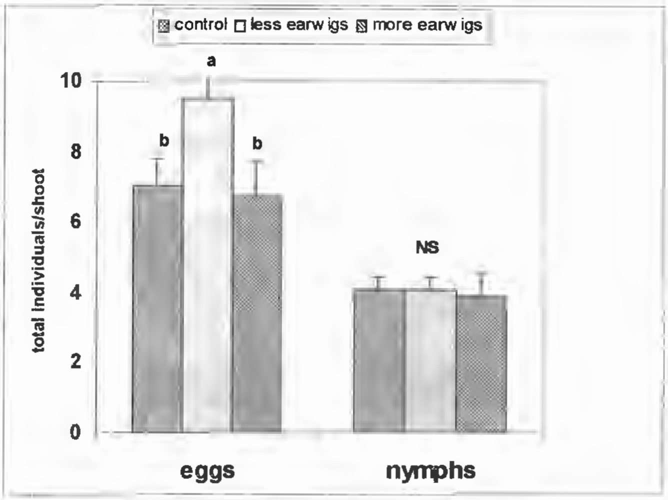

Figure 1. Total number of *C pyri* eggs and nymphs per shoot in each treatment. (Treatment 1= undisturbed control; Treatment 2=earwigs partially removed; Treatment 3= earwigs enhanced). Means with the same letter are not significantly different at $P > 0.05$ (LSD)

Plots where earwigs were enhanced showed a lower number of psylla nymphs (Fig. 1) and adults (Fig. 2) but differences were not significantly different. With a similar methodology based on management of earwig populations, Solomon *et al.* (1999) found, a decrease below 50% of numbers of *C. pyricola* nymphs, in pear trees where earwigs population was enhanced, compared with trees from which earwigs were removed.

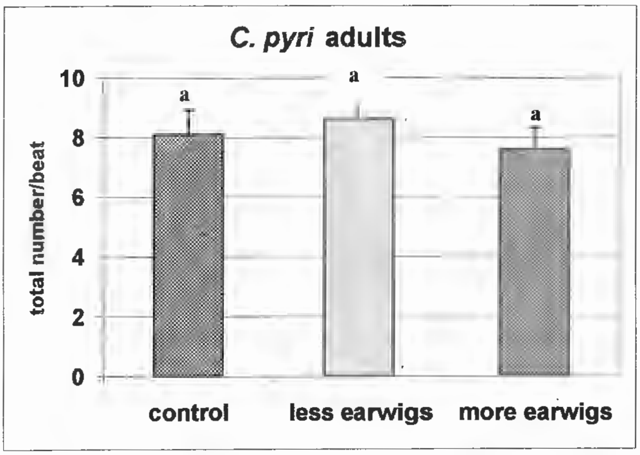

Figure 2. Total number of *C pyri* adults in each Treatment (1=control; 2=less earwigs; 3=more earwigs). Means were not significantly different (LSD)

In conclusion, the predatory role of earwigs on *C. pyri* populations should be considered and they may be one of the main targets in the habitat manipulation to conserve and enhance naturally occurring predators in pear orchards. This is being investigated in our laboratory.

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Sustainable control of the rosy apple aphid *Dysaphis plantaginea*

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Abstract: The rosy apple aphid *Dysaphis plantaginea* Passerini (Homoptera: Aphididae) is a key pest in Western Europe that damages leaves, shoots and fruits on apple trees. These aphid populations are usually controlled by insecticides, which are sprayed as soon as aphids are detected on trees. Aphid densities vary widely from one year to the next, and spraying is not likely to be required every year. The continuous use of insecticide sprays has given the appearance of resistance. Therefore, forecasting tools are urgently needed to predict aphid outbreaks and to optimize the control of this species. Sustainability of fruit production entails a strategic shift in which a 'bottom-up' approach is recommended. This approach is based on a suitable design and management of the orchard to allow a control of pests and diseases based on resistant rootstocks and cultivars, cultural management and biological control. Thus, as a first step in orchard planning, an adequate choice of cultivars is highly recommended. Cultivars with tolerance and little susceptibility to *D. plantaginea* are available at present for both cider- and dessert-apple production. As a second step, biological control should be emphasized. Natural populations of antagonists are not able to control *D. plantaginea* populations. Habitat manipulation (e.g. sowing of flower strips) to increase antagonist populations does not improve biological control to a great extent. However, augmentative releases of *Adalia bipunctata* (L.) larvae just after egg hatch or in autumn may successfully control *D. plantaginea* populations. Lastly, when antagonists are not effective on susceptible cultivars, neem-derived insecticides may be sprayed against this aphid with high effectiveness. The integration of these strategies allows a sustainable control of *D. plantaginea* that is well suited to organic as well as integrated apple production.

Key words: apple, biological control, cultivars, forecasting, neem, tolerance

Introduction

The rosy apple aphid *Dysaphis plantaginea* Passerini (Homoptera: Aphididae) is a key pest in Western Europe that damages leaves, shoots and fruits on apple trees. *D. plantaginea* is a holocyclic dioecious species with apple tree being its primary host and *Plantago* spp. the secondary hosts. This aphid is normally controlled with specific insecticides (e.g. pyrimicarb, imidacloprid). Since damage can be observed in twenty-four hours after infestation (Forrest & Dixon, 1975) the apple grower should be precise in the timing of application to avoid high damages. Graf *et al.* (1999) attempt to determine precisely the egg-hatching moment to time accurately the insecticide applications. On the other hand, insecticides are sprayed independently of aphid abundance despite aphid numbers varied widely from one year to the next (Hemptinne *et al.*, 2004). The continuous use of insecticide has given the appearance of resistance (Delorme *et al.*, 1998) and an increase by 50 % of the number of applications needed (Graf *et al.*, 1999). The development of a model forecasting the variations in abundance of *D. plantaginea* would improve the use of insecticides reducing the threat of resistance and would facilitate a sustainable control of the aphid. Hemptinne *et al.* (2004) attempt to develop a mathematical model to precisely asses damage risk. They concluded that *D. plantaginea* populations are regulated by density-dependence processes while climate may act modulating this fact, but further studies are needed. Molecular markers (Solomon *et al.*, 2003) and *D. plantaginea* sex pheromone (Jean Fitzgerald, personal comment) may help to follow the population dynamics of this aphid.

Sustainability of fruit production entails a strategic shift. A 'bottom-up' approach based on a suitable design and management of the orchard to allow a control of pests and diseases based on resistant cultivars, cultural management and biological control is recommended (Prokopy, 2003, Dapena *et al.*, 2002; 2005).

The correct choice of cultivars

An adequate choice of cultivars is highly recommended as a first step in orchard planning (Prokopy, 2003, Dapena *et al.*, 2002; 2005). Cultivars with tolerance and little susceptibility to *D. plantaginea* are available at present for both dessert- and cider-apple production.

Dessert-apple cultivars
Florina (Rat-Morris & Lespinasse, 1995), *Galarina* and *GoldRush* (Miñarro & Dapena, unpublished) are tolerant to *D. plantaginea*. Other cultivars (e.g. *Liberty*, *Priscilla*) show very low susceptibility (Miñarro & Dapena, unpublished). All these cultivars have the V_f gene of resistance to apple scab *Venturia inaequalis*, and derived from *Malus floribunda* 821. This cultivar is the origin of the V_f gene and is also supposed to be the origin of the resistance to *D. plantaginea* (Rat-Morris & Lespinasse, 1995). Although the V_f gene and the resistance to *D. plantaginea* are inherited independently (Miñarro & Dapena, 2004) other V_f-cultivars could also be resistant to *D. plantaginea* and should be tested against this aphid. The development of molecular markers linked to this resistance would facilitate the analysis and selection of tolerant cultivars. Growing cultivars resistant to scab and tolerant or low susceptible to *D. plantaginea* would reduce the pesticide inputs facilitating a sustainable apple production.

Cider-apple cultivars
In order to improve the durable resistance to diseases and pests, Asturian (NW Spain) cultivars were crossed with *Florina* (Dapena & Blázquez, 2004), which is resistant to scab, little susceptible to the fire blight *Erwinia amylovora* and resistant to *D. plantaginea* (Rat-Morris & Lespinasse, 1995). From the first crossing, performed in 1989, four cultivars have been selected and are in registry at present. All are resistant to scab and three of them are tolerant to *D. plantaginea* (Dapena & Miñarro, 2001; Dapena & Blázquez, 2004; Miñarro & Dapena, 2004). Therefore growers will be able to grow cider cultivars resistant to scab and *D. plantaginea* soon. Progenies of other crosses are being evaluated with high percentages of descendants tolerant to *D. plantaginea* (Miñarro & Dapena, 2004).

However, the resistance of *Florina* has been broken down in a few cases (Rat-Morris *et al.*, 1999). If this is the only kind of resistance employed, the selection pressure of resistance-breaking biotypes of *D. plantaginea* and the threat of the resistance to be overcome will increase. Therefore, new sources of resistance to *D. plantaginea* should be explored.

Tolerance to *D. plantaginea* of local cider-apple cultivars has also been evaluated (Miñarro & Dapena, unpublished). A gradient of susceptibility was observed although none of them resulted tolerant. However, an interesting trend was observed: early-leafing cultivars tended to develop colonies sooner and consequently suffered higher infestation and damage. Thus, growing of late-leafing cultivars could facilitate the control of *D. plantaginea*. Bonnemaison (1959) observed that cultivars which lost their leaves early in autumn suffered lower infestations in the next spring because egg-laying had been lower. Manual defoliation of trees in autumn successfully controlled *D. plantaginea* (Hoehn *et al.*, 2003) although the application of this method to commercial orchards seems difficult.

Natural enemies and possibilities of biological control

Parasitoids and predators are the most important natural enemies of aphids. However, parasitism on *D. plantaginea* colonies is infrequent, probably due to host alternation, ant attendance and/or hyperparasitism (Cross *et al.*, 1999). However, both generalist and specialist predators occur frequently in apple orchards. *Adalia bipunctata* (Coleoptera: Coccinellidae), *Episyrphus balteatus* (Diptera: Syrphidae) and *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) are the three most abundant predators of *D. plantaginea* in European orchards (Wyss *et al.*, 1999[b]; Solomon *et al.*, 2000; Miñarro *et al.*, submitted). However, natural populations of specialist enemies are unable to control *D. plantaginea* abundance probably due to their reproductive behaviour: the numerical response to the abundance of aphids is weak or absent and the predators respond negatively to the presence of conspecifics (Miñarro *et al.*, submitted). Since natural populations of antagonists are not able to control *D. plantaginea* populations, alternative strategies of biological control have been investigated. Habitat manipulation (e.g. sowing of flower strips) to increase antagonist populations does not improve biological control to a great extent (Wyss, 1995; Vogt & Weigel, 1999). However, augmentative releases of *A. bipunctata* larvae just after egg hatch or in autumn resulted efficient in experimental conditions (Wyss *et al.*, 1999[a]; Kehrli & Wyss, 2001). It remains to be seen if this strategy is practically and economically applicable to orchards.

Botanicals: neem-derived insecticides

Bioinsecticides derived from the neem tree *Azadirachta indica* A. Juss (Meliaceae) are a good alternative to control *D. plantaginea* on susceptible cultivars when natural enemies do not guarantee biological control. Satisfactory control has been achieved with applications of NeemAzal-T/S (Schulz *et al.*, 2000, Miñarro & Dapena, 2005) although quite less satisfying results were obtained with other commercial neem insecticides (Miñarro & Dapena, 2002; 2005). The main effect of neem on *D. plantaginea* is the retardation in the aphid development (Schulz *et al.*, 2000). This is a disadvantage against classical insecticides since some damage could be done until the extinction of the aphid colony. Thus, the recommended strategy for controlling *D. plantaginea* with NeemAzal-T/S is an application early in the development of the colony, during the third/fourth larval stage of the fundatrices (Schulz *et al.*, 2000). The application of NeemAzal-T/S before full blooming reduces aphid populations sooner than application after blooming (Miñarro & Dapena, 2005). Since late infestation may be observed when NeemAzal-T/S is applied just once (Schulz *et al.,* 2000), two applications could reduce risk of late damages improving control.

Conclusions: strategy of control

When possible, priority should be given to the growing of resistant cultivars as the most economically and ecologically valuable strategy for controlling *D. plantaginea.* Further investigations to improve biological control seem necessary. Lastly, NeemAzal-T/S applications control successfully *D. plantaginea* without interfering with biological control of other phytophagous arthropods. Moreover, the development of a forecasting method would improve the sustainable control of this aphid. The integration of these strategies allows a sustainable control of *D. plantaginea* that is well suited to organic (Weibel & Häseli, 2003) as well as integrated (Cross, 2002) apple production.

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Preliminary investigation on the natural enemies of the peach twig borer, *Anarsia lineatella* Zeller in northern Italy

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Abstract: A survey has been carried out to assess the status of natural enemies of the peach twig borer (PTB), *Anarsia lineatella* Zeller, which recently increased its importance in Northern Italy.
Among the species more frequently found, are *Paralitomastix varicornis* (Nees) and two species never recorded from *A. lineatella*.
The study has been carried out in orchards in Northern Italy; some of them were organic and other conventional. As great variability has been found in the different situation, a studies will be planned to investigate on the factors that could increase the presence and activity of natural enemies of PTB.

Key words *Anarsia lineatella*, parasitoids

Introduction

Damages by *Anarsia lineatella* Zeller (Lepidoptera: Gelechidae) (PTB) on apricot, peach, almond and plum have different importance in different years, even in the same orchard.

In the last decades the insect has spread to new areas, showing periods with heavy damages followed by some years with a lower incidence.

In 2002 larvae were collected from shoots and fruits and a very significant presence of parasitoids was recorded, especially in organic peach orchards (Molinari F., Zanrei O.,2004) (Tab. 1).

Table. 1 - Parasitization rate of PTB larvae in a previus study (Molinari F., Zanrei O.,2004).

date	site	shoots/fruits	larvae collected	larvae parasitized	% parasitization
7/6	S. Prospero	Shoots	335	16	04.8%
14/6	S. Prospero	Shoots	252	35	13.9%
19/6	Casalfiumanese	Shoots	340	205	60.3%
26/6	Casalfiumanese	Shoots	105	69	65.7%
25/7	PEMPA	Fruits	27	4	14.8%
02/8	PEMPA	Fruits	35	7	20.0%

Larval and pupal parasitoids have been listed from Amerca and Europe among Hymenoptera Ichneumonoidea and Chalcidoidea and some authors report a high degree of parasitization.

For this reason, in 2004 a survey has been planned in order to assess the importance of natural control of PTB in northern Italy orchards.

The present paper is dealing with the first results.

Methods and Materials

In order to assess the presence of parasitoids, larvae and pupae of *A. lineatella* were collected in northern Italy in peach orchards where a significant infestation had been recorded. The orchards chosen had different pest managements: organic, Integrated Fruit Production and conventional.

In an orchard where a high percentage of larval-pupal parasitisation was found, batchs of eggs of *A. lineatella* were exposed to check for the presence of egg parasitoids.

In order to collect overwintering larvae, cardboard bands were exposed in field in the first days of April 2004 and collected before the beginning of the first flight.

Single larvae and pupae from cardboard bands were put in Bio-assay trays and checked daily for adult PTB or parasitoid emergence; farms are listed in table 2.

Table. 2 - Farms in which research on parasitization of PTB was done in 2004.

[illegible] site	management
Casalfiumanese 1(BO)	Organic
Casalfiumanese 2 (BO)	Organic
Casalfiumanese 3 (BO)	Organic
Lagnasco 1 (CN)	IFP
Lagnasco 2 (CN)	IFP
Cavallotta 1 (CN)	IFP
Cavallotta 2 (CN)	IFP
S. Pietro in Vincoli (RA)	IFP-MD
Montenovo di Montiano (FC)	Organic
Gambellara (RA)	IFP-MD
S. Lorenzo in Noceto (FC)	Organic

During the rest of the season the level of infestation in different orchards was monitored in order to collect larvae and pupae from the 1^{st} and 2^{nd} generation.

Results

Cardboard bands yelded a total of 464 overwintering larvae from 7 out of 11 peach orchards. Parasitoids were found in 3 organic orchards at a very significant rate (20%-45,5%) (Tab. 3). The sentinel eggs exposed in Casalfiumanese 2 yelded no egg parasitoid.

In 2004 it was difficult to find PTB infested orchards during summer and from one orchard 38 larvae were obtained from fruits and no parasitoid.

Three species of parasitoids where collected from the overwintering larvae: *Aethecerus discolor* Wesmael (Hym. Ichneumonidae), *Baeognatha armeniaca* Telenga (Hym.

Brachonidae) and *Paralitomastix variicornis* (Nees) (Hym. Encyrtidae). Only the latter is already known to parasitise *Anarsia lineatella.*

A. discolor was found in the three orchards of Casalfiumanese in which organic farming was applied, while the other two species only in one of the above orchards each.

B. armeniaca belongs to Agathidinea, koinobiont solitary endoparasitoids of lepidoptera hidden larvae, which have been recorded from Gelechiidae (Nixon, 1986). It is present in Austria, in the Marmara region and in Turkey and Armenia, where it were obtained from *Anarsia eleagnella* Kuznetzov. The mature larva leaves the host and devours it from the outside, except for head capsule. In the rearing media from which *B.armeniaca* emerged we found the head capsule of the last instar larva of PTB.

Table 3 – Larvae collected in the 2004 and parasitization rate.

location	n° traps	larvae collected	adults emerged	n° parasitized	% parasitization
Casalfiumanese 1(BO)	120	20	11	6	30.0%
Casalfiumanese 2 (BO)	120	15	7	3	20.0%
Casalfiumanese 3 (BO)	120	22	11	10	45.5%
Lagnasco 1 (CN)	120	0	0	0	0.0%
Lagnasco 2 (CN)	191	267	217	0	0.0%
Cavallotta 1 (CN)	120	9	7	0	0.0%
Cavallotta 2 (CN)	60	117	105	0	0.0%
S. Pietro in Vincoli (RA)	120	14	10	0	0.0%
Montenovo di Montiano (FC)	120	0	0	0	0.0%
Gambellara (RA)	120	0	0	0	0.0%
S. Lorenzo in Noceto (FC)	80	0	0	0	0.0%

In the present study a significant presence of parasitoids was recorded in some environments. Natural enemies occurred in organic orchards in a hilly area surrounded by abundant spontaneous vegetation, where PTB had been present for a long time.

In other situations despite the high number of larvae collected in Piedmont (Lagnasco 2, Cavallotta 2), no parasitoids were found. In this area *A. lineatella* is presence is increasing in the last few years and it can be retained that a natural enemy complex is not build up.

Further investigation are planed to asses the possibility of improving natural control.

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Occurrence of the anthocorids *Anthocoris nemorum* and *A. nemoralis* in apple and pear in Denmark

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Abstract: The majority of anthocorids found in three Danish organic apple orchards in 2001 were *Anthocoris nemorum*. The following year there were also more *A. nemorum* in apple, whereas in pear, *A. nemoralis* was dominant. In the apple orchards most *A. nemorum* were found in trees early and late in the summer, while in the mid-summer the density of *A. nemorum* fell in the trees while it increased in the herbal vegetation. 2001 data suggest emergence of a second generation. The observed higher number of *A. nemorum* in herbal vegetation in mid-summer suggests that maintenance of the proper herbal vegetation can help to keep *A. nemorum* in orchards when fewer prey are available in the trees. *Anthocoris nemorum* is normally thought to have only one generation a year in Denmark. However, an unusually warm summer can have caused the emergence of a second generation.

Key words: anthocorid, generations, orchard, occurrence

Introduction

Two of the most abundant predators in apple and pear orchards of Europe are *Anthocoris nemorum* (L.) and *A. nemoralis* (Fabricius) (Anthocoridae: Heteroptera) (Skanland, 1981; Solomon, 1982). Both anthocorids are polyphagous predators preying on aphids, mites, psyllids and lepidopteran eggs and young larvae (Anderson, 1962a; Anderson, 1962b; Collyer, 1967; Hill, 1957; Solomon *et al.*, 2000; Solomon, 1982). Both are found in a wide range of habitats, however *Anthocoris nemoralis* is mostly found on perennials, whereas *A. nemorum* is found on both perennials and annuals (Anderson, 1962b; Collyer, 1967; Fauvel, 1999). An understanding of the mechanisms that influence the distribution of predators will be an important tool to improve biological control in orchards.

Materials and methods

In 2001 arthropods were sampled in three organic apple orchards on Zealand, 'Rørrendegaard' in Tåstrup, belonging to the Royal Veterinary and Agricultural University, a private orchard in Jørlunde, near Slangerup, and a private orchard near Frederikssund. Samples were taken from May to October 2001. Again in 2002 the orchard at 'Rørrendegaard' was sampled. In 2002 a pear and an apple orchard separated by a windbreak on the island of Fejø south of Zealand were also sampled.

A combination of the beating and funnel method was used to sample tree dwelling arthropods. Samples were collected by sharply beating one branch on each of ten randomly selected trees three times with a stick. Arthropods dropping off were collected in a plastic bag attached below a hole in the center of a cloth funnel (45 × 60 cm). Sweep net samples were taken from annual vegetation between trees (Jørlunde and Frederikssund) or –in the case of Rørrendegaard and the two orchards on Fejø where vegetation under trees was cut very short in the annual vegetation along the surrounding windbreak. Each sample consisted of ten double-sweeps. On each sampling occasion 5-10 beating funnel samples and 5-10 sweep net samples were taken from each orchard. Arthropods from beating funnel samples were identified to genus, species or group, while for sweep net samples only anthocorids were identified.

Results

By 3rd of May 2001 the first overwintering *A. nemorum* females were active on apple. Across the season there were (mean ± SE) 1.35 ± 0.09 *A. nemorum* individuals per sample, but only 0.09 ± 0.03 *A. nemoralis*. Other predatory Heteroptera were also encountered, most commonly *Orthotylus marginalis* with an average of 1.09 individual per sample.

Densities of anthocorids in apple at Rørrendegaard in 2002 were similar to those found in 2001, with an average of 1.89 ± 0.09 *A. nemorum* and 0.09 ± 0.04 *A. nemoralis* respectively. In the two neighbouring orchards on Fejø 1.32 ± 0.27 *A. nemorum* and 0.08 ± 0.07 *A. nemoralis* were found. In pear *A. nemorum* occurred with an average of 0.06 ± 0.10 while there was an average of 3.72 ± 0.05 *A. nemoralis*.

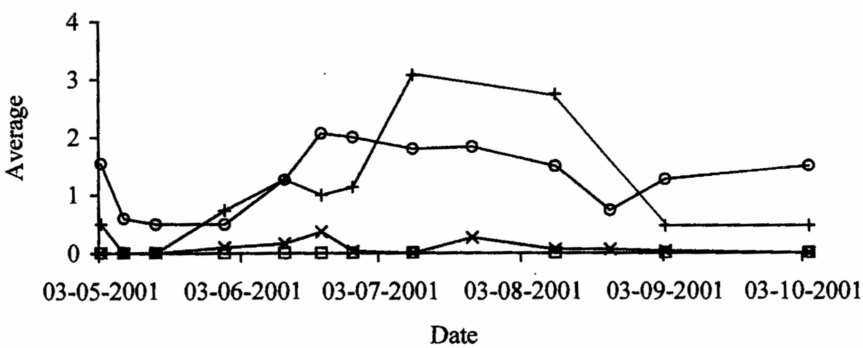

Figure 1. Average number of *A. nemorum* sampled from trees (+) and annual vegetation (O) and of *A. nemoralis* sampled from trees (×) and annual vegetation (□) in three apple orchards in 2001.

Samples from the annual vegetation allowed an assessment of possible seasonal changes in the distribution of anthocorids on annual versus perennial vegetation, although the use of two different sampling techniques only allows for a comparison of relative changes. In 2001 the densities of *A. nemorum* were initially higher in the trees, but from mid-July to late August *A. nemorum* was relatively more numerous in the annual vegetation. This period coincided with a lower density of prey in the apple trees. After mid-end August densities in trees increased again (Figure 1). Especially in two of the orchards (Rørrendegaard and Jørlunde) stinging nettle (*Urtica dioica*) was common in the annual vegetation, and *A. nemorum* was often found in this plant. A similar pattern with more *A. nemorum* found in the annual vegetation in mid-summer was observed in apple 2002. No *A. nemoralis* were found in the annual vegetation in 2001 and only few in 2002.

In two of the tree orchards in 2001 there were indications of two distinct generations of *A. nemorum*. In the third one, Rørrendegaard, where the density of *A. nemorum* was highest, the distinction between generations was less clear, probably because of an overlap between generations. Average from the three orchards is shown in Figure 2. Data from 2002 suggest that two generations also occurred this year.

Data from apple in 2001 and 2002 and from the two neighbouring apple and pear orchards in 2002 show that while *A. nemorum* is by far the most numerous in apple, *A. nemoralis* is more numerous in pear. Even in a case with two neighbouring orchards, and high densities of *A. nemoralis* in pear, movement of *A. nemoralis* into the neighbouring apple orchard appeared limited. Thus, when densities of *A. nemoralis* were highest by mid-September 2002, 0.1 ± 0.1 *A. nemorum* and 16.5 ± 3.1 *A. nemoralis* were found in pear and in the neighbouring apple orchard 0.6 ± 0.4 *A. nemorum* and 0.2 ± 0.2 *A. nemoralis*.

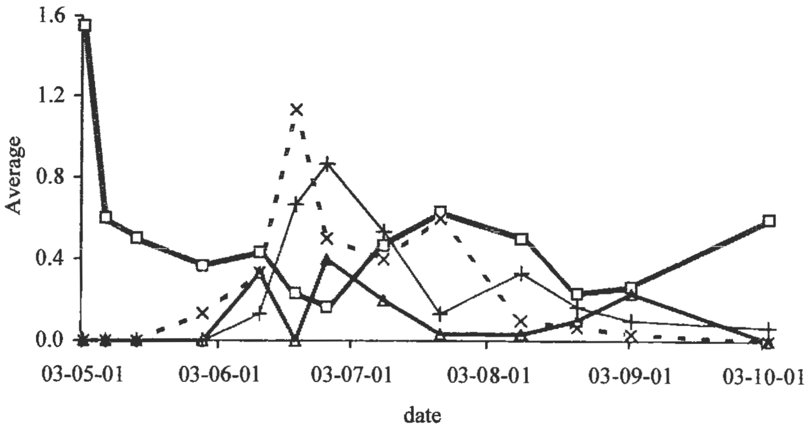

Figure 2. Average number of *A. nemorum* females (□), small (×), medium (+) and large nymphs (Δ) sampled in three apple orchards in 2001.

Discussion

The early presence of antochorids in orchards indicates that they can play an important role in the natural control of insect pests. Generalists can be of key importance if they occur early in the season, controlling or delaying the population development of insect pests. A delay will provide better chances that the later occurring specialists can keep a pest attack at an acceptably low level.

The high density of *A. nemorum* in annual vegetation during mid-summer shows that *A. nemorum* is able to exploit both layers of the vegetation. Further, data suggest that annual vegetation can help maintain or attract anthocorids in an orchard in periods where there is few prey in the trees. A plant such as stinging nettle with much alternative prey is one option. English studies show that cornflower (*Centaurea cyanus*) and corn chamomile (*Anthemis arvensis*), can also serve to attract anthocorids (Fitzgerald & Solomon, 2004).

In Scotland there is normally a single generation of *A. nemorum* a year (Hill, 1957), while there are two in Southern England (Collyer, 1967) and three generations have been reported near Paris, France (Herard & Chen, 1985). Based on this, one or perhaps two generations were expected to occur in Denmark. Data from 2001 and 2002 suggest that in both years there was a second, or at least a partial second, generation of *A. nemorum* in Denmark. Since both summers were warmer than usual, it is likely that in cooler summers only one generation is completed.

Laboratory experiment suggest that the predominance of *A. nemorum* in apple and *A. nemoralis* in pear is affected by oviposition preferences for apple and pear leaves respectively (Sigsgaard, 2004). Further, *A. nemoralis* is an important predator of pear psylla (*Cacopsylla pyri*) and the high numbers of this predator in the pear orchard in 2002 may have been caused as well by a heavy infestation of this pest. For biological control purposes results stress that in pear *A. nemoralis* is the anthocorid of interest, while in apple it is *A. nemorum*.

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Fly-free cherries: utopia or realistic hope?

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Abstract: The European cherry fruit fly (*Rhagoletis cerasi* L.) is presently the most serious pest of cherries in Europe and causes enormous economic losses in sweet and sour cherries. Control of cherry fruit fly up to now was mainly based on broad spectrum insecticides, which are toxic to humans, non-target organisms and the environment. As a result, there is a need to find alternative methods of control, which are safe for both non-target organisms and the ecosystem.
Different potential strategies to control the pest are discussed and first results of own initial experiments for biological control with entomopathogenic nematodes as well as chemical and biotechnical control with bait sprays and potential bait substances are shown as an overview of the current research work of the authors.

Key words: European cherry fruit fly, *Rhagoletis cerasi*, biological control, biotechnical control, entomopathogenic nematodes, bait sprays, bait substances

Introduction

The control of the European cherry fruit fly *Rhagoletis cerasi* L., the most important pest in cherry orchards is very difficult in all production systems, i.e. conventional or integrated cherry production, in organic farming as well as in house gardens (Vogt, 2002, Daniel & Wyss, 2003). Different strategies are conceivable to control the pest, chemically, physically, biotechnologically or biologically.

Dimethoate is the only compound registered for the chemical control of the pest at present in conventional farming in Germany (BVL, 2004, Vogt, 2002). There are two problems concerning the application. One, in using dimethoate, a pre-harvest period of at least 21 days is required. Two, the compound is only registered till the end of December 2004. Within the re-evaluation process underway for pesticides in the EU, this insecticide might be removed because of its environmental impact. Another possibility to control cherry fruit fly with insecticides could be bait sprays. In contrast to cover sprays the amount of insecticides can be reduced with bait-and-kill formulations. This method, mainly using broad spectrum insecticides, is applied since years against different species of fruit flies (Roessler, 1989). First experiences have been made with *Rhagoletis cerasi* (Haniotakis *et al.*, 1987) and using modern environmentally friendly insecticides with other *Rhagoletis* species (Pelz *et al.* 2004).

Physical barriers against the cherry fruit fly are also possible. Netting systems to cover the trees are very expensive and not suitable for high trees and uneven landscape.

There exist several biotechnical approaches to control *R. cerasi*. Yellow sticky traps are used in monitoring and forecasting cherry fruit fly (e.g. Haisch & Boller, 1973, Remund & Boller, 1979, Kapoor *et al.*, 1987, Voigt, 1997, Epp, 1998, Theinert & Dickler, 2000). An increase of efficacy of the traps can be reached with ammonia releasing compounds

(Wiesmann, 1944, Haisch & Forster, 1969, Katsoyannos, *et al.*, 1980, Katsoyannos, *et al.*, 2000, Liburd *et al.*, 2001), but results are not homogenous. As another biotechnological method, the use of host marking pheromone was investigated in the 1970th (Katsoyannos, 1975, Hurter *et al.*, 1976, Katsoyannos & Boller, 1976, Katsoyannos, 1979, Katsoyannos & Boller, 1980). In first experiments with host marking pheromone washed off from cherries an efficacy of 78 % up to 90 % could be reached (Boller *et al.*, 1980). The high costs of complete synthesis of the molecule prevented that method to be used in practice (comm. Boller). Furthermore a high infestation pressure (Fimiani, 1982) and the decreasing effect of the pheromone (Hoffmeister & Boller, 2003) restrict the control of the pest. The sterile insect technique (SIT) as biotechnological control technique of European cherry fruit fly was mainly investigated in the 1960th, 70th and 80th (Boller, 1969b, 1970, Haisch & Boller, 1971, Boller *et al.*, 1970, 1971, Russ *et al.*, 1973, Boller, 1975, Boller *et al.*, 1976, Vallo *et al.*, 1976, Boller, 1978, Haisch *et al.*, 1978, Boller *et al.*, 1980). But there is no suitable rearing method to produce enough specimens for this technique. Another problem for the efficacy of SIT in cherry fruit fly can be a high infestation pressure and the dispersal of only a few males of the natural population into the treated area (Boller *et al.*, 1980). The multiple mating of the flies (own observations) can reduce the efficacy of sterile males.

For biological control of European cherry fruit fly parasitoids, entomopathogenic fungi and entomopathogenic nematodes are potential agents. Parasitoids are of less importance (Hoffmeister, 1990, Balazs & Jenser, 2004). In dependence on the fly stage there are medium and good results in mortality of different species of fruit flies caused by entompathogenic fungi in laboratory (Lezama-Gutierrez *et al.*, 2000, Castillo *et al.*, 2000, De la Rosa *et al.*, 2002, Ekesi *et al.*, 2002). No investigations with entomopathogenic fungi have been made with *Rhagoletis cerasi*. First investigations in laboratory, semifield and field resulted in a high efficacy of entomopathogenic nematodes against *R. cerasi* larvae (Köppler *et al.*, 2003a, 2003b, 2004). Patterson Stark & Lacey (1999) and Yee & Lacey (2003) showed a high susceptibility of the Western cherry fruit fly *R. indifferens* to entomopathogenic nematodes.

Material and methods

Own experiments were carried out for biological control of *R. cerasi* with entomopathogenic nematodes in 2002 and 2003, for biotechnological control with potential bait substances to improve traps and for chemical control with bait sprays in 2004.

Biological control with entomopathogenic nematodes

Materials and methods of using entomopathogenic nematodes against *R.cerasi* are described in Köppler *et al.*, 2003a and 2004.

Biotechnological control with sticky traps combined with bait substances

Materials and methods are only described in general to show the main idea of these experiments. That was to do some first olfactometric bioassays to develop a laboratory method for testing potential bait substances. For this reason, different types of olfactometers were used, a static 4-chamber-olfactometer, a dynamic T-shaped olfactometer and a dynamic Y-shaped olfactometer. As bait substances different NH_3 releasing solutions were tested, NH_3-solution, NH_4-acetate and NH_4-carbonate. Further potential bait substances were yeast hydrolysate and sugar (1:4) in some water, benzaldehyde as a potential compound of unripe fruits and a cherry twig with leaves and unripe fruits. The experiments varied in concentration, combination of bait and potential bait substances, age, sex, nutritional status, number of flies and the combination with a piece of a non-sticky yellow plastic trap.

Chemical control with bait sprays:
In first field cage model experiments (27/07/04 after cherry harvest) GF-120 Naturalyte Fruit Fly bait™ (Dow AgroSciences, UK: 0,02 % Spinosad , 99,98 % sugar, proteins, water) was used. For this trial 3 x 3 trees (approximate height 3,0-3,5m) were coverd with nets (Rantai K net, Rudolf Schachtrupp KG, Gemany). One cage was used for one variant, control (liquid yeast hydrolysate - sugar solution 1:4), GF-120 - water 1:5 and GF-120 - water 1:10. In each cage 3 branches of each tree were sprayed with 50 ml of one of the solutions with a hand sprayer. 119 *R. cerasi* specimens, reared from pupae collected the previous year were released per cage (53 males and 66 females). After 6 days two Rebell ®-traps were hang up in each cage to catch the remaining flies.

Results

Biological control with entomopathogenic nematodes
Results of using entomopathogenic nematodes against *R.cerasi* are described in Köppler *et al.*, 2003a, 2004. In all experiments *Steinernema carpocapsae* and *S. feltiae* achieved the highest efficacies (Abbott). In laboratory, efficacies of 76 % up to 89 % in average and in semi field of 62 % to 71 % were reached. The field trial in 2002/2003 resulted in an efficacy of 88 % and in 2003/2004 of 35 %. There was no significant infection of *R. cerasi* pupae. In laboratory temperatures of 20°C and 24°C and soil types did not influence the infection significantly. The dosage of 10 nematodes per cm^2 was insufficient. No significant decrease of efficacy of nematodes after two weeks in laboratory could be documented.

Biotechnological control with sticky traps combined with bait substances
In all olfactometric bioassays no significant response to any bait substance or their combinations was found.

Chemical control with bait sprays
In this first field trial no fly specimen was recaptured in each GF-120 variant. In the control cage 17 flies were found on the Rebell ®-traps, corresponding a 14.3 % recapture rate.

Discussion

The results of the experiments for biological control indicate a high susceptibility of *R. cerasi* larvae to entomopathogenic nematodes, especially to steinernematid species *S. carpocapsae* and *S. feltiae* in laboratory and semifield. In field the susceptibility was high in the first year. In the second year, the result was less promising. Comparing soil temperatures in a 5 cm depth during nematode application in 2002 and 2003 clear differences could be found. In 2002 the temperature range was 20°C to 24°C. This corresponds to the optimum temperature for infection of the used nematodes (Grewal *et al.*, 1994, pers. comm. Peters). Temperatures above 28°C and more reduce the effectiveness of steinernematid species. In 2003 soil temperatures between 27°C and more than 35°C were documented. Consequently, the infection rate of nematodes decreased and only a low efficacy could be reached. Some more critical aspects must be kept in mind. Results are only based on low recapture rates between 8 % and 11 % and for this field trial no natural population was used. Consequently to assess the effectiveness of entomopathogenic nematodes real field experiments under practical conditions must be carried out.

Results of olfactometric bioassays do not indicate a strong olfactoric orientation of

R. cerasi in the described experimental design. The optical sense of female *R. cerasi* seems to play a more crucial role in finding host fruits (Wiesmann, 1937). Furthermore the contrast is important as optical stimulus (Haisch & Levinson, 1980). But olfactoric perception seems to be important for distant orientation (Wiesmann, 1937). Results of increased attraction of traps with ammonium baits indicate an olfactoric influence (Wiesmann, 1944, Haisch & Forster, 1969, Katsoyannos, *et al.*, 1980, Katsoyannos, *et al.*, 2000, Liburd et al., 2001). For further experiments for testing potential bait substances a different spatial experimental design is needed. Olfactoric responses of fruit flies could be found in wind tunnels combined with optical stimuli (pers. comm. Stelinski 2004, De Cristofaro 2004).

The described bait spray experiment represents a first step for indication of effectiveness of GF-120 bait formulation against *R. cerasi*. Further experiments in laboratory and field are needed.

Fly-free cherries: utopia or realistic hope? Using environmental friendly control strategies, which are safe for non-target organisms and the ecosystem, that question cannot be answered yet. All biotechnological and biological strategies are not sufficiently developed. Futher experiments are needed for both. Furthermore, investigations of some biological aspects are necessary. Detailed knowledge about dispersal and its dependance on host density of cherry fruit flies is important to assess the sustainability of biotechnological or biological methods. Informations in literature are not homogenous and vary between 100 m and 3000 m (Boller, 1969a, Boller & Remund, 1980, Katsoyannos *et al.*, 1986, Kneifl *et al.*, 1997). A further question is the flight range of cherry fruit flies within one cherry orchard depending on different ripening cherry cultivars. Katsoyannos *et al.* (1986) reported about movement of cherry fruit flies after harvest to not harvested trees. For an effective bait spraying or positioning of baited traps the behavior of flies on one tree during a day or the detailed knowledge of preferd areas (e.g. Wiesmann, 1944, Remund, 1971, Epp, 1998) are important, too.

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Effects of plant protection products on *Kampimodromus aberrans* (Oudemans): the dietary effect of airborne pollen.

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Abstract: The purpose of this study was to determine if the dietary effect of airborne pollen is important in improving the survival and reproduction of *K. aberrans* exposed to plant protection products (Steward, Dursban, Ryania, Laser and Trebon). *K. aberrans* population dynamics was studied in a Golden Delicious orchard divided into two adjacent plots (one mown plot and one unmown plot), in wich potential prey was rare.The experimental design comprised four replications per treatment per plot. Treatments were repeated over time on a biweekly basis in order to evaluate population reduction and recovery potential. At the same time, pollen grain concentration was evaluated using a volumetric sporetrap. A relevant difference in selectivity of the tested insecticides towards *K. aberrans* was observed, which seems to be related to the amount of pollen in the plot. In the mown plot, the negative effects of the insecticides were more pronounced than in the unmown plot, and both acute and delayed side effects were observed. The increased effects of the insecticides in the mown plot could be due to the lack of pollen provided by flowers in bloom: the phytoseids were not as well nourished as in the unmown plot characterized by a higher quantity of pollen.

Key words: *Kampimodromus aberrans*, plant protection products, pollen, side effects

Introduction

Kampimodromus aberrans Oudemans is a generalist predator and a recent research showed that it can persist in commercial apple orchards even when prey is scarce (Kennett *et al.*, 1979). Althoug the principal predator-prey theory developed to explain biological control assumes that the best predators are specialists, numerous successfull biological control agents are in fact generalists. The role of the generalist predator *K. aberrans* in the natural control of spider mites has been the the subject of many investigations in recent years (Duso *et al.*, 1989; Girolami *et al.*, 1992). It has been shown that the dietary range of this species is not restricted to tetranychids (*Panonychus ulmi* and *Tetranychus urticae*), but includes also other mites, such as eriophyds. Furthermore, *K. aberrans* can also feed on pollen (Duso *et al.*, 1997; Girolami *et al.*, 2000). Althoug some studies have shown that some phytoseids feed and reproduce on pollen (Kennett *et al.*, 1979), scant attention was given to the role of pollen in the population dynamics of predaceous mites in agricultural systems. In particular, the importance of pollen to the diet of *K. aberrans* and its effect on the survival and reproduction of this mite species in combination with the application of plant protection products are not well-known, althoug various studies suggest that it could be very important (Duso *et al.*, 1997). In this study, we investigated the dietary effect of airborne pollen on improving the survival and reproduction of *K. aberrans* exposed to different plant protection products.

Material and methods

Experimental design

During a one year-study, the population dynamics of *K. aberrans* was investigated in a Golden Delicious orchard; the orchard was divided into two adjacent plots. In one plot, the grass and flowers were mown during the summer, while the other plot was left unmown.
Distance between rows within the plots was 3.8 m, and distance between plants was 1.5 m; apple trees were grown according to the Spindelbusch trailing system. In each plot, five insecticide treatments (details below) and one untreated control treatment were each applied to four areas of 200 m² in size and containing at least 6 trees of 8 year-old . Thus, four replicates were used in each treatment. Treatments were repeated over time (details below).

Pollen

During the trial, pollen grain concentration was measured using a volumetric sporetrap (Lanzoni VPPS 1000), and the different ground management practices (mown and un-mown) and treatments were compared using microscopic analysis.

Test pesticides and time of treatments

The five formulated insecticides used in the trial were: Dursban 75WG® (a.i. clorpirifos 75% WG, 70 g/hl), Laser® (a.i. spinosad 44.2 g SC, 30 g/hl), Trebon ® (a.i. etofenprox 30% 280 g/l EC, 50 g/hl), Steward® (a.i. indoxacarb 30% DF, 16.7 g/hl), and Ryania (a.i. Ryania speciosa SP, 500 gr/hl). Treatments were first applied on June 25, 2002 (t), when 2±0.5 phytoseids per leaf were present. Treatments were repeated after two weeks, on July 09, 2002 (t2). Population density was estimated at t-1, t+3, t+7, t+14, t2+3, t2+7, t2+14, t2+44 days, by randomly collecting 25 leaves per treatment, and counting the number of mobile phytoseiid forms on the leaves using a binocular microscope.

Data analysis

The effects of the different ground management practices and the side-effects of the pesticides on the population dynamics of the phytoseiids were evaluated using the Henderson & Tilton formula (1955).

Results

In both the mown and unmmown plot, all the insecticide applications showed both acute and delayed effects on the the phytoseid populations (reduction of the populations), but effects were more pronounced in the mown plot than in the unmown plot (Figure 1). In the unmown plot, the *K. aberrans* population was during the summer much more numerous than in the mown plot, even though in May, that is before treatments had been applied, fewer *K. aberrans* mites were present in the unmown plot compared to the mown plot (Figure1).
In the mown plot, the negative effect of the insecticides was respectively 49.12% Dursban, 73.26% Laser, 69.49% Trebon, 50.68% Steward and 41.87% Ryania, while in the unmown plot it was respectively 24.44% Dursban, 42.67% Laser, 40.74% Trebon, 20.64% Steward and 16.84% Ryania (Table 1). The increased negative effects of the insecticides in the mown plot could be due to the lack of pollen provided by spontaneous flowers in bloom. Pollen grain concentration in the mown plot was always lower than in the unmown plot (Table2), which suggests that in the former the phytoseids were less well nourished.

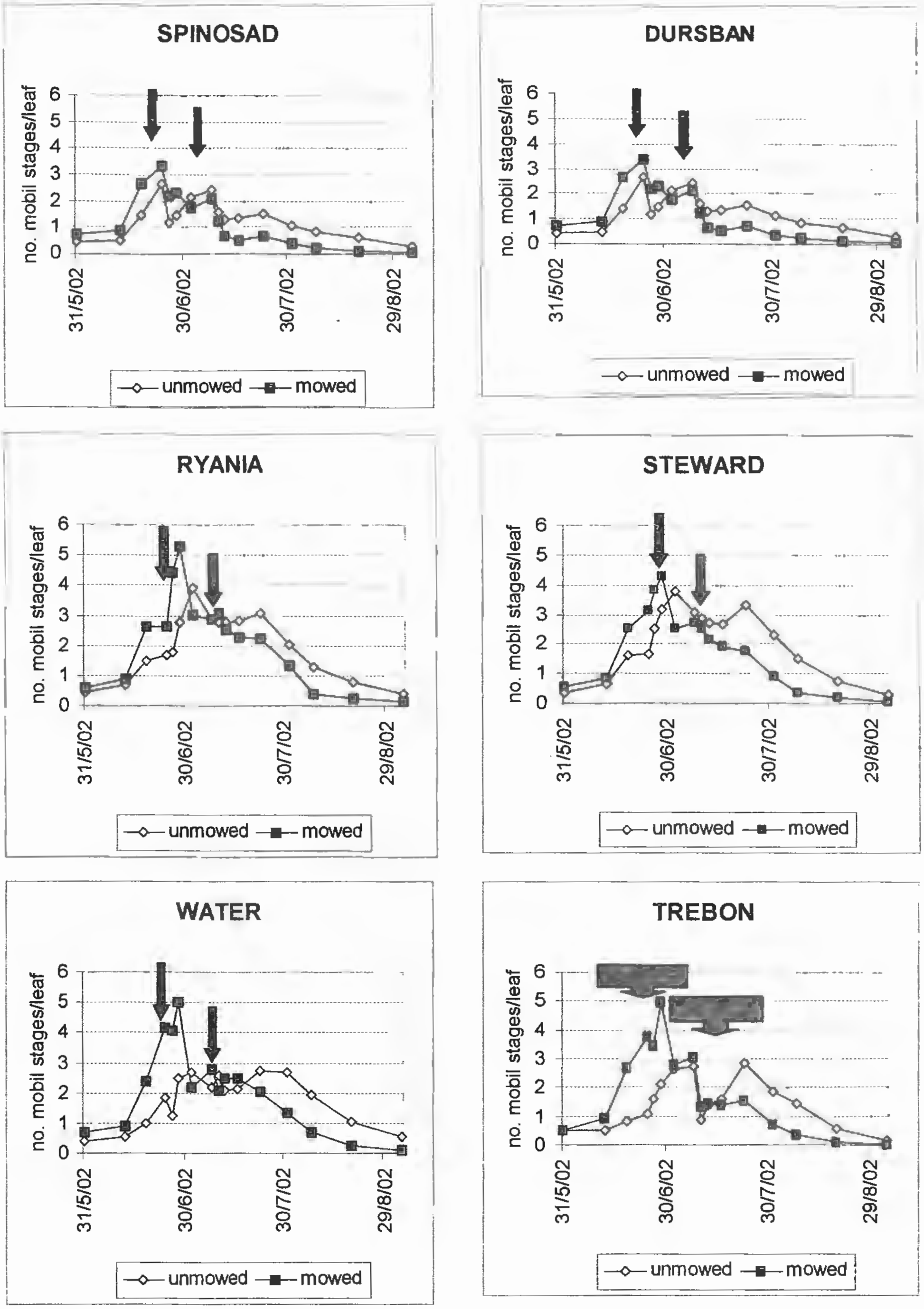

Figure 1. population dynamics of mobil stages and side-effects of insecticide applications in the mown and in the unmown plot.

Table 1. Toxicity effect (%) and classes (IOBC) of the treatments on *K. aberrans* population in the mown and in the unmown plot. (*) only one application

Treatments	Toxicity effect mown (%)	Toxicity effect unmown (%)	Toxicity class mown	Toxicity class unmown
Dursban	49.12 a	24.44 b	2	1
Laser	73.26 a	42.67 b	2	2
Trebon*	69.49 ns	40.74 ns	2	2
Steward	50.68 a	20.64 b	2	1
Ryania	41.87 ns	16.84 ns	2	1

Pesticides were classified according to the 4 IOBC evaluation classes: Class 1, harmless = E < 30%; Class 2, slightly harmful = 30% < E < 80%; Class 3, moderately harmful = 80% < E < 99%; Class 4, harmful = E >99%.Anova and Duncan ($p < 0.05$) test between the toxicity effect of the treatments in the mown an unmown plot.

Table 2. Analysis of pollen grain concentration in the atmosphere

pollen grains/m^3 air	**Plot**	**Sampling date**					
		17/06/02	01/07/02	10/07/02	31/07/02	22/08/02	23/08/02
	mowed	1218	1455	1036	1240	1624	1472
	unmowed	12024	6511	12151	14025	9816	11233

Discussion

For the biological control of *P. ulmi* on apple and grapevine, the generalist predator *K. aberrans* provides some advantages, because it can often persist or even maintain itself at high densities, even when its primary prey is scarce. The phytoseid may thus be able to suppress the prey as it increases in density, without the normal time lag associated with a numerical response. Pollen is one of the major factors affecting the abundance of *K. aberrans* mites in the field, and this mite species could thus be positively influenced by pollen produced by the host plant itself or by certain grass plants (Duso, 1997; Girolami *et al.*, 2000). The relevant difference in the selectivity levels of all the tested insecticides towards *K. aberrans* seems to be related to the amount of pollen present in the plot, and thus to the ground management practices. In order to improve the survival and reproduction of the phytoseids exposed to plant protection products, great care over the management of the spontaneous vegetation in bloom is required.

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Bioassay methodology and resistance to insecticides of pear psylla (*Cacopsylla pyri* L.) (Homoptera: Psyllidae) in Lleida, Spain

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Abstract: *Cacopsylla pyri* L. (Homoptera: Psyllidae) is the key pest of pear orchards in the fruit growing area of Lleida. The selection pressure with only a few insecticides applied repeatedly can involve the appearance of resistance. With the aim of monitoring changes in the susceptibility we established basic bioassays methodology with the adults, adapting slide dip and topical methods to the winter and summer forms. We did a field monitoring of susceptibility, to Cypermethrin, Amitraz and Abamectin, in populations from different areas of Lleida where heavy use of insecticides for the control of *C. pyri* has been the common practice. No evidence of resistance to any of the evaluated insecticides was found. We also started a laboratory strain with a field population from an untreated orchard, to use it as the base line.

Key words: *Cacopsylla pyri,* resistance, bioassays, slide dip, topical application.

Introduction

Cacopsylla pyri L. (Homoptera: Psyllidae) is the key pest of pear orchards in the fruit growing area of Lleida (Spain) (Avilla *et al*., 1996). Chemical control is the most commonly used against pear psylla, but with the implant of the European Unitary Register of Pesticides, the authorized insecticides to control pear psylla have been reduced.

The selection pressure with these few insecticides applied repeatedly in the whole area can involve the appearance of resistance, as it has happened in France (Buès *et al*., 1996) and Switzerland (Schaub *et al*., 1997).

Our objective is to monitor changes in the susceptibility of *C. pyri* to the most frequently applied insecticides Cypermethrin, Amitraz and Abamectin.

Material and methods

Adapting the well known topical application (Van de Baan *et al*., 1989; Buès *et al*., 1996) and slide dip method (Follet *et al*., 1985) to the winter and summer-form adults, we standardized the main aspects of the bioassays and reduced to the minimum the mortality in the control. We compare both methodologies.

Winter and summer-form adults were collected from a pear orchard by jarring trees using the limp-tab method (Retan & Burts, 1984).

We used 6 to 9 concentrations and a control per bioassay, with four replicates of 10-15 adults per concentration.

During 2003, autumn and winter, we did a field monitoring of susceptibility, to the three mentioned insecticides, in populations from different areas of Lleida, where heavy use of insecticides for the control of *C. pyri* has been applied.

In all bioassays, treated pear psylla were kept for 24 hours in humid chambers at 22±1°C and high humidity (95-100%)

In 2004 we started a susceptible laboratory strain with a field population from an untreated orchard that will be for us the susceptible control. The rearing was made into PVC cages, on young pear seedlings.

Data from all the tests were subjected to probit analysis with Polo Pc, LeOra Software (Robertson & Preisler, 1992).

Results and discussion

The results of both methods with winter-form adults are similar for the Cypermethrin, with a ratio of 0.8 (topical/slide dip) but different for Amitraz and Abamectin. These last two products are recommended only against juvenile stages (table 1).

Summer-form adults need to have access to plant, and the mortality in 24 hours is often greater than 20 % in slide dip. Winter-form adults are more resistant to starvation and can survive this method.

For that reason we will use the topical method (greater precision) to obtain basic data during the year and the slide dip method only to apply discriminating doses to winter-form adults because this method is easier to use (Van de Baan *et al.*, 1989).

Table 1: Comparison of topical and slide dip bioassays with Cypermethrin, Amitraz and Abamectin, against winter-form adults from Aitona population in 2003

Insecticides	Method	n[1]	Control mortality	Slope	LC_{50} (ml/l AI)	FL 95%	χ^2	Ratio[2]
Cypermethrin	Topical	440	3.4 %	1.846 ± 0.241	0.178	0.141-0.223	2.957	-
	Slide Dip	448	2.9 %	3.673 ± 0.463	0.219	0.165-0.281	6.706	0.8
Amitraz	Topical	560	12.6 %	1.358 ± 0.202	1.340	0.569-2.419	7.245	-
	Slide Dip	472	4.1 %	1.532 ± 0.189	2.163	1.628-2.930	4.354	0.6
Abamectin	Topical	440	10.0 %	3.355 ± 0.596	0.091	0.075-0.106	2.738	-
	Slide Dip	293	17.5 %	2.705 ± 1.102	0.172	0.132-0.303	3.765	0.5

[1] Number of adults tested

[2] LC_{50} topical / LC_{50} slide dip

In the first monitoring in 2003, populations showed similar susceptibility levels. Only with Amitraz the maximum difference between the higher and the lower LC_{50}, is 3.6 times.

The LC_{50} fluctuates in Cypermethrin from 0.051 to 0.079 (figure 1a), in Amitraz from 0.641 to 2.318 (figure 1b), and in Abamectin from 0.169 to 0.249 (figure 1c).

The variability within populations (fiducial limits) is higher in insecticides normally used against nymphs.

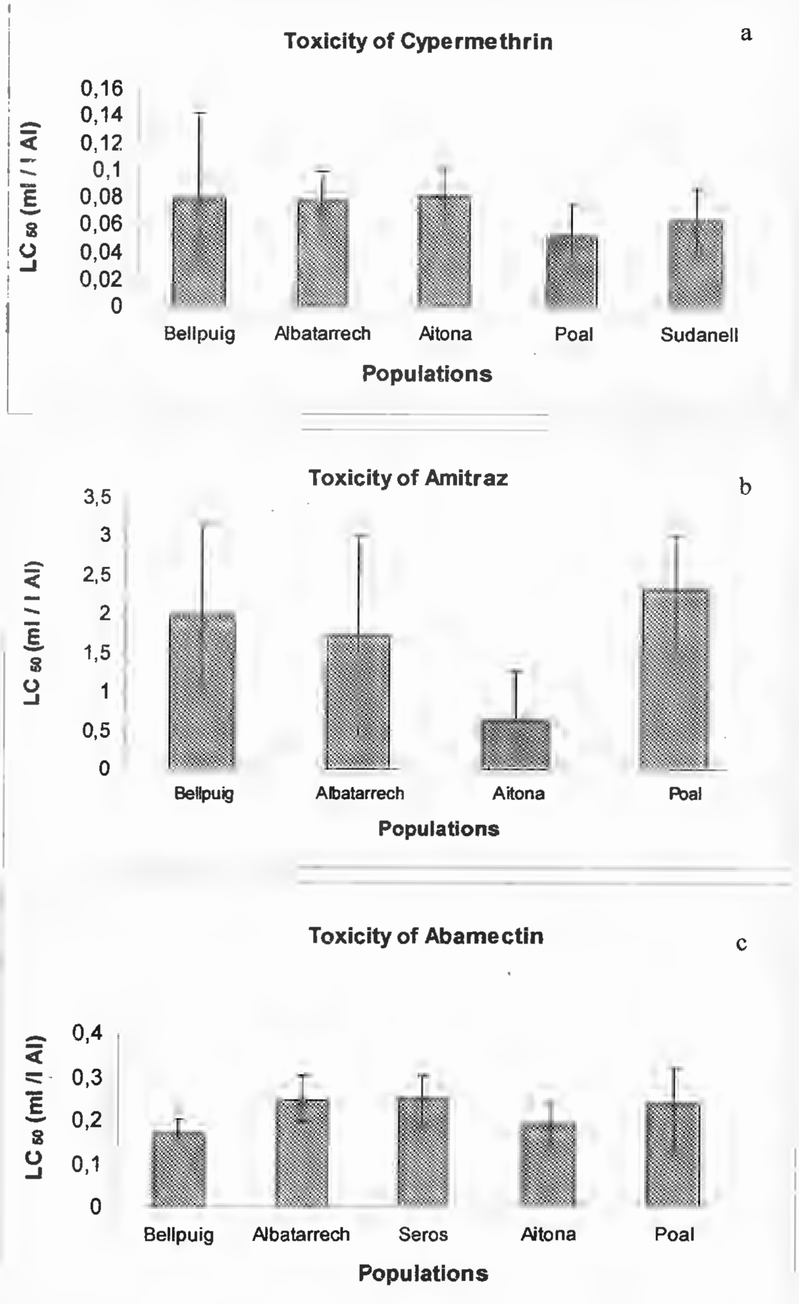

Figure 1: Susceptibility of winter-form adults in different areas of Lleida treated by topical application with Cypermethrin (a), Amitraz (b) and Abamectin (c) in 2003.

The base line data of the laboratory strain, from an untreated orchard, with summer-form adults are in table 2. We need to select for susceptibility in this laboratory strain to detect any resistance level in the fields, because the LC_{50} to the tree insecticides are not very different from the results of monitoring of treated orchards in 2003.

Table 2: Baseline data, LC_{50} and LC_{90} to different insecticides, obtained after topical applications on summer-form adults from an untreated orchard (Gimenells) in 2004

Insecticides	n[1]	Control mortality	Slope	LC_{50} (ml/l AI)	FL 95%	LC_{90} (ml/l AI)	FL 95%	χ^2
Cypermethrin	280	14 %	1,703 ± 0,272	0.136	0.047-0.254	0.768	0.386-1.434	6.051
Amitraz	280	5.9 %	1,899 ± 0,376	0.740	0.360-1.090	3.499	2.453-6.252	4.930
Abamectin	280	7.3 %	1,695 ± 0,247	0.033	0.014-0.052	0.186	0.112-0.501	4.571

[1] Number of adults tested

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Organic cider-apple production in Asturias (NW Spain)

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Abstract: Cider is a key economic factor in the Asturian (NW Spain) agrarian sector, where apple production is principally geared towards cider making. Research on sustainable strategies for managing cider-apple orchards has been performed by SERIDA since 1986. Nowadays, a viable strategy for organic cider-apple production may be recommended. Cultivar selection is of critical importance in the planning of new orchards. In organic production, disease and pest susceptibility is an important consideration. The cultivars selected by SERIDA are highly recommended because of their disease tolerance, productivity and juice quality. Recently, some cultivars resistant to both scab and the rosy apple aphid have been obtained in a breeding programme. Since recommended cultivars are tolerant to fungi, their selection makes the use of fungicides normally unnecessary. Thus, phytosanitary sprays are reduced to those used against the codling moth (granulosis virus) and the rosy apple aphid (neem-based insecticides). Other potential pests are regulated naturally or do not represent a serious problem for cider-apple production. A semi-intensive to semi-extensive system with trees growing on semi-invigorating rootstocks (M.7, MM.106, MM.111 or even seedling rootstocks in markedly sloping sites) has been chosen because trees may be grown without irrigation and trunk support in most cases. Trees are developed in vertical-axis or sole-axe systems. The proper fertilization of trees is achieved with applications of organic fertilizers and/or composted cow-manure. Plant competition in the tree row is avoided by mulching (grass, straw, pine-bark) or mechanically. Fruit thinning is done by hand (extinction technical or by manually removing fruits). Apples may be marketed individually or collectively, dealing directly with the cider producer. Apples produced under organic conditions reach a little higher price than conventionally produced apples. Organic cider-apple production is possible when the orchard is well planned and managed in a holistic manner. Growing of resistant cultivars is a key factor in achieving success. This system is applicable to dessert-apple production with some minor changes (e.g. tree vigour or the planting system) when scab-resistant cultivars are grown.

Key words: bottom-up approach, cider-apple, organic production, resistant cultivars, semi-intensive system

Introduction

Cider is a traditional valuable feature and a key economical factor in Asturian (NW Spain) (43 N, 5 W) agrarian production. Thus, apple production is almost exclusively geared towards cider making. Research on sustainable strategies for managing cider-apple orchards has been performed by SERIDA (Servicio Regional de Investigación y Desarrollo Agroalimentario) since 1986 (Dapena & Blázquez, 2002). This research allows us to recommend a viable strategy for organic apple production which is being adopted by a rising number of growers.

The change from conventional to sustainable fruit production has usually been done under a 'top-down' approach, i.e. starting with a conventionally-managed orchard and reducing progressively the external inputs while ecological processes of natural control are gradually established. However, a 'bottom-up' approach is possible (Prokopy, 2003). This 'bottom-up' approach is based on a suitable design of the plantation, a correct habitat management, and a management of pests and diseases based on the growing of resistant rootstocks and cultivars, cultural management and biological control (Prokopy, 2003). Organic production (Weibel & Häseli, 2003) as well as the IOBC (Cross, 2002; Avilla & Riedl, 2003) greatly recommend the adoption of the bottom-up approach. The SERIDA also adopted this approach for research and production of cider-apples.

A semi-intensive system

In order to take advantage from both traditional extensive and conventional intensive systems of production, a semi-intensive to semi-extensive system has been adopted for producing cider-apples in Asturias (Dapena *et al.*, 2002). This system is based on the growing of trees on semi-invigorating rootstocks (M.7 and MM.106 with vigorous cultivars and MM.111 with cultivars of moderate vigour) with a tree-density of 550-750 trees/ha. MM.111 (550 trees/ha) and seedling rootstocks (220 trees/ha) are used in sites with marked slope. The elevated vigour and height reached by trees are not a handicap for cider-apple production since apples are harvested on the ground. The most important advantage of this system *vs.* intensive production is the few requirements derived from the greater vigour. Irrigation and trunk support are unnecessary in most of the cases and the inputs into the orchard are lower. The main advantage *vs.* traditional big trees is the easier management of trees (i.e. pruning, spraying). Trees are formed in vertical-axis or sole-axis system taking into consideration the natural growing habits of each cultivar.

Cultivars

A proper choice of cultivars is of critical importance in the planning of new orchards. Apart from productivity and the technological and organoleptical features of the apples, resistance to pests and diseases should be a key point in the choice of cultivars for a sustainable production (Cross, 2002; McCarthy, 1994; Avilla & Riedl, 2003; Prokopy, 2003; Weibel & Häseli, 2003). Disease and pest susceptibility is an important consideration especially in organic production, where alternative methods to chemicals are usually less effective and more expensive.

A group of Asturian cultivars with high productivity, juice quality and resistance or little susceptibility to scab, powdery mildew, European canker and monilia have been selected by SERIDA (Dapena & Blázquez, 2002). These cultivars are nowadays among the 22 cultivars accepted in the quality label 'Denominación de Origen Protegida Sidra de Asturias' and all the apple growers who carry out a new orchard use these cultivars. Four to six cultivars are normally grown in a 1-ha orchard.

Apart from these traditional cultivars, some hybrids resistant to both scab and the rosy apple aphid (Dapena & Blázquez, 2004; Miñarro & Dapena, 2004) have been obtained in a breeding programme in the SERIDA and are in registry at this moment. They will be available for growers soon.

Fertilization and groundcover management

A proper fertilization of trees is achieved with organic fertilizers and/or composted cow-manure. Plant competition for water and nutrients in the tree row is avoided by mulching (grass, straw, pine-bark) or mechanically with a disc plough or a rotovator. The grass- and the straw-mulches favour the soil humidity and the tree growth but may be a disadvantage with high densities of voles. Alleys are cleaned when necessary using a shredder.

Fruit thinning

Biennial bearing is a basic problem for both apple-growers and cider-producers in Asturias. 50,000 tons of cider-apple are produced in the 'on' years while only 10,000 tons are produced in the 'off' years. This is mainly a consequence of the bearing habits of these cultivars and is a problem for both organic and conventional production. Fruit thinning is made by manual removing of fruits. A new technical named extinction (Lauri *et al.*, 2000) consisting of a partial removing of complete fruit points is also recommended. However, these two technical require high labour and are difficult to carry out in big commercial orchards. Research on thinning products compatible with organic production has been initiated.

Management of pests and diseases

Asturias has a humid climate in which fungi are the main phytosanitary problem. However, the growing of these cider-apple cultivars resistant to diseases allows an apple production free of fungicides. The risk of phytophthora crown and root rot is diminished by cleaning the herbs around the tree trunk. Fire blight has not been detected in Asturias.

Among arthropods, only the codling moth and the rosy apple aphid are serious problems for cider-apple production in Asturias. Codling moth control is successfully achieved with the granulosis virus: damage is reduced up to less than 2 % with 6-8 sprays (Miñarro & Dapena, 2000). Mating disruption efficacy in Asturias is low, probably due to the small size of the orchards as well as the abundance of isolated trees or semi-abandoned orchards which act as infestation sources of mated females (Miñarro & Dapena, 2000). The rosy apple aphid may be controlled combining the use of tolerant cultivars, biological control and the spray of neem-derived insecticides (Miñarro & Dapena, 2005). Other potential pests are regulated naturally or do not represent such a serious problem for cider-apple production to require an action plain: e.g. the European red mite is controlled by phytoseiid mites (Miñarro *et al.*, 2002). The green apple aphid is generally not a problem which is easily eliminated with Rotenone sprays when necessary. Therefore, the only sprays to produce organic cider-apples in Asturias are those of granulosis virus against the codling moth and those of neem-derived insecticides against the rosy apple aphid when necessary.

Voles (*Arvicola terrestris*, *Microtus lusitanicus*, *M. agrestis*) are an increasing problem in Asturian orchards. Trapping and a proper management of the groundcover can contribute to reduce damage. Research on this topic has been recently initiated in the SERIDA.

Marketability

There are few cider producers interested on elaborating organic cider or other derived products (e.g. juices, vinegar). Apples may be marketed individually or collectively, dealing directly with these producers. Apples produced under organic conditions reach a higher price than conventionally produced apples, which can compensate the higher price of products and the extra labour demand.

Production of organic dessert-apple

The semi-intensive system for cider-apple production presented is applicable to dessert-apple production in Asturias or in other pome production regions. Two possible difficulties to manage dessert-apple orchards could be firstly the selection of appropriate cultivars and secondly the need of limiting tree vigour and height in order to allow an easy collection of fruit. Nowadays a lot of scab-resistant cultivars are available (Crosby *et al.* 1992; Brown & Maloney, 2003), some of which are also tolerant to the rosy apple aphid (Miñarro & Dapena, 2005). Moreover, other minor pests whose aesthetic damage is not a difficulty for cider-apples may become a problem for dessert-apples.

Organic apple production in Asturias

Constituted in 1986, the Council for Organic Production in Asturias registers and certifies organic producers. Nowadays, 20 cider-apple producers are registered in this Council, although the number is increasing. Four organic-cider producers are also registered in Asturias.

Conclusions

This work shows that organic production of cider-apples is possible when the orchard is well planned and managed in a holistic manner. The growing of resistant cultivars is a key point for the success of the bottom-up approach. However, further research (e.g. alternatives to hand thinning) is needed. Most of the strategies presented to produce organic cider-apples could be translated to organic dessert-apple production with minor adaptations and even to conventional cider-apple production.

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Hazelnut fruit quality as influenced by the cultivation system adopted

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Abstract: Preliminary investigations are carried out to detect the effect of the cultivation system (conventional and organic protocols) on hazelnut fruit quality with particular reference to technical and marketable fruit traits. To this end, in the Piedmont hazelnut-growing area, fruit samples from the same cultivar (Tonda Gentile delle Langhe) from 3 orchards under organic cultivation are evaluated and compared with fruit samples from 3 orchards managed conventionally and sited in pedoclimatic conditions similar to the former.
To date, differences in fruit quality seem to be relevant for damages caused by bugs (*Palomena prasina* and *Gonocerus acuteangulatus)*.

Key words : hazelnut, fruit quality, organic production.

Introduction

Although uncertain as to the final destination of their production, some growers in the Langhe area (Piedmont) have undertaken organic cultivation of hazelnut using EU funds available for organic cultivation, in accordance with the Regional Development Programme for 2000 – 2006. It can be claimed that these are deserving pioneers who have not been able to avail themselves of experimental data in this field. Indeed, the few initiatives they can refer to (Franco et al, 2004), concern cultivars and environmental conditions which are completely different from those in the Langhe.
While awaiting the results of specific *ad hoc* experimentation (Scortichini et al, 2004), it was decided to make use of the presence of organic hazelnut orchards – or those which have almost entirely completed the period of transition – and the willingness of their owners, to compare the qualitative characteristics of their nuts with those of conventional hazelnut orchards, located as next as possible, that means in similar pedoclimatic conditions.

Material and methods

The study was carried out in 2 representative areas for hazelnut cultivation in the Province of Cuneo, at Alta Langa and Carrù. In the former, hazelnut orchards in the administrative areas of Bosia-Cortemilia (Alanga1) and Serravalle Langhe (Alanga2) were chosen, while in the latter suitable hazelnut orchards were found only at Carrù. It was thus possible to study 3 pairs of hazelnut orchards, each pair including 1 farm which was organic (ORG) and 1 which was conventional (CON), as close as possible to each other. The distribution and features of the farms are shown in and Tab. 1. In all cases the orchards were planted with the cv Tonda Gentile delle Langhe (TGL) grown as a bush.
In both the conventional and the organic orchards the main cultivation techniques were recorded (see Tab. 2 and 3), with reference to fertilising and pest control. Average yield per plant was calculated from the total yield over the last 2 years.

In 2003, during harvesting, for each orchard and farm, 1–3 samples of 3 kg of fruits were taken from areas representative of the whole orchard.
All the samples underwent the usual series of nut analyses, with special reference to technological and marketable traits. More specifically, for the whole fruit the following were determined: # average weight (g); # empty percentage; # kernel/fruit ratio percentage.
For the kernel the following were determined: # average weight (g); # bug damage incidence (%); # other damage incidences, i.e. rancid, mouldy, double, undersize, dried up (%); # kernel without defects (%) that is seeds of the right size and without any damage, i.e. of quality requested and preferred by the processing industry.

Results

Features of the farms

The farms studied, both conventional and organic, are medium to small in size (Tab. 1). Their most important traits are given in the above mentioned Tab.1. It is noticeable that tree spacing (that leads to plant densities ranging from 278 to 417 bushes/hectare) and bush training system are typical of the area and so not planned specifically with organic cultivation in mind.

Table 1. Features of the 6 farms considered for conventional and organic management.

Farm & orchard traits	CONVENTIONAL			ORGANIC		
	Baudana	Gonella	Salvini	Muzzi	Ravera	Manzone
Height (m)	762	364	560	762	364	490
Farm surface area (ha)	13,62	15,39	7,97	3,94	12,91	14,34
of which hazelnut cultivation(%)	82,2	47,4	25,8	57,4	83,3	39,8
Average age of orchard (years)	21,5	20,9	17,4	34,5	15,6	19,9
Bush spacing (m)	6 x 5	6 x 4	5 x 5	5 x 5	5 x 5	6 x 6
Stems/bush (No)	5	5	5	5	8	6
Distance of 2 compared sites (m)	close	700	2,000	close	700	2,000

Orchard management

In the conventional hazelnut orchards, chemical herbicides and simple and/or NPK fertilizers are used. Pinching out of suckers is manual in late spring and always chemical before harvesting (ammonium glufosinate). Treatments to control pest and disease, carried out from twice to four times per year involves the use of synthesised molecules registered for hazelnut cultivation. Exceptions are sulphur and to a lesser extent copper, which are also permitted in organic cultivation.
In the organic hazelnut orchards (Tab. 3) the ground is occasionally harrowed superficially. The wild grass which grows as a result is checked by repeated cuttings until pre harvest. Only commercially-available organic fertilisers registered for organic cultivation are used. Pinching out of suckers is manual, and on the Ravera farm is carried out every other year. Pest and disease control treatments are never carried out more than twice a year, and almost always involves sulphur, sometimes copper.

Table 2. Summary of conventional orchard management carried on during the last 2 years.

Farm :	*Baudana*	*Gonella*	*Salvini*
Ground management	herbicides on the rows (once); herbicide at pre-harvest (once)	herbicides on the rows (once)+ harrowing between rows; herbicide at pre-harvest (once)	herbicides on the rows (once) + harrowing between rows; herbicide at pre-harvest (once)
Fertilisers	urea; NPK; mixed organic	urea; potassium sulphate	urea; potassium sulphate; superphosphate; pellets
Sucker pinching out	manual (late spring) + chemical (pre harvest)	chemical (late spring and pre harvest)	manual (late spring) + chemical (pre harvest)
Number of treatments	2 (*Phytoptus avellanae* and several bugs)	3 - 4 (*Gleosporium*, scales, *Phytoptus avellanae*, bugs and/or weevils)	3 (*Gleosporium*, *Phytoptus avellanae* and bugs)

Table 3. Summary of organic orchard management carried on during the last 2 years.

Farm:	*Muzzi*	*Ravera*	*Manzone*
Ground managment	spring harrowing + cutting (2 – 3 times)	cutting (2 – 3 times)	spring harrowing + cutting (twice)
Fertilisers	commercial organic	commercial organic	manure
Sucker pinching out	manual (before harvest)	manual (alternate years)	manual (before harvest)
Number of treatments	2	1	2

Yield

The yields recorded in 2002 and 2003 are rather low, for both conventional and organic farms, being no more than 2 tons/Ha (Gonella , 2002) for the conventional cultivation and 0,9 tons/Ha (Muzzi,2002), for the organic one. Also, on average the yield from the organic hazelnut orchards was 43,5 % lower than that from the conventional orchards. The sample year, 2003, was an off bearing year and so with much lower yields than the previous year except for the Salvini farm. However, given that the study only covers 2 years and that one was off bearing year it seems premature to generalise.

Fruit quality

The average weight (Tab. 4) is consistently lower in the organic orchards, and the differences are always highly significant. The highest percentage of empty fruit was found at the Alanga1 organic orchard (3.95%), and the lowest in the Carrù organic orchard (0.86%). Kernel/fruit ratio percentage was noticeably lower in the organic orchards Alanga1 and Alanga2 compared with the conventional orchards, and the relative differences were always highly significant. However, at Carrù this ratio for the organic orchard was higher compared with those for conventional orchard, but the difference was not statistically significant.

Comparing the characteristics of fruit from conventional orchards with those of fruit from organic orchards, irrespective of the location and the farm, it is even clearer that organic cultivation yields significantly inferior fruit.

Kernel quality

The average weight of kernels (Tab. 4) is significantly higher for fruit from the conventional orchards Alangal and Alanga2, while the opposite is the case for Carrù. The occurrence of bugs damages in all of the 3 orchard areas studied was always significantly higher in the organic orchards than in the conventional ones. The occurrence of other damage was always higher and highly significant in the organic orchards of Alta Langa, compared with conventional orchards in the same area. At Carrù, however, the occurrence of this parameter did not differ between the organic and the conventional orchards (Tab. 4). Lastly, the percentage of in-size kernels without any defects was noticeably lower in organic orchards, with negative differences of 29% in ALanga2, 27% in Alanga 1 and 6.6% at Carrù.
As already said for fruit, organic cultivation has not achieved the qualitative results for kernel obtained under conventional cultivation.

Table 4. Comparison for some fruit and kernel properties of nuts yielded in organically and conventionally managed orchards in 3 hazelnut Langhe sites.

Locations, orchard management & owners		*Alanghe1*		*Alanghe2*		*Carrù*	
		CON (Salvini)	ORG (Manzone)	CON (Baudana)	ORG (Muzzi)	CON (Gonella)	ORG (Ravera)
Fruit properties							
average weight	g	2.30	2.18***	2.36	2.13***	2.53	1.98***
empty	%	2.57	3.95 **	1.43	3.43 **	1.43	0.86
kernel/fruit ratio	%	48.65	45.73***	46.22	43.63***	45.50	46.12
Kernel properties							
average weight	%	1.15	1.04***	1.11	0.96***	0.91	1.18***
bug damage	%	0.24	18.38***	0.44	21.56***	2.18	8.49***
other damage	%	2.24	10.22***	1.01	8.30***	2.74	2.72
without defects	%	97.51	71.39***	98.55	70.14***	95.08	88.78 **

Discussion

Although drawing on only 1 year of sampling, this preliminary study shows how organic hazelnut orchards yield inferior fruit at a higher cost, especially due to the requirement that suckers pinching out be manual.
The worst damage found in fruit produced organically is due to bugs; the effectiveness of molecules allowed in organic farming was not tested for.

Lower yield and lower kernel/fruit ratio percentage may be due to the fact that organic fertilising carried out in organic farming does not fully cover the mineral nutritional requirements of the plants (Roversi, 2003)

These first results suggest that possible directions for the development of organic hazelnut cultivation may involve agro-biological and economic lines of research.

Given that an edaphic equilibrium between bugs and their predators does not seem easy to achieve at the moment, from an agro-biological point of view experimental testing of the effectiveness of pesticides compatible with organic farming is required. The possible effects of specific cultivation techniques (e.g. tree spacing and training, pruning and weed management) also need testing.

From a economic point of view, an increased use of *organic hazelnut* in order to increase its market value is to be hoped for, to justify the lower yield of organic orchards.

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Digital analysis of injuries caused by raspberry spider mite *Neotetranychus rubi* (Träg.) and two-spotted spider mite *Tetranychus urticae* (Koch.) on raspberry leaves

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Abstract: The aim of this study was to compare harmfulness of two tetranychid mite (*Tetranychidae*) species numerously occurring on raspberry plantations in Poland: raspberry spider mite *Neotetranychus rubi* (Träg.) and two-spotted spider mite *Tetranychus urticae* (Koch.). The level of damage caused by females of *N.rubi* and *T.urticae* on raspberry leaf disks was estimated with a digital camera and a computer program for image analysis. Significant differences in injury level between tetranychid mite species were observed. An area of brown spots resulting from feeding of females of two-spotted spider mite was significantly larger compared to that caused by females of raspberry spider mite. Because the injury level of studied species of spider mites differs significantly, it has been suggested to consider separately thresholds for them.

Key words: raspberry, *Neotetranychus rubi* (Träg.), *Tetranychus urticae* (Koch.), harmfulness, digital analysis of injuries, Poland

Introduction

The raspberry is one of the most important soft fruit crops in Poland. The majority of plantations has to be treated with acaricides against phytophagous mites. The most important are two species of tetranychid mites: raspberry spider mite *Neotetranychus rubi* (Träg.) and two-spotted spider mite *Tetranychus urticae* (Koch.) (Skorupska 1975, Michalska and Kropczyńska 2002). According to Gajek (2003) both species occurred in relatively high population density on raspberry plants, however *N.rubi* was found to be dominant in most regions of raspberry growing in Poland.

As IPM methods for raspberry protection aim at a substantial reduction of chemicals use, workable thresholds have to be elaborated and implemented to the practice. Presently obligating threshold for the control of spider mites in raspberry plantations in Poland refers to all tetranychid mite specimens found on raspberry leaves, irrespectively of their species status. The goal of the presented research was to assess the degree of leaves injury of raspberry leaves caused by two mentioned mite species. It was a first step of the investigation on an improvement of thresholds which are presentely used for phytophagous mites control in raspberry plantations.

Material and methods

The study was carried out in June of 2003 on two raspberry cultivars: 'Veten' and 'Malling Seedling'. Both are commonly grown in Poland. A samples of 30 leaves, in good health and free of mites, were taken randomly from plants of each cultivars. Three discs of an area of 3

cm^2 were cut from each leaf. One of the discs was infested with 10 adult females of raspberry spider mite whereas the second with 10 adult females of twospotted spider mite. A third leaf disc constituted the check.

All discs were placed inside Petri dishes on moist cotton and put into environmental chamber (24^{O}C; L;D=16:8). After three days of mites feeding, leaf discs were worked out chemically according to Warabieda and Olszak (2003). Then, digital pictures of the discs were taken with Panasonic Colour Digital Camera GP-KR222. A computer program Lucia G was used to assess a level of generated leaf injuries.

Results and discussion

After three days of mites feeding, significant differences in injury level between tetranychid mite species as well as between raspberry cultivars were observed (table 1). In general, an area of light spots due to feeding of females of two-spotted spider mite was significantly larger compared to that caused by females of raspberry spider mite. It was especially visible in the case of cultivar ‚Malling Seedling', which seemed to be more susceptible to damages created by *T.urticae* in comparison with cultivar "Veten'. However, cultivar ‚Malling Seedling' has been viewed as a less numerously infested with *T.urticae* than cultivar "Veten" (Łabanowska 1986, Gajek 2003b).

Unexpectedly, in the case of both studied cultivars, raspberry spider mite has been found to generate quite limited damages of raspberry leaf discs. An intensity and scale of light coloured spots caused by its females was only slightly higher than that observed on check leaf discs, in which damage of leaf tissue resulted from natural, physiological process. Nevertheless, no significant differences in injury levels were ascertained here.

The results obtained showed that the damage degrees and presumably harmfulness of the studied species of spider mites is not comparable. Therefore thresholds for raspberry protection against *N.rubi* and *T.urticae* have to be considered separately.

Table 1. A level of injuries caused by adult females of twospotted spider mite (*T. urticae* Koch.) and raspberry spider mite (*N. rubi* Träg.) on raspberry leaf discs after three days of feeding

Species	A degree of injury of raspberry leaf discs (%)	
	cv. "Veten'	cv. ‚Malling Seedling'
Tetranychus urticae (Koch.)	6.63 c*	9.67 d
Neotetranychus rubi (Träg.)	4.40 b	4.06 ab
Check	3.29 ab	2.29 a

* Means in rows and columns followed by the same letter are not significantly different at P = 0,05, Duncan's multiple range t - test

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Temperature model of the flight activity of *Adoxophyes orana* (Lep.: Tortricidae) for timing of control

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Abstract: In 1992 – 2002 the flight activity of *Adoxophyes orana* was investigated using pheromone traps placed in apple orchards in five localities in Central and Eastern Bohemia. The cumulative catches of AO were plotted against time expressed as the sum of degree-days (D°) above 8 °C and approximated by a Richards′ function. This model was constructed using the numbers of *A. orana* males caught by pheromone traps from 1992 – 1998 at all the localities. The flight of the overwintering generation of *A. orana* began when 200 D° had accumulated, culminated at 300 D° and ceased at 430 D°, irrespective of locality. The high predictive ability of the model was validated using data from one locality for 2001 - 2003. It was impossible to construct a similar model to predict the flight activity of the summer generation because its dependence on D° varied greatly between localities and years.

Key words: *Adoxophyes orana*, apple orchards, flight activity, pheromone traps, temperature model, degree-days

Introduction

Summer fruit tortrix moth, *Adoxophyes orana* (Fischer v. R., 1834) is the most important leafroller in Europe, especially in Belgium, south Netherlands, Austria, south Switzerland, Germany and the Balkans (van der Geest and Evenhuis 1991). Recently, *A. orana* became the most important leafroller in the Czech Republic. Autochthonous *A. orana* populations in the Czech Republic didn't cause important damage because they were controlled by the pesticides such as phosalone and chlorpyrifos-methyl, used against other pests. In 1999, aggressive populations of *A. orana* were found at several localities, which were not controlled by these insecticides and caused serious damages. Since these aggressive populations occurred in the neighbourhood of fruit stores for apples from Western Europe, it is likely that these *A. orana* populations were imported into the Czech Republic. In 2002 and 2003 these populations spread into the orchards in Eastern Bohemia, part of Central Bohemia and Central Moravia and caused serious damage, mainly because chemical control was unsuccessful.

In this paper we present a temperature model of the flight activity of the overwintering generation of *A. orana* constructed on the basis of data from pheromone traps from 1992 – 1998, which revealed the phenology and relative abundance of this species in apple orchards in the Czech Republic. The model was verified using data collected in 2001, 2002 and 2003.

Material and methods

Adult male flight activity of *A. orana* was monitored by pheromone traps from 1993 to 2003 (except of 1999 and 2000) in apple and plum orchards with different pest control strategies. The orchards were placed at five localities and were maintained using different technologies of fruit growing and plant protection: 1) Ruzyně – experimental apple orchard of the Research Institute of Crop Production; no chemical treatment, experimental area 1 ha, different cultivars, 2) Doksany – formerly an intensively cultivated apple orchard, no chemical treatment since 1992, experimental area 1 ha, cultivar Golden Delicious, 3) Slaný - intensively cultivated apple orchard employing an integrated pest management (IPM) strategy, experimental area 1ha, cultivar Idared, 4) Holovousy – experimental apple orchard, no chemical treatment, experimental area 1ha, different cultivars, 5) Roudnice – intensively cultivated plum orchard employing a long-term regime of IPM, experimental area 1 ha, cultivars Cacanska Lepotica and Cacanska Najbolja.

A lower developmental threshold (LDT) of 8 °C was used for calculating the sums of day-degrees (DD°; accumulation of daily effective temperatures). The daily effective temperature was calculated as: $[(t_{min} + t_{max})/2]$ – LDT and summed from the 1st January for calculating sums of day-degrees. Weekly trap catches of the overwintering generation of *A. orana* were expressed as percents of total trap catches of the overwintering generation. Richards′ function was fitted into a plot of cumulative percentage of catches of males of the overwintering generation and day-degrees (DD°) and calculated as:

$$y = 100/[(1 + c_3 * e^{-c_1(x-c_2)}) * 1/c_3],$$

where c_1, c_2, c_3 are parameters of the Richards′ function (Fircks &Verwijst, 1993), y is cumulative percentage of captured moths and x is day-degree.

Results and discussion

Pheromone trap catches

The flight curves indicated that *A. orana* is bivoltine in the Czech Republic. The flight activity of the overwintering generation began usually in the end of May and ended in the fourth week of June. The period of maximum catches was usually very short. The flight of the individuals of the summer generation varied considerably between years and localities. Total catches of the overwintering and summer generations of *A. orana* varied mainly between localities (Table 1). There was no significant difference between the numbers caught in chemically treated (Roudnice and Slaný) and untreated orchards (Ruzyně, Doksany, Holovousy) ($p=0.9302$) in the period 1992 - 1998. Importantly the catches of *A. orana* males at Slaný increased in 2001 – 2003 despite intensive chemical control. The share of the total catch made up of the summer generation fluctuated between years and localities from 10.5% (Slaný 2002) to 90.6% (Slaný 2003). The average share of the summer generation did not differ significantly between localities ($F=0.60$, $p=0.6712$) but markedly between years.

The construction and verification of the flight activity curve of the overwintering generation of A. orana

The cumulative catches of the overwintering and summer generations of *A. orana* males relative to DD° are shown in Fig. 1. The flight begins at 200 DD°, peaks at 300 DD° and ceases at 430 DD°. The flight of the summer generation is prolonged and differs not only among localities but also years. It was impossible to construct a model of the flight activity of this generation, which flies from 640 DD° to 1580 DD°.

The model of the flight activity of the overwintering generation of *A. orana* was validated against the pheromone trap catches at Slaný in 2001 - 2003. The difference between the prediction of the model and the catches were tested when 20%, 50% and 80% of the moths had emerged. In 2001, the flight occurred several days later than predicted by the model (Table 2). However, the beginning of the flight and the first flight peak were predicted sufficiently accurately for insecticide application. The difference between the predicted and observed was least for 20% of the moths emerged (3 days) and greatest for 80% emerged (10 days). In 2002, the difference throughout between predicted and observed was only 1 day (Table 2). The best prediction was for 2003, when the predictions were accurate in all terms of 20%, 50% and 80% of moths emerged (Table 2).

Table 1. Total catches of the overwintering and summer generation of AO males in pheromone traps, W = overwintering generation, S = summer generation, T = total

Year	**Ruzyně**			**Doksany**			**Roudnice**			**Holovousy**			**Slaný**		
	W	S	T	W	S	T	W	S	T	W	S	T	W	S	T
1992	-	-	-	-	-	-	84	53	**137**	-	-	-	-	-	-
1993	13	4	**17**	13	67	**80**	19	20	**39**	-	-	-	-	-	-
1994	9	3	**12**	20	16	**36**	18	5	**23**	-	-	-	-	-	-
1995	3	8	**11**	14	30	**44**	20	17	**37**	41	64	**105**	39	20	**59**
1996	9	31	**40**	-	-	-	-	-	-	100	103	**203**	58	119	**177**
1997	-	-	-	-	-	-	-	-	-	124	73	**197**	-	-	-
1998	-	-	-	-	-	-	-	-	-	25	62	**87**	-	-	-
2001	-	-	-	-	-	-	-	-	-	-	-	-	119	21	**140**
2002	-	-	-	-	-	-	-	-	-	-	-	-	324	38	**362**
2003	-	-	-	-	-	-	-	-	-	-	-	-	39	376	**415**
Average			**20**			**53**			**59**			**148**			**231**

Table 2. The correspondence between the observed and the flight model as prediction of timing in D° and date of the 20%, 50% and 80% cumulative flight of the overwintering generation of AO

		***A. orana* moths occurrence**					
		20%		**50%**		**80%**	
		Real term	Model	Real term	Model	Real term	Model
Slaný 2001	D°	269	243	301	301	431	363
	Date	**31.5.**	**28.5.**	**15.6.**	**8.6.**	**27.6.**	**17.6.**
Slaný 2002	D°	256	243	306	301	357	363
	Date	**24.5.**	**23.5.**	**1.6.**	**31.5.**	**7.6.**	**8.6.**
Slaný 2003	D°	244	240	304	302	370	365
	Date	**22.5.**	**22.5.**	**28.5.**	**28.5.**	**3.6.**	**3.6.**

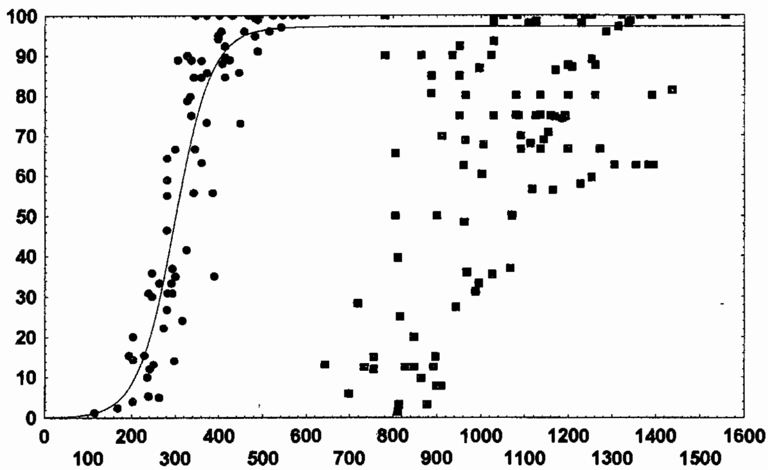

Figure 1. The dependence of cumulative pheromone trap catches of the overwintering and summer generations of AO on D°. Flight curve of the overwintering generation was fitted using Richards´ function.
„x" line - cumulative percentages of males caught in pheromone traps at Ruzyně, Doksany, Roudnice, Holovousy and Slaný in 1992 – 1998, „y" line – day-degrees
„•" – males of the overwintering generation, „▪" - males of the summer generation

This paper reports that the dependence of the flight pattern of the overwintering generation of *A. orana* on DD° was similar in all years and localities in a region. The flight patterns of the summer generation of *A. orana*, however, differ significantly between localities and years in one region. In contrast, Flückiger & Benz (1982) developed models of the flight activity of *A. orana* for both the overwintering and summer generations, wich have a good predictive reliability. It is possible to construct models of flight activity based on catches of pheromone traps for some generations of some species of tortricids (Beránková *et al.*, 1988; Glen & Brain, 1982; Stará & Kocourek, 2001; Hrdý *et al.*, 1996). These can simulate the flight activity of a pest, either in a whole region or only in the locality from which the pheromone trap catches were obtained. It is impossible to construct a temperature-driven model for the flight activity of the summer generation of *A. orana* in Czech Republic and therefore the timing of insecticide application against this generation is dependent entirely on data from pheromone traps.

Acknowledgements

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Monitoring of San Jose scale (*Quadraspidiotus perniciosus* Comst.) by pheromone traps and timing of control on crawlers

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Abstract: Three pheromone components were tested for monitoring males of San Jose scale (SJS). Combination of two specific pheromone components were the most effective for trapping SJS males. The monitoring of SJS by pheromone traps was conducted in orchards at one locality in North Bohemia and one locality in South Moravia in 1996 - 1999 and at one locality in South Moravia in 1996 - 2003. The date on which the first generation crawlers start to migrate can be determined according to the sum of effective temperatures from 350 - 400 °C above 7.3 °C, calculated as from the date on which the first males of the owervintering generation have been found in the pheromone traps. This sum of effective temperatures indicates the optimum period for timing of insecticide application. In orchards where insecticides were applied against the crawlers, population density of SJS decreased and the damage to fruits was eliminated.

Key words: San Jose Scale, *Quadraspidiotus perniciosus*, apple orchards, pheromone traps, day degrees

Introduction

Since 1995, San Jose scale (*Quadraspidiotus perniciosus* Cumstok, 1881) population densities increased in fruit orchards in warm regions of the Czech Republic. SJS gradation was first recorded in the Czech Republic in fifties and sixties. SJS didn't occurred in intensive orchards till nineties. It survived in hardwood forests and old gardens. In the period of 2nd gradation in 1995 – 2000 SJS spread to intensive orchards. Application of oleo-organophosphate sprays in early spring isn't effective sufficiently. Control of crawlers in summer hasn't been already practised. According to Flint (1991), crawler emergence begins about 405 day degree (°F) after the first male flight. Optimum timing for crawler spray is in California 600 – 700 day degree (°F) after the first male scale is collected. The aim of this work was to develop the method of timing of insecticide applications against crawlers on the basis of monitoring of flight activity of San Jose scale (SJS) males by pheromone traps and sum of effective temperatures.

Material and methods

The monitoring of SJS by pheromone traps was conducted in orchards at one locality in North Bohemia, one locality in South Moravia in 1996 - 1999 and at one locality in South Moravia in 1996 - 2003. In Bohemia, pheromone traps were placed in one old garden and one nontreated apple orchard. In Moravia, pheromone traps were placed in one garden, one nontreated apple orchard, one apple orchard with IPM and one apple orchard with conventional protection against pest. Funnel traps lined with Bird Tangelfoot adhesive were used.

First, the efficacy of catching by 3 pheromone components of synthetic sexual attractants: I. *(2E)–3,7–dimethyl–2,7–oktadienyl propionate*, II. *3-methylem-7 methyl-7-okten-1-ol propionate,* III. *(Z)-3,7dimethyl-2,7-octadien-1-ol propionate* and their combination was tested (Fig.1). Sexual attractants synthesized dr. L. Streinz (*Streinz L. et al., A simple method of isomerisation of terminal double bond in terpene chain. Coll. Czech. Chem. Commun. 50, 2174 (1985)*).

On the basis of previous results (Fig.1), the combination of pheromone components II. + III. at a ratio 1 : 1 was used for the monitoring in all years and localities. The lures contained 0,1 mg of synthetic sexual attractant. The pheromone traps were replaced in weekly or two times a week during the whole growing season.

Air temperatures were recorded 2m above ground by automatic meteorological station. Calculation of sums of temperature was made using 7.3 °C development threshold. The calculation started at the date of first catch of males of the overwintering generation in the pheromone traps.

Results and discussion

Combination of pheromone components II. *3-methylem-7 methyl-7-okten-1-ol propionate* + III. *(Z)-3,7dimethyl-2,7-octadien-1-ol propionate* at a ratio 1 : 1 was the most effective for trapping SJS males (Fig.1). When the component I. *(2E)–3,7–dimethyl–2,7–oktadienyl propionate* was added to this combination, no increase of efficacy of catching was recorded. Simple components of pheromone and their another combination had lower efficacy of catching.

Two SJS generations were recorded in 1996 - 2002. Third generation was recorded only exceptionally in warm year 2003 (Fig. 4). Sum of effective temperatures 827 day degrees was necessary for the development of the summer generation. This was calculated as a difference between the emergence dates for males of the first and the second generations (Fig. 2) for localities in south Moravia in 1993 - 2003. Sum of effective temperature increased in warm years. Generation time for SJS is 770 °C above 7.3 °C for conditions of central Europe (Huba 1961) and 583.3 °C above 10.6 °C for conditions of California (Rice *et al.* 1982). Lower value of lower threshold of development for SJS population from Europe can be a geographical adaptation. We determined the beginning of crawlers occurrence as 350 - 400 day degrees above 7.3 °C calculated from the first males in the pheromone traps (Fig. 3). This sum of effective temperatures indicates the optimum period for timing of insecticide application. According to Rice *et al.* (1982), crawler emergence begins about 380 - 400 day degree (°F) above 51 °F after the first male flight. Optimum timing for crawler spray is in California 600 – 700 day degree (°F) above 51 °F after the first male scale is collected. Using of biofix as the first occurrence of males in pheromone traps is necessary for determining of the beginning of crawlers occurrence. Start of males flight was recorded in particular years at different day degrees from 175 to 497 °C in south Moravia in 1996 – 2003.

The method of SJS monitoring using pheromone traps was able to detect the presence of SJS even at low population densities. Population density of SJS increased markedly in intensive orchards without chemical control and in orchards with conventional control, while in the gardens it was stable. In the old garden in Židovice and in the garden in Velké Bílovice the SJS populations were stable in 1996 – 2001. The share of total catches of the 1st and 2nd generation males was approximately 1:1. In contrast to this, in the untreated orchards the gradation of SJS was found in Bohemia as well as in Velké Bílovice. This gradation ceased since 1997 to 2000 (Fig. 4). In time of gradation peak the catches of the 2nd generation males were much higher than the catches of the 1st generation males. Total catches of the 2nd generation males ranged from 1.5 to 8 thousand of individuals per trap. In the orchard in Velké Bílovice with IPM including application of selective insecticides (phenoxycarb, phosalone, etofenprox, chlorpyriphos-methyl), population density of SJS remained low. In the orchard with conventional chemical control in Nosislav (deltamethrin, methidathion) without application aimed against crawlers, population density of SJS remained high. Maximum weekly catches of males from 500 to 1000 individuals corresponded with stable harmful occurrence of SJS. In orchards where insecticides were applied against the crawlers population density of SJS decreased and the damage to fruits was eliminated.

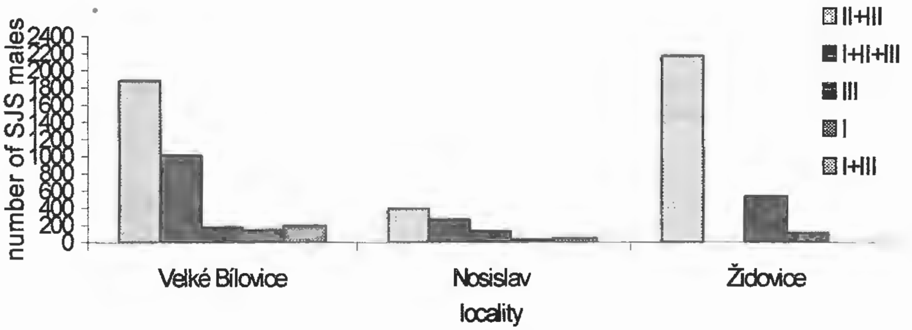

Figure 1. Total annual captures of males of SJS in feromone traps with 3 types of pheromone components and their mixtures (I. (2E)-3,7-dimethyl-2,7-oktadienyl propionate, II. 3 methylem-7 methyl-7-okten-1-ol propionate, III. (Z)-3,7dimethyl-2,7-octadien-1-ol propionate) in Velké Bílovice, Nosislav and Židovice in 1997

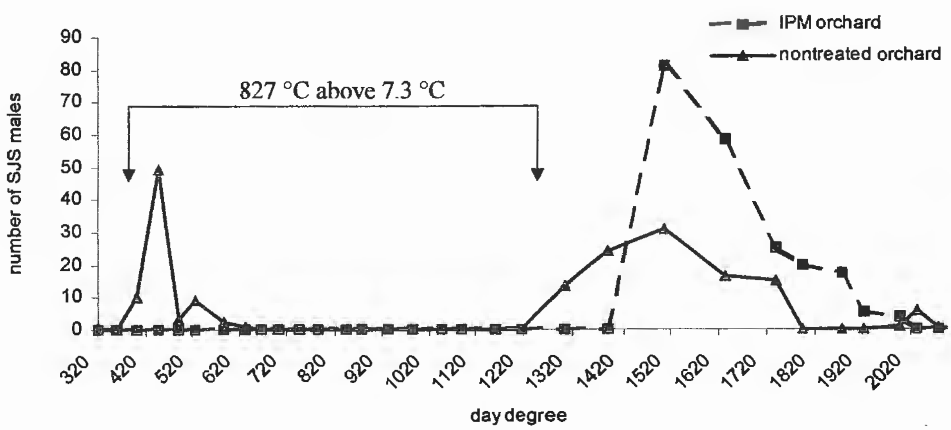

Figure 2. Sum of effective temperatures for the development of summer generation of SJS. Week captures of SJS males in traps placed in old garden and orchard in Židovice in 1996.

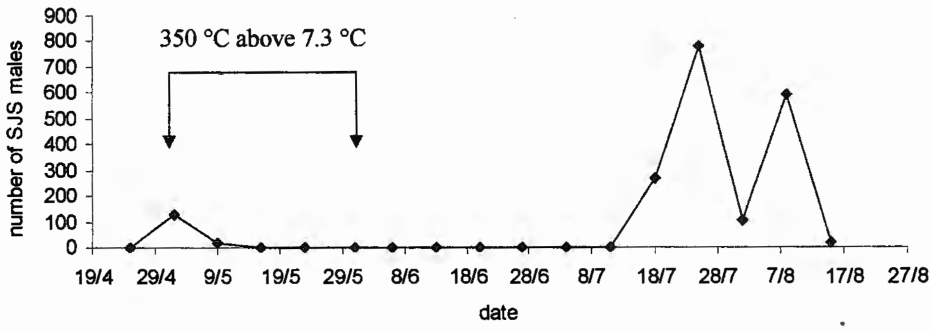

Figure 3. Sum of effective temperatures for the timing of insecticide application against SJS crawlers in nontreated orchard in Velké Bílovice in 1999.

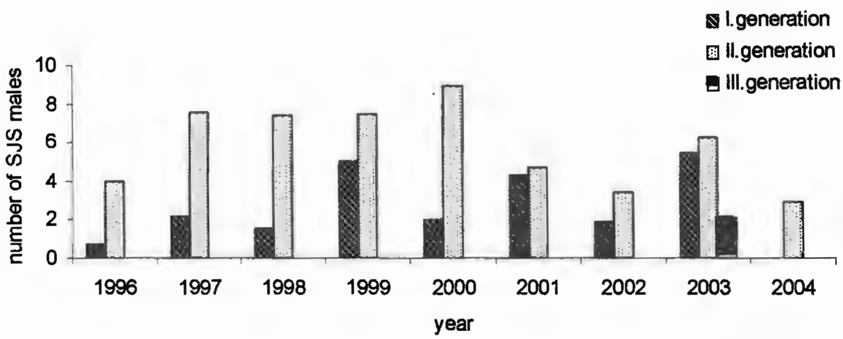

Figure 4. Total annual captures of males of the I. generation (owerwintering), II. generation (summer) and III. generation of SJS in pheromone traps in nontreated orchard in Velké Bílovice in 1996 – 2004.
No. – ln (x + 1), x – total males for generation

Acknowledgements

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Secondary dissemination of *Bacillus subtilis* (BD170 - BioPro) against *Erwinia amylovora* on apple flowers by means of pollinators, *Apis mellifera* and *Osmia cornuta*

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Abstract: The efficiency of two pollinators, *Apis mellifera* Linneaus (Hymenoptera Apidae) and *Osmia cornuta* (Latreille) (Hymenoptera Megachilidae) as secondary disseminators of *Bacillus subtilis*, strain BD170 (BioPro) for 'fire blight' control on 'Golden Delicious' apple flowers was evaluated by means of field and semifield trials. In two apple orchards (one in a plain area and the other on the hill) the two pollinators were introduced together, while in an analouge couple of fields *A. mellifera* was introduced alone. Two fields were sprayed once with the BCA (100 g/hl) and the others two times during apple's flowering. The pollinators' efficacy in transferring the biocontrol agent from sprayed flowers to the stigmas of newly opened ones at different elapsed times after the spray treatment was evaluated. The detection of the BCA was performed by PCR analyses based on molecular markers. The percentages of positive PCR flower samples were higher in the internal sprayed areas of the fields, but the high colonisation's level found in the non-sprayed areas and in the flowers opened several days after the treatment(s) demonstrated that pollinators can play an important role in secondary spreading. In semifield trials, the two insect species were also studied as secondary BCA disseminators on apple plants in pots. Their approaching behaviour to the apple flower and their capacity to deposit the BCA in the most receptive parts of the flower, were compared both by washing the flower organs and diluting and plating the samples on a recovery medium and through PCR analyses. Preliminary results are presented and discussed.

Key words: biocontrol agent, *Bacillus subtilis, Erwinia amylovora, Osmia cornuta, Apis mellifera*

Introduction

A new topic in phytoiatric research is the role that pronubial insects can play in microbiological control as efficient carriers of beneficial microrganisms against pathogens and arthropod pests (Bazzi et al, in press; Bilu et al., 2004). Their use as vehicles for BCA could result in a very efficient system for the control of several diseases, whose preferential way of inoculum is represented by flower organs, like the bacterial fire blight (*Erwinia amylovora*) for apple and pear crops. Up to now, there is a lack of knowledge concerning the efficiency of the different distribution techniques, with special reference to the role that pollinators can play also as secondary disseminating actors during flowering.

The laboratory efficacy of *Bacillus subtilis*, strain BD170 (Biopro) against *E. amylovora* has been recently demonstrated, and a method for the detection and tracking of the BCA by a PCR based molecular marker was developed (Broggini et al., 2004). The aim of this study was to evaluate the role that two pollinators *Apis mellifera* Linnaeus (Hymenoptera Apidae) and *Osmia cornuta* (Latreille) (Hymenoptera Megachilidae) can play also as secondary BCA disseminating actors in apple orchards ('Golden Delicious') during flowering.

Materials and Methods

Antagonist bacterial strain

Biocontrol strain *Bacillus subtilis* BD170 was provided in its commercial powdery formulation (BioPro) ($2x10^{11}$ spores/gram).

Semifield Trials

The efficiency of individuals of *A. mellifera* and *O. cornuta* in secondary BCA dissemination was studied on apple plants in pots under net screened tunnels. In a separated chamber, one plant was sprayed till drop with a BCA water suspension (100 g/hl). When dried, the treated plant and an untreated one were introduced in the tunnel. One insect per time was allowed to forage on both and the visited flowers of the non-sprayed plant were collected, according to the visit order. The foraging flights of 31 *A. mellifera* and 39 *O. cornuta* were observed. Quantitative BCA analyses were run on the first flower visited on the untreated plant (washing, diluting and plating of the flower stigmas on a recovery medium, incubation at 36°C and countings of the colony forming units); qualitative analyses were run on all the other flowers visited by the insect by means of PCR analyses of the flower stigmas, based on the BCA molecular markers. Untreated and treated open flowers acted as controls.

Field Trials

In four 'Golden Delicious' apple orchards, two in the Trento plain, two in Val di Non, (height 800 m) an internal area measuring 620±100 m² was delimited with labels. In two fields, one in the plain and the other in the hill, two honeybee colonies and one nesting shelter with 400 *O. cornuta* females and 800 males were introduced together in the center of the internal area, while in the other two fields only *A. mellifera* was used as pollinator. Apple bloom was monitored, and at 15-20% of flowering all the four fields were sprayed with BioPro (100 g/hl) in water suspension. In two fields a second treatment was applied at 48-55% of flowering. The efficacy of pollinators in transferring the BCA from sprayed flowers to the stigmas of newly opened ones was estimated at different elapsed times after treatment, till the end of blooming, by sampling 80 flowers both in the internal sprayed area, either in the external non-sprayed area, according to the protocol summarized in table 1. The detection of the BCA was performed by PCR analyses of the flower stigmas. Untreated and treated flowers acted as controls.

Table 1. Treatments and sampling protocol in the two sites.

Site	N. sprays	Sampling intervals							
Plain	1	T-1day	T	T+1day	T+3days	T+6days			
Plain	2	T-1day	T	T+1day	T+3days	T2	T2+1day	T2+4days	
Hill	1	T-1day	T	T+1day	T+3days	T+5days	T+8days	T+11days	
Hill	2	T-1day	T	T+1day	T+3days	T2	T2+1days	T2+4day	T2+7days

T= 1° treatment; T2=2° treatment

Results and discussion

Semifield trials

The BCA reisolation from the first visited flowers on recovery medium showed a significantly higher level of contamination of the flowers visited by *O. cornuta* (9.6 x10^3 ±18.9 x10^3) with respect to those visited by *A. mellifera* (1.4 x10^3 ±2.1 x10^3) (Mann-Whitney U Test, P=0.0006). The distribution of the flower samples in classes of contamination (fig. 1) indicates that the most of *O. cornuta* individuals contaminated the first visited flower with a number of CFU pertaining to a two or three times higher order of magnitude.

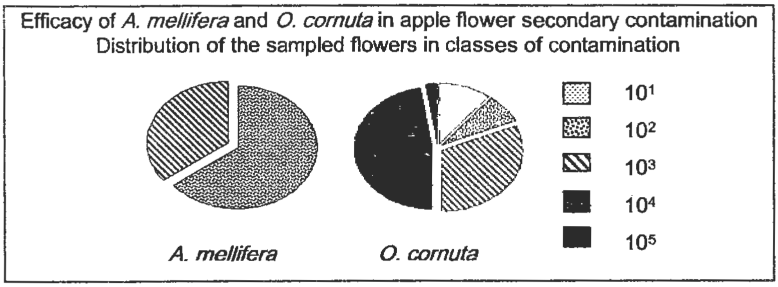

Figure 1. Percentage of first visited flowers pertaining to each class of contamination (order of magnitude of the number of Colony Forming Units)

The PCR analyses run on the stigmas of all the visited flowers after the first, confirmed the high contamination capacity of both species. Anyway, the percentage of positive samples found for the flowers visited by *O. cornuta* (85.3±18.9) was significantly higher with respect to that one registered for *A. mellifera* (66.3±31.4) (Mann-Whitney U Test; P=0.0084).

Field trials

In figure 2, the percentages of flower samples in which the presence of the BCA was detected at the PCR analyses are reported. The graphs show that a significant portion of the flowers sampled before the BCA sprays resulted positive to the PCR analyses. This unexpected finding needs further investigation to uscertain which *B. subtilis* strains are present in the experimental area and to assess the suitability of the molecular markers to detect the presence of the BD170 *B. subtilis* strain. At present, this finding suggests us not to run any statistical analysis, but some considerations are indeed possible.

As expected; after the first spray treatment the percentage of secondarily contaminated flowers was higher in the treated internal areas.

In the fields on the hill, the percentage of contaminated flowers in the non-treated external areas increased and reached that of the internal ones (4 days were necessary in the field with 1 BCA spray, 6-7 in the field treated twice) and reached satisfactory levels of protection (>85% of opened flowers contaminated with BCA).

In the plain areas, the percentage of contaminated flowers in the untreated external areas remained always lower with respect to the internal treated ones. In the field treated twice.the level of protection was higher in the second part of the flowering, with respect to the field with 1 treatment, even if it remained uncomplete.

Two factors appeared to affect the level of protection of newly opened flowers that can be gained: the weather conditions (with more total rains and a higher RH registered for the hill areas) and, related to this, the flowering duration (8 days for the fields in the plain, 11 days for the hilly ones). The combination of these two factors seems to be favourable to the flower colonization by the BCA and allow pollinators to perform very efficiently their BCA carrying activity.

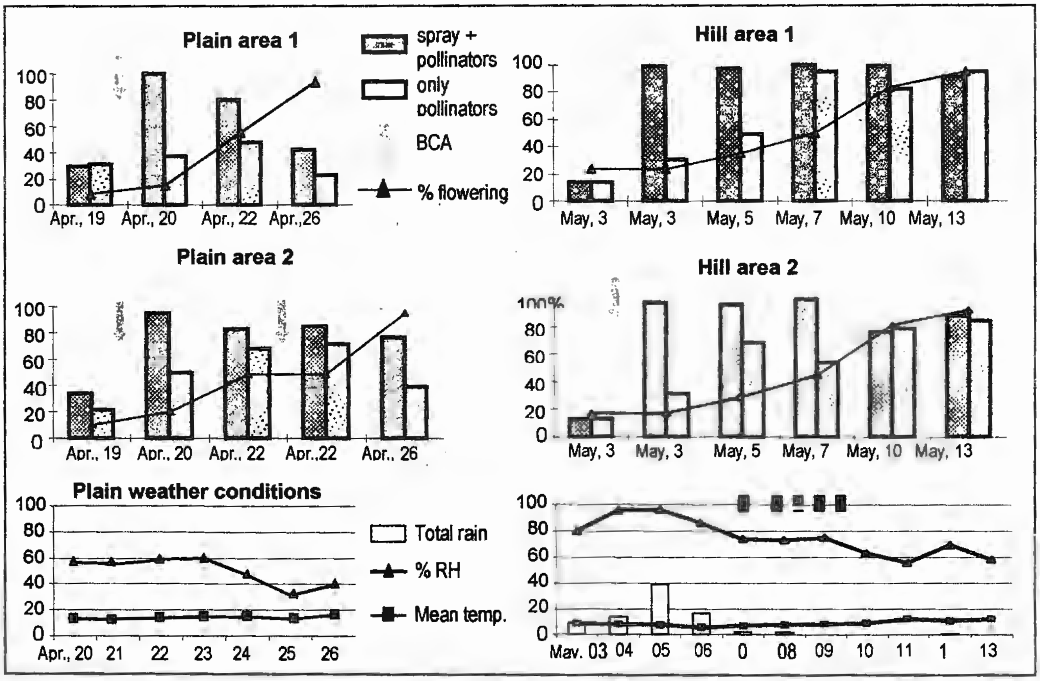

Figure 2. Percentage of newly contaminated flowers in the four experimental fields and weather conditions registered in the hill and in the plain sites.

Acknowledgments

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Biological efficacy of kaolin against the pear sucker *Psylla pyri* in winter and summer applications

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Abstract: The pear sucker *Psylla pyri* is an important pest in European pear orchards. In 2001, 2002 and 2003, we conducted extensive field trials to test the biological efficacy of formulated kaolin (Surround WP) against the pear sucker in Belgium. We compared its use against the winter (3-4 applications during February and March) and summer generations (3-4 applications during May and June). Surround is highly effective (90% reduction compared to water treatments) in the control of the winter generation, preventing egg-laying and subsequent larval development in early spring. Shifting the applied dosage gave variation in efficacy. Control of summer generations was less pronounced (up to 70% efficacy). The application of Surround WP in orchards has an effect on the population of the predatory bug *Anthocoris nemoralis* , though we cannot distinguish whether this is due to repellency or the lack of prey (*Psylla* nymphs) .

Key words: pest control, side effects, pear orchards, Surround WP, climate stress

Introduction

In pear orchards, multiple generations of *Psylla pyri* damage tree and fruits on specific levels (Bylemans 1996). High infestations of consecutive generations weaken tree growth and hamper the development of future flower buds, while important honeydew secretion allows secondary infestation of sooty mould that blackens branches and fruits. As adult *Psylla* can fly in from neighbouring orchards, proper control must address each generation in turn. However, control of the first generation is primordial to prevent damage to flowers and increased russetting of fruits. Furthermore, good control of that generation can reduce pressure of subsequent generations. Currently, Belgian fruit growers face increasing problems with *Psylla*, with the withdrawal of amitraz from 2005 onwards. As a result, growers' demands for alternative *Psylla* control is high.

In 2001, 2002 and 2003, we conducted extensive field trials to test the biological efficacy of formulated kaolin (Surround WP) against the pear sucker in Belgium. Kaolin is a natural clay compound that has a variety of industrial and medicinal uses. It has several uses in horticulture, especially in insect control (Glenn *et al.* 1999) and reduction of climate stress (Glenn *et al.* 2002; Jifon & Syvertsen 2003; Wisniewski *et al.* 2002). We performed experiments to test the biological efficacy of kaolin against the pear sucker, *Psylla pyri*, and the side effect against its main predator, *Anthocoris nemoralis*.

Material and methods

We tested formulated kaolin (Surround WP) in field trials with winter and summer applications (Feb-Mar and Jun-Jul respectively). Surround WP was consecutively applied 1-6

Water controls and Surround were each applied in 4 replicates laid out in a randomized block design. Standard dose rate was 20kg of active ingredient (a.i.) per ha leaf wall area (LWA), in two winter experiments a dose rate range was tested (7kg, 14kg and 20kg ai/ha LWA). The amount of water used was equivalent to 500l/ha standard orchard in winter and 1500 l/ha standard orchard in summer. Trial execution was in accordance with EPPO guidelines. In each trial, the presence of pear sucker adults & nymphs were recorded at a specific time after first application. We present data as the % reduction in numbers compared to water treatment controls (Abbott value =C-T)/C x100; C = average infection in the untreated object; T= average infection in the treated object). In 7 trials, the presence of predatory bugs (adults & nymphs) was sampled with a knocking method up to 4 months after first application.

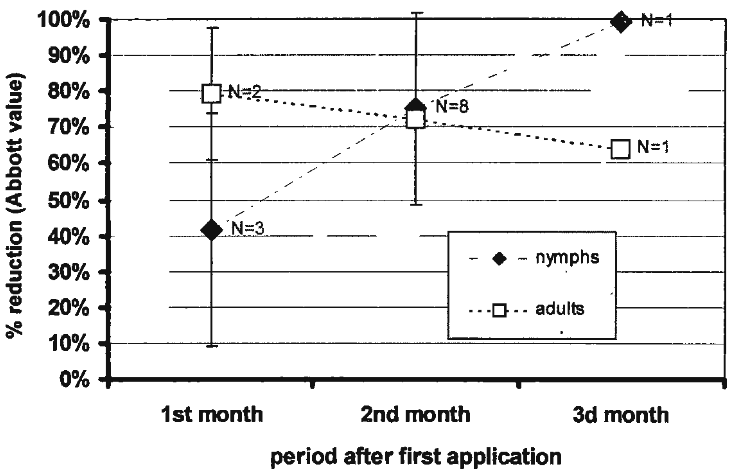

Figure 1. Biological efficacy of formulated kaolin on adults and nymphs of *Psylla pyri* measured 1 to 3 months after first application. Kaolin was applied 3-4 times with 10 days interval in a winter treatment. (N= number of trials).

Results

Surround has a strong effect on the presence of adult *Psylla pyri* that lasted up to three months (Fig. 1). This is likely due to the repellent nature of the kaolin powder. As a result, the number of nymphs developing on treated trees was greatly reduced. We see a slight increase in efficacy on nymphs between 1 and 3 months after first application. This suggests that surround is less effective against nymphs (or hatching eggs) that are already present, but rather limits the build up of nymphs through prevention of adult egg laying. Biological efficacy was sufficient from three consecutive treatments onwards in winter treatments (Fig. 2), with efficacy ranging from 70%-99% (average 90±12%). Summer applications are less effective, although acceptable efficacy (75%) was obtained after 6 consecutive treatments. Trials with variable dose rates suggest that efficiency is lost only at the lowest concentration of 7kg/ha leaf wall area (Fig. 3).

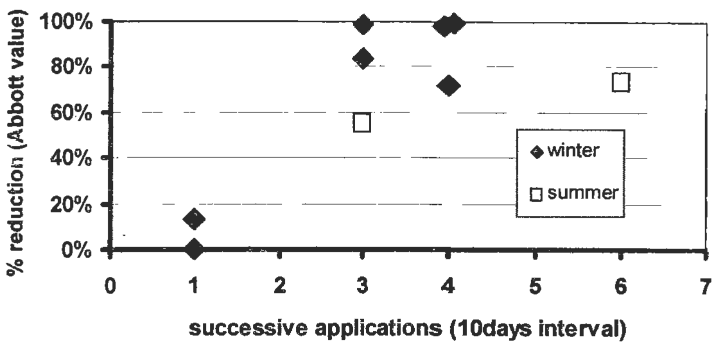

Figure 2. Biological efficacy of formulated kaolin on pear sucker nymphs related to the number consecutive applications in winter and summer treatments

The application of kaolin reduces the population of the key predatory bug *Anthocoris nemoralis* with 50±8% in orchards with sufficient population (>10 individuals average per sample) for confident data interpretation. Here we suspect a combination of a repellent effect of kaolin and a drop in prey availability as only few *Psylla* larvae remain (see Fig. 2). Further tests with specific focus on prey-predator relations are needed to distinguish these effects.

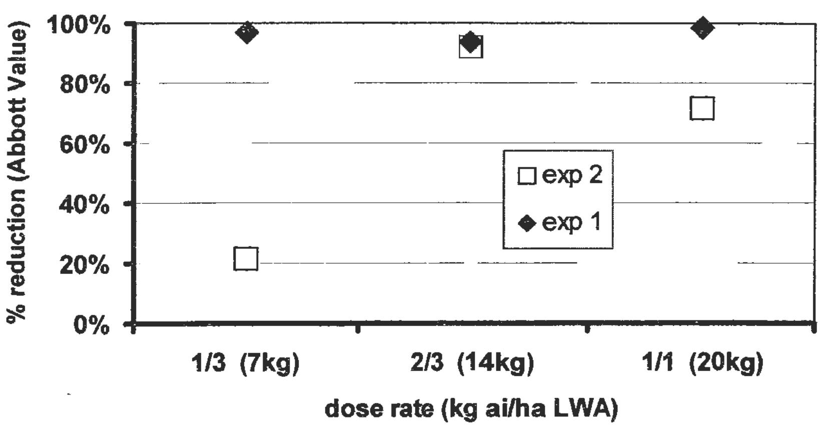

Figure 3. Biological efficacy of different dose rates of formulated kaolin on nymphs of *Psylla pyri,* measured after four applications (10 days interval).

Discussion

The application of formulated kaolin (Surround WP) is very effective at reducing the occurrence of *Psylla pyri* adults in pear orchards in early spring. This is likely due to a repellent effect as found in Silverleaf Whitefly (Liang and Liu, 2002), Pecan Aphids (Cottrell *et al.* 2002) and Boll Weevils (Showler, 2002). Adult insects suffer from accumulation of particle film on their body when in contact with kaolin, and avoid kaolin-treated surfaces based on colour-differences.

Our field results on *Psylla pyri* confirm an earlier laboratory study in which adults did not settle or oviposit on kaolin-treated leaves (Glenn et al. 1999). In their experiment, kaolin residue found on the legs of untreated adults proved that they attempted to settle on treated leaves but were repelled. Repelling *Psylla* adults prevents massive egg-laying and reduces subsequent suction feeding by nymphs on flower buds in the field (Pasqualini *et al.* 2002). Spring feeding causes important damage to flowers and young fruits. Furthermore, lowering nymph numbers reduces secondary damage caused by sooty mould growing on excreted honey dew. Care should however be taken to allow recovery of predatory bug populations, e.g. by in-flight from untreated shrubs or woodlands.

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Effects of some botanical pesticides on pests and beneficial Arthropods

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Abstract: The effect of extracts from *Melia azedarach* L., *Artemisia absinthium* L., *Anona cherimola* Mill. and *Quassia* spp. on adults of *Frankliniella occidentalis* (Pergande) and *Monosteira unicostata* (Mulsant et Rey) was evaluated in laboratory tests. The side effects on the predators *Orius* spp. and *Cheiracanthium mildei* L. Koch were also considered. Both the pesticide effect and the deterrence activity were tested for each compound. A Computer Controlled Spraying Apparatus was used to ensure a homogeneous treatment and the accuracy of pesticide doses. The statistical analyses showed that among all the pesticides tested on *M. unicostata*, the *A. absinthium* extracts presented significant differences on the third, fourth and fifth days after spraying ($P \leq 0{,}01$), comparing both the control and the trial treated with soap. Among the compounds tested on *Orius* spp., significant differences ($P \leq 0{,}01$) were evident between the control and *A. cherimola* and between the control and *A. absinthium*. *A. absinthium* was the unique product that showed a repellent effect only towards *F. occidentalis*. None of the tested compounds showed side effects both on adults and spiderlings of the spider *C. mildei*.

Key words: botanical pesticides, side effects, *Frankliniella occidentalis, Monosteira unicostata, Orius, Cheiracanthium mildei.*

Introduction

The studies aimed at identifying botanical substances with insecticidal properties and no harmful effect on beneficial organisms and vertebrates date back to several decades ago. One of the substances possessing these qualities is the azadirachtin, extracted from the seeds of Meliacea *Azadirachta indica* A. Juss (neem tree), the botanical insecticide most studied at the world level (Mordue & Blackwell, 1993; Schmutterer, 1987; Tsolakis *et al.*, 2001).

The activity of the extracts of vegetable origin is very diversified, ranging from repellence to antifeedant activity (Jacobson *et al.*, 1978; Klocke & Kubo, 1982; Murray *et al.*, 1999), from the sterilizing action to the regulation of growth and physiology of insects (Di Ilio *et al.*, 1999), from deterrence to oviposition (van Randen & Roitberg, 1998) to severe toxicity.

The present study is part of an Italian research project aimed to assess the insecticidal or repellent activity and the side effects on beneficial Arthropods of botanical extracts obtained from plants widespread in Italy or easily available on the market.

Materials and methods

The botanical substances taken in consideration in the present trial were *Anona cherimola* Mill., *Artemisia absinthium* L., *Melia azedarach* L. and *Quassia* spp.

The insecticidal and/or repellent effect of each substance was tested on pests *Frankliniella occidentalis* (Pergande) (Thysanoptera Thripidae) and *Monosteira unicostata*

Mulsant et Rey (Rhynchota Tingidae) and on the predators *Orius* spp. (Rhynchota Anthocoridae) and *Cheiracanthium mildei* L. Koch (Araneae Clubionidae).

Trials were run in three different periods in 2003 according to the supply of the substances to test and the availability of the pests and predators. The first trial was started at the end of June on *F. occidentalis* only, the second at the end of September on *F. occidentalis, M. unicostata, Orius* spp. and *C. mildei,* the third was started at the end of November on *M. unicostata, Orius* spp. and *C. mildei.* The aqueous solutions used in the trials of late June and late September were obtained by soaking previously crushed and filtered botanical substances. In November trial the solutions were obtained by dilution of concentrated extracts in distilled water added with Marseilles soap. Both the crushed botanical substances and the concentrated extracts have been supplied by Palermo University. The extraction methods, the analyses of the extracts and the dilutions are described in Ragusa Di Chiara *et al.* (2002).

The adulticidal effect of each substance was evaluated by a factorial design, considering the different botanical substances as first factor, and the death of arthropods 1, 2, 3, 4 and 5 days from the treatment, as second factor. The experimental units were constituted by boxes obtained upturning a 9-cm basis of Petri dish on a second basis of the same size, joining the two halves by adhesive tape. For the three species of insects under test, 10 adults were released in each box on a leaf disk of about 3 cm laid over a rectangle of cotton wool soaked with distilled water. In the case of the spider *C. mildei* the experimental units were the above cited boxes, without leaf disks and containing a single individual, either adult or spiderling, so as to prevent cannibalism. Aeration was ensured making on each box two holes covered with an unwoven fabrics disk. Leaf disks were obtained from bean leaves for the trials on *F. occidentalis* and *Orius* spp., and from almond leaves for the trials on *M. unicostata.* A Computer Controlled Spraying Apparatus (Burkard Manufacturing Co. Ltd., England) was used to spray the solutions.

A. absinthium and *Quassia* solutions were applied at a rate of 1.0 mg/cm^2, those of *M. azedarach* and *A. cherimola* at 1.2 mg/cm^2. The control was the leaf disks treated only with distilled water. When the concentrated extracts were used, a second control was constituted by the aqueous solution treated with Marseilles soap.

In the case of the three species of insects, five replicates were made for each treatment, in the case of the spider *C. mildei* replicates were 10, i.e. 10 boxes, containing each a spider. Mortality was daily monitored till the fifth day after the treatment.

The repellent effect was tested only towards insects, following the same procedure as for the adulticidal effect; the difference was that two leaf disks were placed in each box: one being treated with the formulate at the same doses as above, the other being sprayed with distilled water. Moreover the 10 individuals released in each box were only females. Seven days after the introduction of females the number of eggs laid on the two leaf disks was counted.

The elementary data were submitted to the analysis of variance; when the F values resulted to be statistically significant, the post-hoc comparisons were made using Tukey's test.

Results and discussion

Frankliniella occidentalis

The analysis of variance of the first trial data relative to the adulticidal effect of the extracts towards *F. occidentalis* did not show any significant difference between the treatments compared on all the 5 days of mortality observation.

In the second trial, instead, some differences between treatments appeared only on the third day after the treatment (tab. 1). Tukey's test proved that only the *M. azedarach* extracts showed a significantly different mortality for $P \leq 0.01$, as compared to the control.

Table. 1. Mean cumulative mortality (mean of the 5 replicates) of *F. occidentalis* adults during the second trial (30.IX.03-5.X.03)

Test	1st day	2nd day	3rd day	4th day	5th day
A. cherimola	0.0	0.8	0.8 AB	1.2	1.8
A. absinthium	0.6	0.6	0.6 AB	1.2	1.6
M. azedarach	0.0	0.0	0.0 A	0.2	0.4
Quassia spp.	0.4	0.6	0.8 AB	2.0	2.2
Control	0.6	1.0	1.8 B	1.8	2.4

Note: values having a common letter are not significantly different ($P \leq 0.01$) by Tukey's test.

As a whole it may be stated that the plant extracts used have shown such a mild adulticidal effect against *F. occidentalis* making its use non recommended.

The data on the extracts' repellent activity towards *F. occidentalis* are shown in table 2.

Table. 2. Mean number (mean of the 5 replicates) of eggs laid by *F. occidentalis* on leaf disks during the two trials to evaluate the repellent effect

	First trial (26.VI.03-1.VII.03)		Second trial (30.IX.03-5.X.03)	
Test	treated disk	untreated disk	treated disk	untreated disk
A. cherimola	7.0	19.2	0.4	2.4
A. absinthium	7.2	69.6	0.8	5.7
M. azedarach	24.0	9.6	2.2	1.4
Quassia spp.	13.8	15.6	1.0	3.2

In the first trial, the number of eggs laid on treated disks was shown to be non significantly different in the four treatments concerned. Comparing, instead, the number of eggs laid on treated disks and on the controls of each treatment, significant differences were found for $P \leq 0.01$ only in the case of *A. absinthium*. The statistical analyses conducted on the data of the second trial have fully confirmed the above considerations.

It may be concluded that only *A. absinthium* has resulted to be somewhat effective in reducing the oviposition of *F. occidentalis*.

Monosteira unicostata

The data analysis of the first trial on the assessment of the adulticidal effect of extracts towards *M. unicostata* has not shown any statistically significant difference between the tested substances and the control.

As to the second trial the major data are the following (tab. 3): the mean mortality induced by *A. absinthium* showed statistically significant differences (for $P \leq 0.01$) on the third, fourth and fifth days after the trial (respectively 6.8, 7.6 and 8.2 dead adults per box) both as compared to the control (respectively 1.6, 1.8 and 1.8 dead adults per box) and to the treatment with Marseilles soap (respectively 0.6, 0.8 and 1.2 dead adults per box).

Table. 3. Mean cumulative mortality (mean of the 5 replicates) of *M. unicostata* adults during the second trial (26.XI.03-1.XII.03)

Test	1st day	2nd day	3rd day	4th day	5th day
A. cherimola	3.2 A	5.0 A	5.0 AC	5.2 AB	5.4 AB
A. absinthium	3.2 A	5.0 A	6.8 C	7.6 B	8.2 B
M. azedarach	0.0 A	0.4 B	0.6 B	1.2 A	1.2 A
Quassia spp.	3.6 A	3.6 BA	4.0 BAC	4.4 AB	4.4 AB
Marseilles soap	0.0 A	0.6 BA	0.6 B	0.8 A	1.2 A
Control	1.2 A	1.6 BA	1.6 BA	1.8 A	1.8 A

Note: values having a common letter are not significantly different ($P \leq 0.01$) by Tukey's test.

It should be underlined that: 1) the concentrated extracts showed a much greater adulticidal effectiveness towards *M. unicostata* as compared to the aqueous extracts obtained from the crushed plant substances ; 2) among concentrated extracts *A. absinthium* , in particular, induced a definitely higher adult mortality compared to the other substances.

It was not possible to test the repellent effect on *M. unicostata* because any egg was found on none of the leaf disks made available to females.

***Orius* spp.**

During the first trial the mortality of *Orius* spp. resulted to be very similar in the five treatments; actually the analysis of variance did not show any statistically significant difference. In the second trial, instead, statistically significant differences in mortality were observed for $P \leq 0.01$, both between the control and *A. cherimola* and between the control and *A. absinthium* on all the five days of the monitoring (tab. 4).

Table. 4. Mean cumulative mortality (mean of the 5 replicates) of *Orius* spp. adults during the second trial (26.XI.03-1.XII.03)

Test	1st day	2nd day	3rd day	4th day	5th day
A. cherimola	9.0 C	9.6 B	9.8 B	9.8 B	10,0 B
A. absinthium	5.6 B	7.6 B	8.0 B	8.6 B	9.0 B
M. azedarach	0.0 A	0.8 A	1.6 A	2.2 A	4.8 A
Quassia spp.	0.8 A	1.8 A	3.0 A	4.0 A	5.0 A
Marseilles soap	2.0 A	2.8 A	3.6 A	4.0 A	4.6 A
Control	1.6 A	2.4 A	2.8 A	3.4 A	4.4 A

Note: values having a common letter are not significantly different ($P \leq 0.01$) by Tukey's test.

The analysis of data relative to the repellence effect showed that there was no statistical difference in the number of eggs laid by females of *Orius* spp. on the treated leaf disks and on the controls (tab. 5).

Table. 5. Mean number (mean of the 5 replicates) of eggs laid by *Orius* spp. on leaf disks during the two trials to evaluate the repellent effect

	First trial (30.IX.03-5.X.03)		Second trial (26.XI.03-1.XII.03)	
Test	treated disk	untreated disk	treated disk	untreated disk
A. cherimola	4.4	5.6	1.0	0.4
A. absinthium	0.0	5.2	1.8	0.2
M. azedarach	9.2	14.6	2.6	0.8
Quassia spp.	4.0	6.0	3.0	5.6
Marseilles soap	---	---	1.0	6.0

It should be emphasized that *A. cherimola* and *A. absinthium* were shown to be harmful towards the predator, although their efficacy as adulticides was found only with the concentrated extracts.

Cheiracanthium mildei

The extremely low mortality of the spider adults observed in the first trial both in treated treatments and in the control did not reflect any statistically significant difference between the treatments being compared.

During the second trial, all *C. mildei* adults of the treatments and of controls survived till the fifth day of the monitoring. Hence, it may be concluded that both the aqueous extracts prepared from crushed plant substances (used in the first trial) and concentrated extracts (used in the second trial) proved to be actually harmless towards the adults of *C. mildei*.

The tests conducted on *C. mildei* spiderlings using only the concentrated extracts induced a very low mortality (an individual treated with *M. azedarach* died on the fifth day, and another one treated with soap died on the fourth day) thus showing that these substances are not toxic to the immature forms of the spider.

Conclusions

The investigations on the efficacy of the extracts of *A. cherimola* Mill., *A. absinthium* L., *M. azedarach* L. and *Quassia* spp. towards the two pests showed that none of these substances showed a satisfactory adulticidal effect on *F. occidentalis*, whereas only *A. absinthium* was found to have an insecticidal effect on *M. unicostata*. As to the side effects of extracts it resulted that both *A. cherimola* and *A. absinthium* were very toxic to the Anthocoridae *Orius spp.*, whereas none of the tested substances showed a significant mortality on the spider *C. mildei*. *A. absinthium* was the unique product that showed a repellent effect only towards *F. occidentalis*. Concentrated extracts have shown a greater efficacy than the aqueous solutions towards *M. unicostata* and *Orius* spp.

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Monitoring of the AP subtypes spread in Trentino

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Abstract: Apple Proliferation (AP) is a phytoplasma-associated disease spread in all countries of central and southern Europe. In 2000, we demonstrated that *Cacopsylla picta* was a vector of the phytoplasma in the apple growing area of Trentino. Later, one goal of our research was to estimate the genetic variability of a large number of AP isolates from naturally AP-infected apple trees and insect samples collected around the same region. Plant sampling included different cultivars and rootstock-scion combinations as well as apple trees showing AP symptoms since different times. Thousands of psyllids were gathered periodically in orchards and uncultivated areas. PCR-RFLP analysis was carryed out to establish the AP subgroups using the DNA extracted from the main nervation of leaves and from groups composed by 10 insects split up by species and development stages. The analysis produced three different RFLP profiles for the local AP population with hardly any sample belonging to the subtype AP. We noticed a correlation between AT2 type and the sub-area of origin for plant and insect samples.

Keywords: sub-type, psyllids, PCR, RFLP, apple cultivars, rootstock.

Introduction

Apple Proliferation (AP) is a disease caused by a phytoplasma which is spread in the main European fruit areas. In our region (Trentino) the common symptoms of AP have been observed since fifty years (Rui, 1950). More recently AP disorder severely spread in Val di Non and Val di Sole valleys where it struck the cv. Golden delicious, Florina and Renetta Canada grafted on various rootstocks (Vindimian & Delaiti, 1996).

It was proved that psyllid *C. picta* plays a role as a vector of the AP in this area (Frisinghelli *et al.*, 2000), therefore we started a plan to assess the frequency of AP hosting insects based on molecular detection.

AP phytoplasma is in a close phylogenetic association with Pear Decline (PD) and European Stone Fruit Yellows (ESFY) phytoplasmas, which are classified in the same AP cluster (Seemüller *et al.*, 1998). In 2000 Jarausch et al. characterized two point mutations in a non-ribosomal DNA region, so defying three different strains of the AP phytoplasma, AT1, AT2 and AP.

We applied this method to describe the strain composition of AP population in Trentino looking for correlation with the cultivars, the period of symptoms appearance and the type of rootstock.

Materials and methods

Leaves of plants showing the symptoms of the disease and psyllids were picked up periodically in orchards and uncultivated fields in 7 different areas of Trentino as reported in Table 1.

PCR analysis was performed to detect AP. Nucleic acids were isolated from the main nervation of leaves (Doyle & Doyle, 1990) and from groups composed by 10 insects split up by species and developmental stages (overwintering adult, youth form, adult of new generation).

A first PCR amplification carried out with primer pair AP8/AP10 was followed by a nested-PCR with primer pair AP13/AP10 (Jarausch *et al.*, 2000).

Afterwards amplification products (6 µl) were digested with BspH I and Hinc II restriction enzymes (according to the manufacturer's instructions of New England BioLabs) and their digests were analysed by 2% agarose gel electrophoresis.

Results

The Table 1 reports the percentage of samples resulted positive to AP. Not all the plants showing typical AP symptoms generated the PCR fragment with primers AP13/AP10 even if they were positive to the test with primers fAT/rAS (Smart *et al.*, 1996).

Table 1. Samples of plants and psyllids picked up in 7 main areas of Trentino and their percentage of infection to AP phytoplasma.

Area	Symptomatic plants (n)	Infected samples (%)	Samples of psyllids (n)	Infected samples (%)
1- Val di Sole	30	80,0	78	14,1
2- Val di Non	92	79,3	545	21,8
3- Val di Cembra	2	100,0	10	0,0
4- Val d'Adige	15	73,3	283	9,9
5- Valsugana	16	93,8	38	7,9
6- Val del Sarca	12	75,0	21	4,8
7- Bleggio	10	80,0	6	0,0

One hundred forty two plants and 160 samples of insect AP positives were used to estimate the genetic variability of the local population of AP phytoplasma by RFLP analysis.

AT1 and AT2 strains were the most frequent type found in the apple tree samples. The AP type was present only in one plant of the Renetta Canada cultivar. In fig. 1 the distribution of AP sub-types is reported for each investigated area.

Results related to the rootstocks and the time of appearance of symptoms in the plants are showed in fig. 2 and fig. 3.

AT2 strain was identified in 108 batches of *C. picta* while only 3 and 1 samples of this insect species were infected with AT1 and AP sub-type respectively. About 54% of *C. melanoneura* samples were AT2 positive while AT1 has been detected in 46% of the cases.

As far as the developmental stages, only overwintering adults presented AT1 (7,3%) whereas the other stages showed AT2 sub-type only.

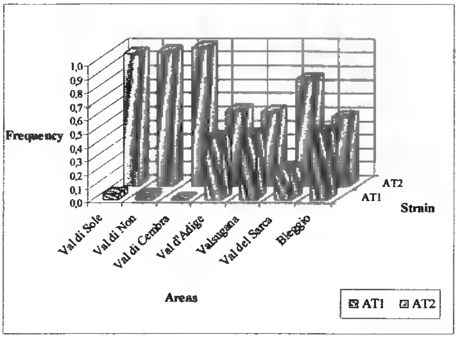


Fig. 1. Distribution of AT1 and AT2 strains in apple trees in the different investigated areas.

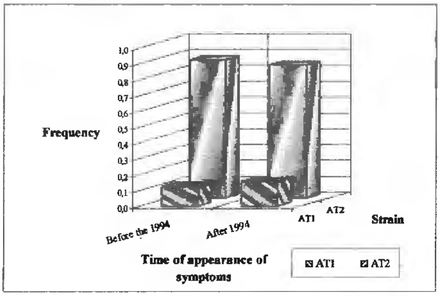


Fig. 2. Distribution of AT1 and AT2 strains in apple trees grafted on different rootstocks.

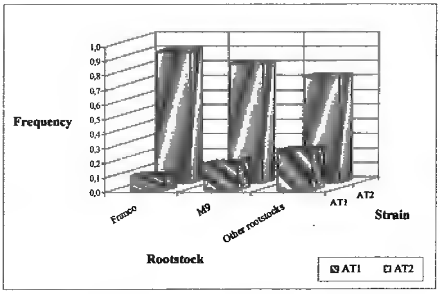
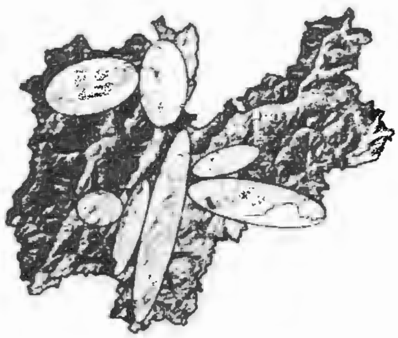


Fig. 3. Distribution of AT1 and AT2 strains in apple trees that showed the symptoms of disease before or after 1994.



Conclusions

We didn't see any connection between the period of first symptoms appearing and the frequency of AP sub-type or with the type of rootstock while it seemed more evident a high correlation between the strain and the geographic area. Starting from North towards South the increasing of AT1 frequency reached the ratio 1:1 with AT2 (Fig. 4).
Fig. 4. Location of sampled area in Trentino. 1= Val di Sole, 2= Val di Non, 3= Val di Cembra, 4= Val d'Adige, 5= Valsugana, 6= Val del Sarca, 7= Bleggio.

C. picta overwintering was found more infected than *C. melanoneura*. We suppose that after the winter period *C. picta* is already infected when it comes back to apple trees while *C. melanoneura* acquires the AP phytoplasma later. The few presence of phytoplasma in insects of new generation could be bound up with the time required for the incubation of the phytoplasma and the time of gathering the insects. The analysis of the variability of the population of the phytoplasma in the psyllids confirms the correlation between the strain and the geographic area found with plants.

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Resistance rootstocks as a strategy to control AP disease

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Abstract: Research on phytoplasma resistance started about 20 years ago following the observation that the phytoplasmas causing apple proliferation (AP) and pear decline cannot persist in the aerial parts of the tree under German climatic conditions. They can only overwinter in the roots. From this finding it was concluded that both diseases can be controlled by the use of resistant rootstocks. Resistance to AP was found in some experimental stocks based on crosses between the apomictic Malus species *M. sieboldii* and *M. sargentii* with genotypes of *M. domestica*, the cultivated apple. In order to understand the genetic background and inheritance of resistance, we started a cooperative genetic program over a three-year period based on several crosses between apomictic resistant and M. domestica susceptible parents. The effort involved the Istituto Agrario di San Michele all'Adige (I), the Centrum Grüne Gentechnik, Neustadt an der Weinstrasse (D) and the Biologische Bundesanstalt für Land – und Forstwirtschaft, Dossenheim (D). Different approaches were followed to evaluate the compatibility of apomicts with *M. x domestica* genotypes and to produce information about the genetic composition of the progenies. First conclusions based on 750 seedlings from all major populations are reported.

Key words: apple proliferation, molecular markers, ploidy level, apomictic rootstocks.

Introduction

Apple proliferation (AP) resistance was discovered in some experimental apomictic apple rootstocks obtained in crosses of *Malus domestica* or *M. domestica*-related genotypes with the apomictic species *Malus sieboldii* or *Malus sargentii*. Trees on these rootstocks show, following experimental inoculation, mild symptoms for 1 or 2 years and then recover (Seemüller et al., 1992). In contrast, all trees on established or more recent rootstocks which all are largely based on *M. domestica* or *M. domestica*-related species are much more affected by the disease and do not or only temporarily recover.

The genetic background and inheritance of resistance are poorly understood. Thus, the aim of our breeding work is to genetically characterize apomictic rootstocks, identify genetic markers linked to resistance and develop resistant rootstocks that are superior to the existing apomictic rootstock selections which are often too vigorous and mediate lower yields than the standard stock M 9. As very little was known about compatibility of apomictics with *M. x domestica* genotypes and about the genetic composition of the progenies, a wide range of crosses made over a three-years period was characterized with DNA markers. Moreover, a study was started in order to establish the ploidy level of some selections and the vitality of their pollen.

Material and methods

Plant material

We used wild *Malus* species, apomictic selections and M9 stocks produced and maintained at BBA in Dossenheim by Prof. E. Seemüller. The wild apomictic *M. sieboldii* and *M. sargentii* are polyploid while the progenies resistant to Apple Proliferation selected from their past crosses e.g. H0909 are supposed to be allopolyploid. M9 is a diploid rootstock widely employed in apple orchards but susceptible to AP. It was used as a parent in the majority of the cross combinations planned in this study.

Molecular markers

Genomic DNA was extracted from leaf tissue and PCR amplified at selected SSR loci. Microsatellite fragment sizes were determined by capillary electrophoresis applying Genescan and Genotyper software with the ABI 3100 sequencer (Bisognin et al. 2003). The pattern of segregation of SSR alleles was used to assign the progenies to three different classes: 1) true recombinant hybrids, 2) hybrid having the full complement of the alleles of the mother plus one allele added from the sexual pollen parent, and 3) mother like genotypes on which no allele from the male parent were detected.

Flowcytometry

In order to establish the ploidy level of the parents and the progenies and thus validate the classification of the seedlings based on the SSR analysis using an independent technique, nuclei were isolated from young leaves of in vitro plants, added to DAPI fluorochrome and processed with a FACStar Plus flow cytometer thanks to the collaboration with S. Citterio and Prof. Sgorbati of Milano Bicocca University.

Pollen vitality test

A study of the apomictic apple pollen was started to evaluate the vitality, compared to other apple varieties. This analysis was done in collaboration with C. Cristofolini and E. Gottardini (IASMA). Following the method reported in Shivann and Rangaswamy (1992) which is based on the reduction of tetrazol salt (TTC) due to the respiratory activity, pollen vitality is revealed by its red colouring.

Results and discussion

All parents and progenies were examined using SSR markers. A set of primer pairs (Gianfranceschi et al., 1996; Liebhard et al., 2002) was applied in order to find segregating polymorphisms suitable to establish the most likely pedigree of each progeny (Tab. 1).

This method allowed genetic characterization of the plant material. In particular, significant information of genome composition of the seedlings could be obtained that enabled classification of the progenies in the major categories 'true hybrids', 'hybrids' and 'mother-like' (apomictic) plants. The results obtained through the SSR tests (Table 2) are thus an important contribution to understand inheritance in apomicts.

Table 1. Microsatellite loci analysed for the determination of the pedigree of the progenies obtained from different crosses made in 2001 and 2002

	SSR markers								
Cross	CH01 b11	CH01 c11	CH01 f02	CH01 f03b	CH02 a08	CH02 c02a	CH02 h07	CH04 g07	CH05 e06
4551 x M 9	√		√			√			√
4608 x M 9	√					√		√	
H0909 x M 9	√	√			√	√			√
M. sieb. x M 9					√				
D2212 x M 9			√					√	
M 9 x D2212			√					√	
M 9 x 4551	√	√						√	√

Table 2. Characterization of seedlings from the main crosses carried out in years 2001, 2002 and 2003 based on the SSR markers pattern of segregation

CROSS COMBINATION	*CLASSES OF SEEDLINGS*		
	mother like	hybrid	true hybrid
1. Malus sieboldii * M 9	76,4 %	19,6 %	4,0 %
2. Malus sieboldii * M 27	88.8 %	11,2 %	0,0 %
3. Malus sieboldii * R. gala	63,1 %	18,9 %	18,0 %
4. Malus sargentii * M 9	84,7 %	12,7 %	2,6 %
5. apom. selection 4551 * M 9	56,2 %	43,8 %	0,0 %
6. apom. selection 4608 * M 9	54,0 %	46,0 %	0,0 %
7. apom. selection D2212 * M 9	74,6 %	25,4 %	0,0 %
8. apom. selection H0909 * M 9	54,7 %	24,6 %	20,7 %
9. M 9 * apom. selection 4551	85,7 %	0,0 %	14,3 %
10. M 9 * apom. selection D2212	32,1 %	0,0 %	67,9 %

Substantial numbers of true hybrids could be obtained in the crossing combinations 8 and 10, the progenies of which are planned to be used in future genetic studies on the inheritance and nature of AP resistance. On the other hand, hybrids which exhibit a good resistance against AP are interesting parents for further breeding programs.

To better understand inheritance of resistance in apomictic *Malus* genotypes, the SSR data have been complemented by information on the ploidy status of both parents and progenies. First flow cytometric results for the parental lines (Table 3) were in agreement with the pattern of inheritance deduced from molecular typing of the seedling populations.

Table 3. Ploidy level of parental lines as determined using the FCM analysis

Genotype	*Ploidy level*
M 9	2n
R. gala	2n
M. sieboldii	4n
D2212	4n
H0909	4n

Suitability of the selected genotypes as parents differed significantly. The results not only depended on the genetic background of the partners including ploidy level but also whether apomicts were used as female or male parents. First results on pollen evaluation (Fig. 1) confirmed previous information that the apomictic pollen is much less vital than pollen from *M. domestica* varieties. This may explain in part the poor fruit set in crossings were *M. domestica* genotypes were pollinated with apomictic pollen.

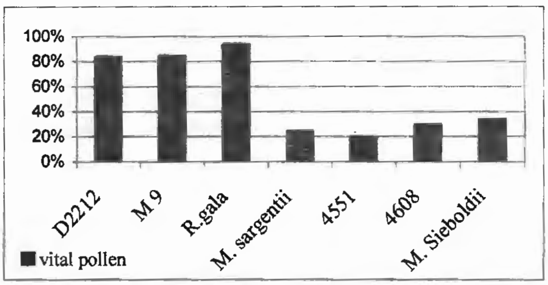

Figure 1. Percentage of vital pollen observed for each parental line

Conclusions

Our planned genetic program requires the availability of progenies from crosses between resistant and susceptible parents. In order to obtain such progenies, crosses between resistant apomictic parents and susceptible *M. domestica* genotypes were made over a three-years period.

Some of the crossing combinations tested in the breeding with apomicts proved to be unsatisfactory due to lacking compatibility and/or unsuitable pollen properties. From other combinations the resulting progenies consisted of many seedlings which, however, contained no or only few true hybrids. Substantial numbers of true hybrids could be obtained in two crossing combinations, the progenies of which are planned to be exploited in future studies on the inheritance and nature of AP resistance.

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The use of a 'visual+temperature' method in timing of cherry fruit fly (*Rhagoletis cerasi* L.) control

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Abstract: Cherry fruit fly (*Rhagoletis cerasi* L.) is recently one of the most important pests in European cherry or morello-cherry plantings (Skaar et al. 1999, Jaastad et al. 1999, etc.). Analogous to other fruit boring insects this species is difficult to control because of the short time interval during which the hatching larvae can be targeted before they become protected by the fruit tissues. The effectiveness of spraying depends on accurate assessment of the application date. Larvicides should be applied when the black spots are visible on the eggs. For this purpose the monitoring of egg laying and laboratory observation of embryonic development must be carried out. Although this way of timing is exact and reliable it can appear difficult and time-consuming for some growers.

That is the reason why we have investigated the possibility of using a more practical approach in the protection against cherry fruit fly. Our timing model is based on the observation of cumulative temperature sums above 10 °C coupled with monitoring of pest flight. Temperatures are recorded from the beginning of continuous daily catches of adults found on yellow sticky traps. Sweet cherries should be sprayed when the temperature sums rise up to 2200 hourly degrees while the sums calculated for treatment of morello cherries correspond to 3000 HD. However, owing to exclusion of formothion from spectrum of pesticides in the Czech Republic and to frequent use of active ingredients with shorter residual effect (thiacloprid, acetamiprid, fenitrothion, etc.) a second application is usually needed after 10 -14 days (but respecting pre-harvest intervals of applied chemicals) to avoid the occurrence of larvae in fruits.

Key words: sweet cherry, morello cherry, cherry fruit fly, chemical control

Introduction

The most of cherry growers does not go without chemical protection against cherry fruit fly in the recent market conditions. Although the alternative methods of *Rhagoletis cerasi* control have been studied since early 80s of the last century (e.g. Boller and Remund 1980) the protection is more or less dependent on insecticide treatments. These are necessary especially in the cases of plantings situated in the neighbourhood of extremely infested plots (private gardens or other untreated plots). Recently, one of the most effective way appears to be the rational use of environment friendly pesticides showing excellent in-depth larvicide effect. However, treatment timing must be very precise even if such preparations used because the larvae are most sensitive to insecticide application only close before or after their hatching from eggs. Therefore the aim of this research was to investigate flight activity and embryonic development of cherry fruit fly in relation to temperature conditions. According to this model the optimum time of insecticide applications can be predicted.

Material and methods

The development of the 'visual+temperature' method was realised on the base of four research activities carried out during the experimental period (1997-2000):

1) Monitoring of the beginning of adult flight. For this purpose the yellow sticky traps treated with long life transparent glue 'Bird Tanglefoot' were used. Three of these traps were installed in the orchards before hatching of adult flies i.e. in the early May. This date corresponds to our long-term experience with cherry fruit fly activity in climate conditions of Holovousy (320 m, 8.3 °C). The traps were placed on the south edge of crowns of the 'Kordia' cv. plantings kept free of chemical treatments against cherry fruit fly. Our daily observations started immediately after the trap installation and we carried on until beginning of continuous pest flight.
2) Calculation of temperature sum for harmfull emergence of eggs in fruits. During the period of continuous pest flight 100 fruits were sampled on the south edges of crowns. The percentage of pest eggs in fruits was discovered under binocular microscope. Samples were collected every day until infestation higher than 1% appeared. The temperature sums were obtained from automatic meterological station 'METEOS-3' having been installed cca 300 m from experimental plot. The output of the appropriate software were sums of effective temperatures (SET) above 10°C in hourly degrees. On the base of these sums the period between flight beginning and the emergence of economicaly harmful fruit infestation was specified.
3) Assessment of the termic constant for embryonic development of cherry fruit fly. The larvae needed for trials were collected from attacked fruits in the coarse of foregoing summer. After larvae pupation the puparia (200-300 pcs) were placed into isolator. In the following year the hatching flies (both males and fe-males) were removed from isolator and put into insectary. During their breeding in the insectary the flies were kept on artificial diet (water + honey solution). The adults were left here for 10 days without possibility of egg laying. After these period the fruit bearing cherry branches were exposed to fe-males incubated in the temperature 23-25°C. After 30 minute exposition the fruits were removed and placed under binocular. Sample consisting of 10 infested fruits was sélected. The fruit skin was carefully detached to made visual observation of eggs possible. The tips of fruit pedicles were placed into Erlenmayer flask filled with water to avoid drying up of the samples. Finally, the samples were placed into meterological box we had installed on the experimental plot. The egg development was observed every day 2x (morning, afternoon) until larvae hatched from eggs. The data needed for the calculation of sums were obtained from the meteorological station 'METEOS-3'. The sums for embryonic development were calculated as SET >10°C measured every year from egg laing to larvae hatching in nature conditions.
4) Verification of the first treatment timing universal for cherries and morello-cherries. For this purpose beginning of larvae hatching in cherries (see 3) and in morello-cherries ('Morela pozdní') was assessed. The method of observation and the data processing are described in 2 and 3. SET values measured for the both of crops were compared and the termic constant representing period from egg laing to hatching of larvae in fruits of cherries or morello-cherries was assessed.

Results and discussion

The 'visual+temperature' method of treatment timing in protection cherries and morello-cherries against cherry fruit fly is based on the use of yellow sticky traps and on the measurement of cumulative sums of effective temperatures (SET) above 10 °C in hourly degrees (HD).

Yellow visual traps were used to monitoring of pets flight. We have found out that the discontinuous or sporadic flight activity can be observed before the major part of the pest population appears in orchards. These discontinuous catches have not exceeded thresholds of economic harmfulness (=2 catches/trap and day). On the contrary, the first harmful emergence of cherry fruit fly adults (>>2 catches/trap and day) coincided with the beginning of continuous flight activity. Described temperature model is functionable provided the SET values we have calculated (table 1, figure 1) are related to this date (i.e. to the beginning of continuous flight).

Another aspect we had to take into consideration was the assessment of the SET needed for beginning of egg laying. This value corresponds approximatelly to 400 HD (table 1). In addition, the termic constant for embryonic development was calculated. It was measured that the length of development cherry fruit fly larvae in eggs is cca 1800 hourly degrees (table 1). The total SET value corresponding to beginning of larvae hatching in sweet cherries is 2200 HD from the start of continuous pest flight (figure 1). In comparison with sweet cherries the tha larvae in morello-cherries are hatching by 800 HD later. The SET value for treatment of morello-cherries is calculated as follows: 400 HD (beginning of egg laying) + 1800 HD (embryonic development) + 800 HD (delay of egg laying in morello-cherries) = 3000 HD (figure 1).

The insecticides legally used against cherry fruit fly (thiacloprid, acetamiprid, fenitrothion) provide maximum 8 to 12 day protection. After this period the second treatment must be done depending on weather conditions, infestation and respecting pre-harvest intervals. The whole model of the 'visual+temperature' treatment timing is presented in figure 1.

Table 1. Sums of effective temperatures above 10°C (in hourly degrees) for the period from the beginning of continuous *Rhagoletis cerasi* flight to egg laying (A) and embryonic development of the pest (B) measured in Holovousy, the Czech Republic

Year	A) Flight beginning-egg laying (HD)	B) Length of embryonic development (HD)
1997	398	1781
1998	432	1777
1999	400	1791
2000	364	1787
Mean	**398.4**	**1784.0**

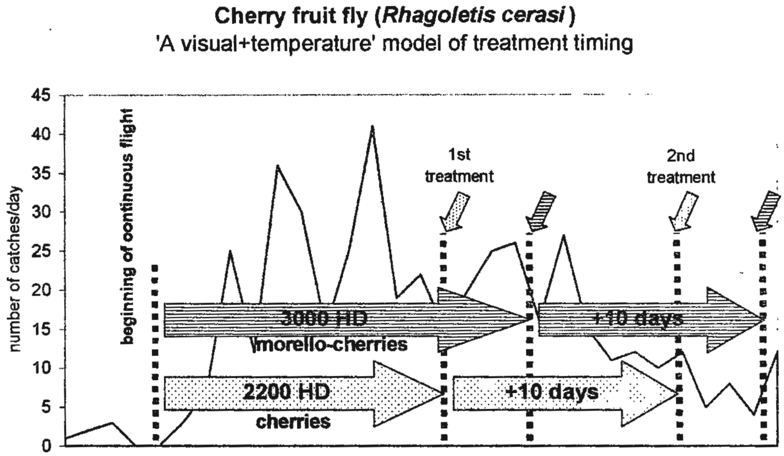

Figure1. Model of a 'visually+ temperature' timing of treatments against cherry fruit fly.

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Control of phytophagous mites on strawberry in Europe by predatory phytoseiid mites or heat treatment

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Abstract: *Tetranychus urticae*, the two spotted spider mite, and *Phytonemus pallidus* the strawberry tarsonemid mite are important pests of strawberry throughout Europe. Experiments in UK showed that the phytoseiid mites *Neoseiulus californicus* and *N. cucumeris* effectively controlled *P. pallidus*, and that *N. californicus*, and to a lesser extent *N. cucumeris*, could also control *T. urticae*. Trials in Norway and Finland with *N. cucumeris* also gave promising results. In Belgium *T. urticae* were controlled for 5 consecutive years by naturally occurring *N. californicus*. In Finland, where the effects of various biological mulching materials on *P. pallidus* control were tested in combination with predatory mite releases, best control was achieved in buckwheat husk and green mass mulch. Treating cold stored plants with 30 min warming up plus 42 min at 43°C in hot air killed all *P. pallidus* present. The treatment had no serious effect on plant vigour on green plants but diminished the number of flowers produced by cold stored plants.

Keywords: *Tetranychus urticae, Phytonemus pallidus,* phytoseiids, mulches, heat treatment

Introduction

The two-spotted spider mite, *Tetranychus urticae*, and the strawberry tarsonemid mite, *Phytonemus (Tarsonemus) pallidus*, are serious and common pests of strawberry throughout Europe and can cause reductions in yield when population densities are high. There are a restricted number of pesticides approved for control of these pests so effective alternative control methods are needed. The experiments reported in this paper were undertaken to determine the effectiveness of the predatory phytoseiid mites *Neoseiulus cucumeris, N. californicus, Anthoseius rhenanus* and *Euseius finlandicus* as biocontrol agents for the two pest species. The effect of different mulches on subsequent biocontrol was also investigated.

As an alternative to control using predators, hot air treatments were used on planting material in an attempt to achieve 100% mortality of *P. pallidus* without damaging the plants. The tolerance of *P. pallidus* to heat is described by Hellqvist (2002).

Methods

Phytoseiids to control T. urticae

1. UK. In 2000, *N. californicus* naturally colonised a strawberry planting at East Malling. A pesticide application was used to reduce numbers of *N. californicus* in half the experimental plots; the pesticide used was not toxic to *T. urticae*. Numbers of *T. urticae* and *N. californicus* were counted on samples of 25 leaves per plot 11 days after pesticide treatment. In a potted

plant experiment in the glasshouse release rates of 2 or 4 *N. californicus* were compared with no-release for control of *T. urticae*. All mites present were counted 41 days after predator release by inspecting every leaf under a stereo microscope mounted on a stand.
2. Belgium. During an acaricide efficacy trial in 1997, *N. californicus* were found naturally colonising the strawberry planting. From 1998-2002 leaf samples were taken during the growing season from different strawberry plantings at Gorsem, and phytoseiids identified and counted. Numbers of *T. urticae* were also recorded.

***Phytoseiids to control* P. pallidus**
1. UK. Potted strawberry plants were infested with *P. pallidus* in July and grown in a glasshouse. In early August plants in treatment 1 received 30 *N. cucumeris* per plant, treatment 2 30 *N. californicus* per plant and treatment 3 no predators. All leaves were removed 26 days after release of predators and mites present counted under a stereo microscope. In a separate potted plant experiment the effect of release ratios of 1:10, 1:20 and 1:40 *N. cucumeris* to *P. pallidus* were determined. Plants were sampled as above 28 days after predator release.
2. Norway. Four plots (3 rows x 10 m) were used in each of seven fields (3 organic + 4 conventional), and *N. cucumeris* were released in May-June in two of the plots, randomly chosen, in each field. Release rates were approximately 100 mites per metre single row, except for one conventional unsprayed field, which received 1000 *N. cucumeris* per metre single row. *Phytonemus pallidus* were sampled before, and one and two months after release, on samples of 30 folded leaflets per plot.
3. Finland. Plants were infested with *P. pallidus* in the planting year 2000. *Neoseiulus cucumeris* (13.4/plant), *Anthoseius rhenanus* (4.5/plant) and *Euseius finlandicus* (9/plant) were introduced in May-June 2001. In 2002, only *N. cucumeris* was introduced (14/plant). Unopened small leaves, 10/plot, were collected and mites counted at approximately 3 week intervals in May-September 2001 and 2002.
Hot air method to control P. pallidus
A growing chamber was used for the hot air treatment. Treatment time varied between 30-90 min, temperature between 40-47°C, and relative humidity 80-100%. Runner cuttings, cold stored potted and bare rooted plants, and potted runner plants were tested. The plants were infested with 50-100 *P. pallidus* two-three weeks before treatment. One and four weeks after treatments live mites were counted from young unopened leaves. Effects of the treatments on plant vigour and yield components were determined.

Results

***Phytoseiids to control* T. urticae**
1. UK. The pesticide applied did not remove all *N. californicus* (Table 1) but differences between treatments were significant. Numbers of *T. urticae* were significantly lower in the plots that had larger numbers of *N. californicus*. In the glasshouse experiment both rates of *N. californicus* release significantly reduced *T. urticae* numbers; mean numbers of active stages 41 days after release were 230.1, 85.8 and 54.6 in the 0, 2 and 4 *N. californicus* per plant treatments.

Table 1: Control of *T. urticae* with *N. californicus* in field grown strawberry 11 days after treatment with pesticide

Treatment	Mean nos *T. urticae*		Mean nos phytoseiids	
	eggs	actives	eggs	actives
N. californicus present	38.9	26.8	5.0	1.72
N. californicus removed	100.0	94.2	1.3	0.82
P value	<0.01	<0.05	<0.05	<0.01

2. Belgium. High numbers of *T. urticae* (250 per leaf) were present in July 1997. By August *N. californicus* had increased to 3.5 per leaf, and *T. urticae* numbers were eliminated from the planting. In 1999 *T. urticae* numbers increased to 36 per leaf in early June. This was followed by an increase in *N. californicus* to 1.4 per leaf, and subsequent reduction of the pest. In 2000 there were very low numbers of *T. urticae* on all plantings, possibly due to the cold and humid spring. There were also few *N. californicus*. In 2001 in May *T. urticae* were present at a maximum of 4.5 per leaf, and *N. californicus* kept numbers low throughout the season.

***Phytoseiids to control* P. pallidus**

1. UK. Numbers of *P. pallidus* were significantly reduced by predator release. Numbers of active stages 26 days after release were 49.3 per plant in the *N. cucumeris* treatment, 30.2 in the *N. californicus* treatment and 229.8 in the untreated. 28 days after release of *N. cucumeris* control of *P. pallidus* was significantly better at a predator prey ratio of 1:10 (mean number of active stages 7.3 per plant) compared with 1:20 (10.5) and 1:40 (41.3).

2. Norway. Numbers of *P. pallidus* increased in all plots during the experiment. In the conventional unsprayed treatments the four plots in the field were kept unsprayed with pesticides and the rest of the field was sprayed. In the organic fields the predatory mites were able to disperse to the untreated plots more easily. In four of the experimental fields the difference between numbers of *P. pallidus* on predator release and no-release plots increased over the two-month period, indicating that *N. cucumeris* was reducing *P. pallidus* numbers. There was no reduction of pest numbers in the other treated plots. Only one of the organic fields had more than one *P. pallidus* female/leaflet at any time. The two conventional unsprayed plots with no predator release had the highest number of *P. pallidus*. In the two conventional fields where plots were sprayed before predator release it is uncertain if *N. cucumeris* established at all.

3. Finland. In 2001, peak population of *P. pallidus* in plastic mulch was 10 mobile mites/leaf, whereas in buckwheat husk (0.5/leaf) and green mass (0.9/leaf) numbers were significantly lower. Highest numbers of predatory mites, mostly *N. cucumeris*, were found in black plastic mulch, pine chips and green mass, and the lowest numbers in flax fibre mat and birch chips. *Phytonemus pallidus* was kept under control throughout 2001; this was apparently due to *N. cucumeris* releases as the two endemic species were rarely found. In 2002, *P. pallidus* increased out of control in plastic and straw mulches but was well controlled by *N. cucumeris* in green mass and buckwheat mulches, in spite of the later predator introduction.

***Hot air method to control* P. pallidus**

The first tests indicated that plants were injured at temperatures over 44°C, measured amidst vegetation. On potted runner plants warming up time plus 30-40 min treatment at 43°C killed all *P. pallidus*. The treatments injured the outer leaves, but the effect on later plant vigour was not serious. In shorter treatments some mites survived.

Table 2: Effect of heat treatment on subsequent plant growth of cold stored bare rooted plants

	No of flowers/plant	Saleable yield g/plant
Untreated	19.2a	223a
Hot air 41.5°C for 36 min	6.9b	-
Hot air 43.1°C for 34 min	-	142b
Hot water 46.0°C for 10 min	8.0b	178ab

Both hot air at 40-42°C and hot water treatments at 46°C reduced numbers of flowers of bare-rooted cold stored plants (Table 2); there was no significant effect on flowers in potted cold stored plants. Longer exposures at 42-43°C reduced yield in both bare rooted and potted cold stored plants.

Discussion

Results of experiments in four European countries demonstrated the effectiveness of biocontrol of two pest mite species on strawberry using predatory phytoseiid mites. Other experiments in UK not reported here are described in Easterbrook *et al.*, 2001. In Norway, *N. cucumeris* release was as cost effective as spraying with methiocarb (Mesurol), the only insecticide approved for use against *P. pallidus* in that country.

An alternative technique using hot air to eradicate *P. pallidus* from planting material was shown to be as effective as the hot water treatment and could be used on potted plants. However, some loss of yield was noticed in the year of planting (Tuovinen, 2000).

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Prey preferences of *Anthocoris nemoralis* and *A. nemorum* (Heteroptera: Anthocoridae) and their predation behaviour towards Pear psyllid, *Cacopsylla pyri*

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Abstract: Prey preferences of the two anthocorids *Anthocoris nemoralis* and *A. nemorum* were tested in a series of two-choice experiments to evaluate their preference for young pear psyllid, *Cacopsylla pyri* or apple psyllid, *C. mali*, green apple aphid, *Aphis mali* nymphs and female fruit tree red spider mites, *Panonychus ulmi*. Preferences were assessed in terms of the number of prey eaten and biomass consumed. The predation behaviour of the two anthocorids towards 4th instar pear psyllid was observed in a separate experiment. *Anthocoris nemoralis* preferred pear psyllid to green apple aphid. In contrast *A. nemorum* preferred green apple aphid to pear psyllid. Both anthocorids preferred psyllids to female fruit tree spider mites. *A. nemoralis*' predation on spider mites was not increased by prior exposure to this prey. Though preference for female spider mites was low, predation on spider mites may be important in the absence of more attractive prey or with younger instars of spider mites. Observations of predation behaviour towards pear psyllids showed that *A. nemoralis*, the smaller of the two anthocorids, had a higher success rate. Results stress that *A. nemoralis* is of special relevance for biological control in pear, where pear psylla is an important pest, whereas *A. nemorum*, which is the most common of the two in apple, is the more relevant to consider for augmentative releases in apple.

Key words: anthocorid, pear psyllid, apple psyllid, green apple aphid, spider mite, preference, behaviour

Introduction

Anthocoris nemorum (L.) and *A. nemoralis* (Fabricius) are important predators in apple and pear orchards. They are polyphagous, preying on aphids, mites, psyllids and lepidopteran eggs and young larvae (Hill, 1957; Anderson, 1962; Collyer, 1967; Solomon, 1982; Solomon *et al.*, 2000). *A. nemoralis* is particularly important in controlling pear psyllids (Solomon *et al.*, 2000). *Cacopsylla pyri* L. (Homoptera: Psyllidae) the most common in Denmark, is a preferred and high quality prey for *A. nemoralis* (Dempster, 1963; Fauvel *et al.*, 1984). *A. nemorum* has been considered to show little preference for any prey, but a recent study documented differences in its preferences for some aphids of importance in greenhouses (Meyling *et al.*, 2003). This paper presents data on the two anthocorids' preference for different orchard pests and a study of their predation behaviour. Knowledge of prey preferences is of importance to be able to predict their predation in orchards.

Materials and methods

Anthocoris nemorum females were collected from organic orchards and adjacent habitats five to ten days prior to the experiments and were kept in thermo cabinets (L16:D8 photoperiod, 20 ± 1°C) until use in assays. *Anthocoris nemoralis* were laboratory reared in thermo cabinets under the same light and temperature regime. Anthocorids were kept in transparent plastic cages 7 cm in diameter and 8 cm high. Leaves of *Pilea peperomiodies* Diel (Urticaceae) were provided for oviposition and additional moisture. Twice a week, fresh leaves were provided and old leaves removed. Leaves with eggs were moved to new cages ensuring equal age cohorts in individual cages. At the same times *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) eggs were provided. A maximum of ten large nymphs or adult anthocorids were kept in each cage. In all experiments field collected *A. nemorum* of unknown age were used. In the case of *A. nemoralis* one week old individuals were used.

C. pyri was collected from a greenhouse rearing established that year. Apple branches with apple psyllid, *C. mali*, eggs and young nymphs, were field collected and kept in the greenhouse prior to experiments. Green apple aphids, *Aphis mali* were obtained from a laboratory culture established from field collected individuals a month prior to experiments and reared on pear leaves. Fruit tree red spider mites, *Panonychus ulmi*, were field collected.

Preference was assessed in small units (30 ml plastic cups). To provide water and support for pear leaves the base of the cages were covered with 3% agar, into which the petiole was inserted. Fifteeen individuals of each prey were used in preference experiments, except in the experiment with *C. mali* where 20 individuals were used. Prey was allowed to settle for at least 20 min. before a predator was introduced. Instars of prey species were selected to obtain comparable sizes. Experimental durations were 1h or 3h, adjusted after prey consumption. Preferences were assessed in terms of the number of prey eaten and biomass consumed. Anthocorids were starved for 24h prior to experiments. Controls were used to assess mortality in the absence of predators. Low control mortality allowed analysis without corrections.

Preference experiments included: a) The two anthocorids preference for 1-2rd instar *C. pyri* and *A. mali* (3h), both n = 15. b) *A. nemoralis*' preference for female *P. ulmi* and 1-2nd instar *C. pyri* (1h) (n = 19). This experiment was later repeated with c) half the *A. nemoralis* having had 24-h exposure to surplus *P. ulmi* prior to the 24-h starvation period (n = 12). d) A comparison of *A. nemorum's* preference for 1-2nd instar *C. mali* and female *P. ulmi* (3h) (n = 9). d) Stage-specific preference of *A. nemoralis* for eggs or young 1-2nd instar nymphs of *C. pyri* (1h) (n = 11).

Predation behaviour of the two anthocorid species towards *C. pyri* was observed in cages measuring 30 × 30 × 60 cm (*A. nemorum*: n = 15, *A. nemoralis*: n = 13). Cages were transparent with a netted sliding door of 30 × 60 cm. In each cage a fresh-cut pear twig with three fully developed leaves was placed. Securing the twig with the basal part through a hole in the lid of a water-filled jar provided support and water. The twig was infested with 20 4th instar *C. pyri*. By the onset of the experiment one 24-h starved adult female anthocorid was carefully released onto the twig. Each anthocorid was observed for at least one hour. After an hour any ongoing feeding was observed until it ended to assess the full duration of feeding. Observations ceased if an anthocorid left the plant.

Results

Anthocoris nemoralis ate significantly more *C. pyri* than *A. mali*, while *A. nemorum* killed (dead + eaten) more green apple aphid than pear psylla (both, Wilcoxon, $p < 0.05$) with significant difference between the proportions killed by the two species (t-test, $p < 0.05$) (Figure 1). In terms of biomass *A. nemoralis* ate an average of 0.04 mg *C. pyri* and no *A. mali* while the slightly larger *A. nemorum* ate an average of 0.02 mg *C. pyri* and 0.03 mg *A. mali*.

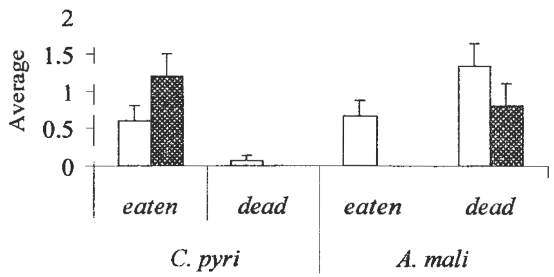

Figure 1. Average number (mean ± SE) of *C. pyri* and *A. mali* eaten and dead in preference experiment with *A. nemorum* (white) and *A. nemoralis* (grey).

Anthocoris nemoralis preferred *C. pyri* nymphs with 3.5 ± 0.4 killed to female *P. ulmi* (0.6 ± 0.2) (Wilcoxon, $p < 0.05$). In terms of biomass consumed the preference for nymphs was even stronger, as nymphs were approximately four times heavier than the spider mites. Adding surplus spider mites to *A. nemoralis*' diet for 24h before the 24h starvation period did not lead to increased predation. Though experienced *A. nemoralis* killed 0.8 ± 0.2 mites and inexperienced 0.6 ± 0.2 (both n=12), the effect of experience was not significant and seemed to be the reverse in terms of prey consumed: one mite was visibly deflated by the experienced *A. nemoralis* while inexperienced females had visibly deflated four mites. Like *A. nemoralis*, *A. nemorum* preferred psyllids. It killed an average of 4.1 ± 0.7 female fruit tree spider mites and 7.9 ± 1.3 *Cacopsylla mali* (Wilcoxon, $p < 0.05$). Finally, *A. nemoralis* ate significantly more *C. pyri* eggs (5.3 ±1.1) than nymphs (2.6 ±0.6) (t-test, $P < 0.05$). However, since nymphs were heavier than eggs, the biomass consumed of nymphs was higher than that of eggs, though not significantly so.

No significant difference was found in the two anthocorids' predation rate towards 4th instar pear psyllids (*A. nemorum*: 1.6 ± 0.2 nymphs, *A. nemoralis*: 1.7 ± 0.4 nymphs). However, the success rate (prey eaten/encounters) was significantly higher for *A. nemoralis* and the handling time (minutes spent to eat one prey) almost double that found for *A. nemorum* (Figure 2) (both GLM, $p < 0.05$). The more 'restless' behaviour of *A. nemorum* was also seen from the fact that significantly more nymphs left twigs with *A. nemorum* than with *A. nemoralis*.

Discussion

While *A. nemoralis* preferred pear psyllid to green apple aphid it was the reverse for *A. nemorum*. Both anthocorids and particularly *A. nemoralis* strongly preferred psyllids to female *P. ulmi*. Prior exposure to spider mites did not increase *A. nemoralis*' preference. Though preference for female *P. ulmi* was low, predation on spider mites may be important in the absence of more attractive prey or with younger instars or eggs of spider mites. The stage specific preference of *A. nemoralis* for *C. pyri* eggs shows that this predator readily feeds on immobile prey. However, in terms of biomass it did not eat more eggs than nymphs.

Though being the smaller of the two, *A. nemoralis* had a higher success rate with the large 4th instar pear psyllids. The longer handling time may reflect a better use of prey by this predator.

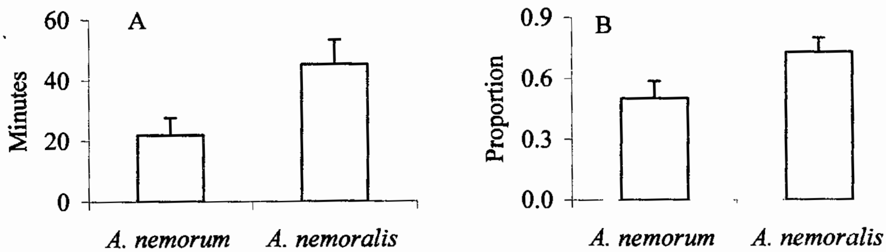

Figure 2. A. Handling time (mean ± SE) (i.e. time to eat a 4th instar *C. pyri*) of *A. nemorum* and *A. nemoralis*, B. Proportion successful encounters (± SE) with *C. pyri*.

Results stress that *A. nemoralis* is of special relevance for biological control in pear, where pear psylla is an important pest, whereas *A. nemorum*, which is the most common of the two in apple, is also the more relevant to consider for augmentative releases in apple, where aphids are a more important pest.

Acknowledgements

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The effect of different growing methods on the incidence of cane pests in Hungarian raspberry plantations

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Abstract: The most important problem in the protection of raspberry is usually cane death, which has a special group of symptoms. Our aim was to search for pests probably responsible for cane death, and we also studied the natural enemies controlling the population of the most important cane pests observed. Research was carried out in an autumn-fruiting (*Autumn Bliss*) and two traditional (*Malling Exploit* and *Fertődi Zamatos*) raspberry plantations in Nagyréde, Hungary between 2002 and 2003. The results show that the difference between the growing methods and the cultivars plays an important role in the incidence of pests. In the plantations the most frequent pest damaging canes was raspberry cane midge (*Resseliella theobaldi* Barnes). We found that the larvae of the pest were parasitized considerably. The reared parasite was a chalcidoid species belonging to the genus *Aprostocetus*. The life history of this species was also investigated. Another important pest damaging canes was rose stem girdler (*Agrilus aurichalceus* Redt.) in the plantations, but the results of our research show that the pest is less dangerous in annual growing method (*A. Bliss*). Further research is needed in connection with the parasite chalcidoid species (*Baryscapus agrilorum* Ratzeburg and *Tetrastichus heeringi* Delucchi), which were reared from the *A. aurichalceus* larvae, for their role in the control of the pest population. Other pests of raspberry canes were found to be of low significance in contributing to cane death.

Key words: raspberry, growing methods, cane death, pests, *Resseliella theobaldi*, *Agrilus aurichalceus*, cultivars, *Malling Exploit*, *Fertődi Zamatos*, *Autumn Bliss*, parasite, *Aprostocetus*

Introduction

The investigation of integrated plant protection methods to defend raspberry canes against pests and diseases has always meant an important task for researchers and growers alike. Our main objective was to search for pests probably responsible for cane death, and we also studied the natural enemies controlling the population of the most important cane pests observed.

One of the most dangerous pests of raspberry canes is raspberry cane midge (*Resseliella theobaldi* Barnes). It is a well-known insect from European raspberry plantations causing extensive cane death due to the larvae feeding on first-year canes and pathogenic fungi (e.g. *Leptosphaeria coniothyrium*) invading the larval feeding sites. Woodford and Gordon (1978) made a review of the history and world-wide distribution of *R. theobaldi*. In Hungary, the pest was first reported from Fertőd, 1958 (Hódosy et al., 1964), and not long afterwards it became the main pest of raspberry plantations. Through many decades, the most popular cultivated variety was *Malling Exploit*, which is very susceptible to the midge, as it has an extremely splitting bark under which midge imagines can oviposit and larvae can develop and feed. Nowadays, as new raspberry cultivars appear in large-scale production the importance of this pest is changing, because some varieties are similarly or more, while others are much less

susceptible to the damage of *R. theobaldi* than *M. Exploit*. *Fertődi Zamatos* seems to be the most promising Hungarian cultivar in respect of cane protection against the pest and subsequent pathogens, as its bark hardly splits.
The other widely distributed and important cane pest in raspberry plantations is rose stem girdler (*Agrilus aurichalceus* Redt.). Imagines of this buprestid beetle lay eggs on canes and the larva gets into the stem after hatching and girdles the cane. Due to this feeding damage a gall-like swelling (pseudo-gall) appears on the stem above which the plant tends to dry causing a severe loss in fruit yield. The rate of damaged canes is changing year by year, but sometimes it can be very high. Kuroli (1996) reported that 64% of the canes were infested by the larvae in Mosonmagyaróvár, Northwestern Hungary, 1995.
Other pests like *Aulacaspis rosae* (Bouché), *Oecanthus pellucens* (Scop.), *Atrococcus bejbienkoi* (Kozár et Danzig) and *Lasioptera rubi* (Schrank) and their damage were also observed in the plantations.

Materials and methods

Our research was carried out in Nagyréde, one of the largest raspberry growing regions of Hungary, between 2002 and 2003. An autumn-fruiting (*Autumn Bliss*) and two traditional (*Malling Exploit* and *Fertődi Zamatos*) raspberry plantations were examined. In case of *A. Bliss* all canes are cut at the end of each vegetation period (annual growing method), while in *M. Exploit* and *F. Zamatos* there are primocanes and fructocanes every year (biennial growing method). The samples were collected for laboratory work every second week from May to September, 2002 in case of *M. Exploit*, from September to October, 2002 in case of *A. Bliss* and from May to October, 2003 in case of *A. Bliss* and *F. Zamatos*. All the samples consisted of 25–25 canes chosen at random from the plantations.
The cut canes were carried to the laboratory of the Department of Entomology where, first of all, the pests and damages well visible to the eye (e.g. pseudo-galls of *A. aurichalceus* or scales of *A. rosae* etc.) were observed and recorded. After this procedure the canes were examined more closely with the help of a stereomicroscope. The bark of the split canes was turned back with a needle to find the living, orange-coloured and died, brown, parasitized larvae of *R. theobaldi*. All types of larvae were counted. Some parasitized specimens were put aside to rear imagines of the parasitic wasp from the larvae.

Results and discussion

Observations on the larval population dynamics of* R. theobaldi *and its parasite
During the time of observations a huge number of living and parasitized midge larvae were found on the collected canes, and this number was changing considerably in the vegetation period. In the *M. Exploit* experiment (2002) it has been found that there were at least two peaks of midge larval emergence between May and September, one at the end of June and another one in the middle of August. A third, smaller peak seems to be in the second half of September. The first parasitized specimens appeared in the middle of June, and there was a smaller peak of wasp larval emergence in the middle of July and a higher one at the beginning of September. After the end of September, the number of *R. theobaldi* larvae quickly decreased, while parasitic larvae remained on canes to overwinter under the protection of skins of died midge larvae. Each died midge larva consited of one parasitic larva.
In the 2003 experiment there were some differences in the midge and wasp larval population dynamics between cv. *F. Zamatos* and *A. Bliss*. The first midge larvae appeared at the beginning of May in case of both cultivars. After that there was a peak of larval emergence at

the beginning of July and a smaller one in the middle of August in *F. Zamatos*, whlie one high peak only in August and a lower one in September in *A. Bliss*. The lack of July peak might be explained by the lower rate of split *A. Bliss* primocanes collected in this month that contained, as a consequence, small number of larvae. On the other hand, the September peak may be due to fresh, vigorous canes, typical of autumn-fruiting cultivars (e.g. *A. Bliss*), that were suitable for midge larvae to feed, opposing the strongly lignifying canes of *F. Zamatos*. Nevertheless, the peaks of larval emergences of the parasite species followed that of the pest's by about two weeks, as in case of the 2002 *M. Exploit* experiment.
Many parasite wasps were reared to identify the chalcidoid species exactly. In 1952 Pitcher referred to a hymenopterous species that parasitized considerably the midge larvae. We asked specialists to give the taxonomic position of the wasp reared by us. The identification is now in process. So far, it has turned out that the species belongs to the genus *Aprostocetus*, and might be new to the Hungarian fauna.

Observations on the damage of* A. aurichalceus *and other cane pests

In the 2002 *M. Exploit* experiment the changing rate of primocanes damaged by *A. aurichalceus* was followed continuously during the vegetation period. The first stems with pseudo-galls appeared at the end of July, and the rate of damage was 32%. From this time, the number of infested canes was increasing week by week until it reached 68% in the middle of September. At this time the rate of damaged canes was 16% in the *A. Bliss* plantation. (Note that the eight-year-old, unkempt *M. Exploit* plantation was in very bad condition, while the *A. Bliss* was a young, well-cultivated one.) In 2003, when cv. *F. Zamatos* and *A. Bliss* were observed the number of *Agrilus*-damaged canes was quite small. The highest rate was 16% again in case of *A. Bliss* in September. It is supposed that the considerable difference in the rate of damage between *M. Exploit* and *A. Bliss* is largely due to the different growing methods. This means that the annual growing system provides good results in the protection against *A. aurichalceus* because of the method in which all canes are cut and burnt after final harvesting preventing larvae from overwintering inside canes and beetles from emerging the next year. On the other hand, growers should keep their plantations in good condition with appropriate cultivation steps.
Further research is needed in connection with the parasite chalcidoid species (*Baryscapus agrilorum* Ratzeburg and *Tetrastichus heeringi* Delucchi), which were reared from the *A. aurichalceus* larvae, for their role in the control of the pest population.
Other pests like *Aulacaspis rosae*, *Oecanthus pellucens*, *Atrococcus bejbienkoi* and *Lasioptera rubi* also occured in the observed plantations, but their number was high only in *M. Exploit*. The reason for this is probably the bad condition of the plantation and the lack of plant protection methods that allowed many insects to appear in large numbers there.

Acknowledgement

We thank Dr. Cs. Thuróczy for identifying the parasite species. We also thank Dr. L. Kollányi and J. Fail for their co-operation in our survey and the growers who allowed access to their plantations.

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Use of a new generation of horticultural oils for mite management in fruit orchards.

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Abstract: Three horticultural oils: JMS Stylet oil, Damoil, and Mite E-oil and various methods of applications (complete sprays vs. alternate row middle applications) were evaluated under commercial fruit orchard conditions. In two orchards multiple applications of Stylet oil were compared with the grower's standard mite management program while in the third orchard various horticultural oils were used to compare their mite control efficacy. Under high mite pressure the horticultural oil treatments applied as complete sprays were effective in controlling mite populations. Applications of oils as alternate row middle sprays with reduced volume of water did not provide adequate mite control. No negative effects (phytotoxocity) on fruit finish were observed in orchards treated with summer oils.

Key words: phytophagous mites, horticultural summer oil, beneficial mites

Introduction

The European red mite (ERM), *Panonychus ulmi* (Koch) is one of the most significant foliar pests in many fruit orchards in Pennsylvania and the mid-Atlantic region. Both, immature and adult mites feed on apple leaves, drastically reducing the photosynthetic capability of the tree. The most serious injury occurs in early summer when trees are producing fruit buds for the following season. Infested trees produce fewer and less vigorous fruit buds. Mite feeding can also reduce the sizing of fruit during current growing season (Hull et al., 1985).

Effective ERM control is often difficult to achieve. The small size of mites and tree-clustered distribution in orchards make mite monitoring a challenge. Their high reproductive potential (8 – 10 generations per year) and a high number of eggs produced by females create a potential for uncontrollable outbreaks. Even a properly identified mite problem might represent a difficult issue from the management perspective.

The utilization of horticultural oils for mite control represents a reduced risk approach to control pests in keeping with the movement towards more biologically based systems. During studies conducted in orchards located in New York State, Agnello (1996) documented good protective ability of summer oils under moderate mite pressure, but not under high mite pressure. Summer oils also proved to be less toxic to mite natural enemies than to various stages of mites. A potential drawback for the program was observed when the use of higher rates of oil (2 and 3 gal/acre) resulted in phytotoxic effects on foliage and fruit.

With the average cost of acaricides running as high as 25 (or more) percent of grower's pesticide program (≈ $40 per single acaricide application/ acre), the use of less expensive horticultural oils should allow a reduction in mite management costs paid by a grower each year. Oils control plant feeding insects by modifying insect feeding and egg laying behaviors and by suffocation (Walsh et al., 2000).

The 1997 Pennsylvania Fruit Chemical Use Survey indicates that 72.6 percent of apple growers consider the protection of beneficial organisms as an important factor for pest control decisions and 88.2 percent would choose pesticides that are less harmful to beneficial organisms. Despite such high level of awareness about biological control of mites, the necessity to control other pests (i.e., internal fruit feeders such as Oriental fruit moth or codling moth) forced growers to apply pesticides that are toxic to natural enemies (i.e., pyrethroids) (Hull et al., 1997). During the 1998-2003 seasons close to 1800 fruit loads were rejected by Pennsylvania fruit processors due to the presence of live larvae in the fruit.

The objective of this project was to involve growers who are interested in reducing their pesticide input for mite control while creating an effective biologically based mite management program. To achieve this goal we evaluated different horticultural oils and various methods of applications under commercial fruit orchard conditions.

Materials and Methods

The experiment was located on three individual fruit farms located in south-central Pennsylvania during the 2002 season. In each orchard, the early season application of dormant oil was applied in early April. Blocks used for the oil experiment on Grower A farm consisted of one apple cultivar 'Red delicious' (15+ year old). On Grower B farm, a 10 acres orchard with 'Yorking' and 'Golden delicious' was divided into two similar blocks and the oil program was applied in one block. The experiment at Grower C site involved 4 separate apple bocks (≈ five acres each) with three of the blocks being treated with different horticultural oils and the fourth block being used as a control. Each block had two apple varieties 'Yorking' and 'Golden delicious'. During the summer oil applications Grower A, B and C used Stylet oil (JMS Flower Farms, Vero Beach, FL) while Grower C also used Damoil (Drexel Chemical Co., Memphis, TN) and Mite-E-Oil (Helena Chemical Co., Memphis TN)

Due to various past experiences with the mite pressure in orchards, the horticultural oil programs were different at each site. While the dormant oil application was applied in each orchard as a standard, the later in-season applications varied among orchards. In the Grower A orchard, the JMS Stylet oil was applied on 11 and 29 May, 10 and 20 June, and 10 July.

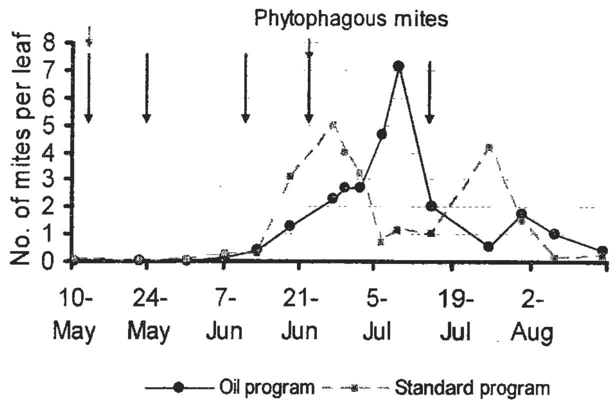
The Standard block received an application of clofentezine on 11 May and an application of pyridaben on 20 June. In the Grower B standard program no conventional acaricides were applied the entire season while the JMS Stylet oil block had applications of the oil on 30 May, 13 and 27 June. In Grower C orchards all 4 treatments received an application of clofentezine on 6 May and then the oil program blocks received various oil treatments on 3, 13 and 18 June. No conventional acaricides were needed in the standard block during the season. All oil applications by Growers A and B were done as complete sprays using 100 gallon of water per acre (≈ 934 l/ha), except for the Grower A 10 July application when 200 gal of water was used per acre. Grower C applied tested oils as alternate row middle applications using 50 gallons of water per acre. At each farm, blocks used for oil efficacy evaluations were treated with the same insecticide and fungicide programs as the standard blocks.

Mite and mite predator densities were evaluated on a weekly basis and compared to mite populations in blocks with standard program. During each observation leaves for counts were collected from eight trees scattered throughout each block. At least 25 leaves per tree (200 leaves per block) were evaluated using a leaf brush machine for the presence of phytophagous mites, predatory mites (*Amblyseius fallacis* and *Zetzellia mali*) and mite eggs. Each variety was also visually evaluated and rated during the harvest for phytotoxicity of oils on fruit and foliage.

Results and Discussion

During the 2002 season the efficacy of three new horticultural oils were tested in commercial fruit orchards. Harvest phytotoxicity evaluations of all fruit varieties used during the study did not detect any negative effect of summer oils on fruit finish. Due to differences in mite population among evaluated sites each orchard situation will be discussed separately.

In the Grower A orchard the standard mite program included two applications of conventional acaricides. During the same time the oil block received 4 complete applications of Stylet oil. The last oil application was done using 1 percent oil solution delivered in 200 gallons of water per acre (1864 l/ha). The seasonal changes in mite and mite predator populations in Grower A orchard are presented in Figure 1.



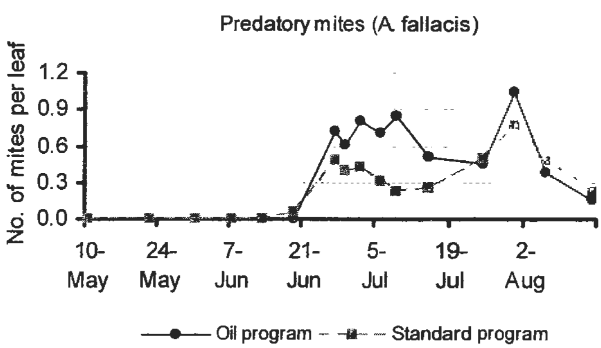


Figure 1. Seasonal change in population of phytophagous mites and predatory mites in Grower A blocks treated with horticultural oil (JMS Stylet oil) and standard acaricides. Arrows indicate the timings of acaricide/oil applications.

The early season oil applications suppressed the mite population until the beginning of July when number of mites increased to more than 7 mites per leaf in oil treatment. The "emergency" oil application on July 10 lowered the number of mites to 2 motile forms per leaf during the July 15 evaluation. The mite population in oil treated block remained at a low level for the rest of the season. In the standard block the pyridaben application lowered the mite population immediately after the treatment but they recovered in late July reaching again more than 4 mite per leaf during the July 25 evaluation. Although the predatory mites were present in both programs, the higher population (per leaf) was observed in the oil treated block. It appears that in both control programs the abundance of predatory mites contributed to successful control of late season mite populations.

In Grower B orchard during the entire season the mite population on "Golden delicious" in both programs never reached more than half mite per leaf. On "Yorking" trees located in the same orchard the phytophagous mite populations reached over 8 mites per leaf in standard program where no acaricide was applied except during the dormant period (Figure 2). At the same time three complete oil applications applied in late May and June suppressed the mite population to less than 2 mites per leaf throughout the season. The population of predatory mites was similar in both blocks reaching the highest levels on July 23 (0.9 predatory mite per leaf in oil block and 0.8 of predatory mite per leaf in no oil block).

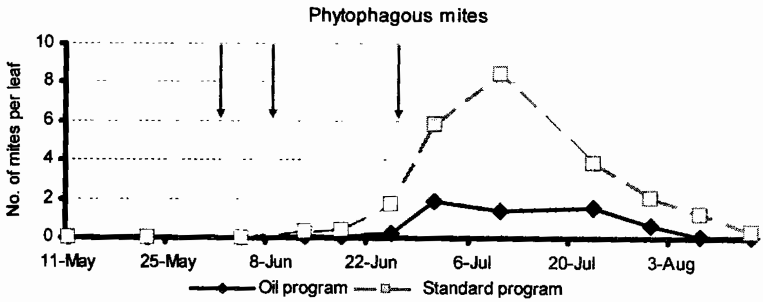


Figure 2. Seasonal mite populations in Grower B blocks after 3 complete applications of horticultural oil (JMS Stylet oil). Arrows indicate the timings of oil applications.

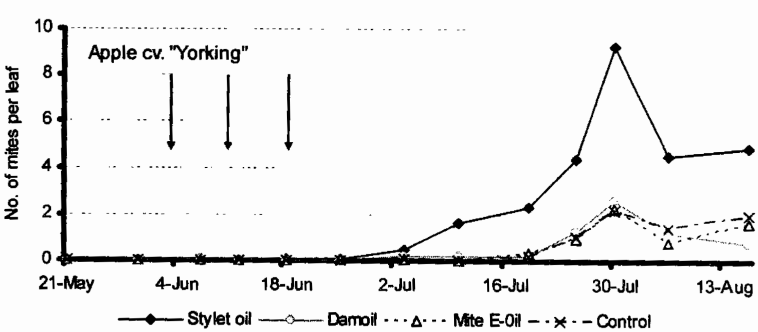


Figure 3. Seasonal mite populations in Grower C blocks treated with three different horticultural oils. Arrows indicate the timings of oil applications.

The mite populations on 'Golden delicious' in the Grower C orchards also remained at a very low level throughout the season, never exceeding more than 1.3 mite per leaf. The mite population on 'Yorking' trees remained at a low level except for the late season (July 30) surge in numbers on "Yorking" trees treated with Stylet oil early during the season (Figure 3).

Due to an almost non-existent early season mite populations in the Grower C blocks the oil treatments were stopped by June 18 and no oil or acaricide was applied after that date on any treatment including the control. The late July increase in number of mites on trees treated with Stylet oil can not be clearly associated with the early season oil applications. When the oil applications were discontinued (late June), mite and mite predator populations were at similar levels in all treatments. Some of the possible explanations for this late season increase in mite numbers in the Stylet oil treatment might be related to the fact that the oil applications were made as the alternate row middle applications using a lower volume of water. But at the same time, the reasons mentioned above do not explain why mite populations did not increase in other oil treatments. Mite populations on both varieties in the control treatment, which received only the pink and petal fall clofentezine applications also remained at a very low level throughout the season.

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The judgement of the population regulating effect of *Zetzellia mali* (Stigmaeidae)

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Abstract: *Zetzellia mali* is frequently a member of Acarina populations communities in the abandoned apple orchards. Its specimens occur from time to time in low number in apple orchards treated with broad-spectrum insecticides, too. Nevertheless it is not ranked among the significant predators of the phytophagous mites populations in the orchards, where the applications of broad-spectrum insecticides ceased. In the following years when the population density of phytoseiid mites increased, the individual number of *Z. mali* gradually decrease. In the IPM orchard it becomes again a member of the population communities, in low population density.

Key words: *Zetzellia mali,* Acarina population communities, integrated control

Introduction

Zetzellia mali (Ewing) as a predator of fruit tree spider mite has been known since the beginning of 1950s (Parent and LeRoux 1956; Böhm, 1960). Its populations are present frequently in variable densities in the different types of apple orchards (Parent and LeRoux, 1956; Dosse, 1957; Berker 1958, Strickler at al., 1987; Karg, 1990; Vogt et al., 1990). In order to establish the real effect of *Z. mali* in the Acarina population communities authors carried out short-term examinations regarding its biology (White & Laing, 1977), the effect of the host plant (Santos, 1984), its prey preference (Clements &Harmsen, 1993), the interrelation between phytoseiid mites and its populations (Croft & MacRae, 1992; Gerson 1992; Clements & Harmsen 1993). After all it is known as one of the predators but it is not ranked among the significant beneficial arthropods of the phytophagous mites. Over a period of ten years we had the possibility to follow the changes of the population density of the phytophagous and zoophagous mites with attention to conditions of apple orchards,
during ecological-faunistic investigations and in the experiments for the sake evaluating IPM system in apple orchard, in Hungary. Consequently, we try to outline the significance of the *Z. mali* populations in developing the IPM in the orchards.

Material and methods

The Acarina populations were studied in:
- an abandoned apple orchard of half ha (Nyíregyháza-Sóskút),
- an commercial apple orchard treated with broad-spectrum (mainly organo-phosphorous) insecticides (Újfehértó),
- back-yard apple orchard treated with broad-spectrum (mainly organo-phosphorous) insecticides and later diflubenzuron (Dimilin) was applied for the last three years (Nyíregyháza – Füzesbokor)

- the IPM experimental apple orchard (Kecskemét – Szarkás), where examinations were carried out in two plots for three years:
- treated with conventional pesticides, mainly organo-phosphorous insecticides (2 has),
- treated with selective insecticides as diflubenzuron, fenoxycarb, Bacillus thuringiensis insecticides and fungicides harmless to predacious mites (2 has).

Orchards were sampled by collecting twenty-five leaves each from four trees. The samples were taken biweekly in the abandoned, in the back-yard orchard and in the commercial apple orchards, and weekly in the plots of IPM experimental orchard.
The samples were taken to the laboratory where the micro-arthropod species, like phytophagous and zoophagous mites were selected under dissecting microscope.
Insecticides used in the IPM plot were: Sulphur + vaselinoil (Nevikén), fenoxycarb (Insegar), diflubenzuron (Dimilin 25 WP), lufenuron (Match), pirimicarb (Pirimor 50 WG), fenbutatin-oxid (Torque 55 SC), Bacillus thuringiensis (Dipel). Sulphur + vaselinoil were used every year as dormant spray. The acaricide fenbutation-oxide was apply in one occasion in the first year of the experiment.
Insecticides used in the conventional plot were: dinitro-orto-cresol (Novenda), methidation (Ultracid 40 WP), methylparathion (Danatox 50 EC), phosphamidon (Dimecron 50), chlorpropilate (Rospin 25 EC), propargite (Omite 57 E).

Results and discussion

In the abandoned apple orchard some phytophagous and predacious mite species (*Tetranychus viennensis, Brevipalpus pulcher, Amblyseius finlandicus, Zetzellia mali, Tydeidae*) were present in low population density. One of them was *Z. mali*. (Fig. 1).

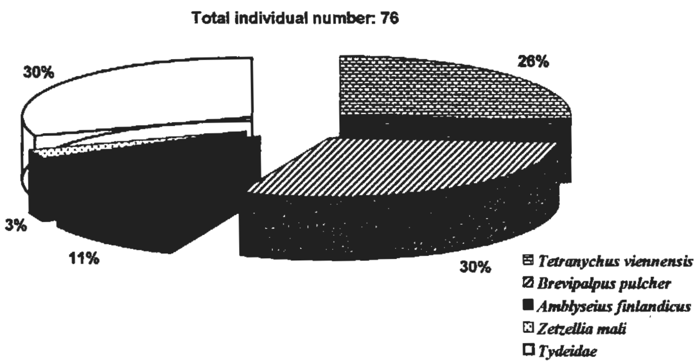

Fig. 1. Composition of Acarina populations in an abandoned apple orchard (Nyíregyháza – Sóskút, 1987)

In the commercial apple orchard regularly treated with broad-spectrum insecticides and acaricides, the dominant or practically the single mite species was the fruit tree red spider mite (*Panonychus ulmi* Koch). From to time a few specimens of *Z. mali* were present. (Fig. 2).

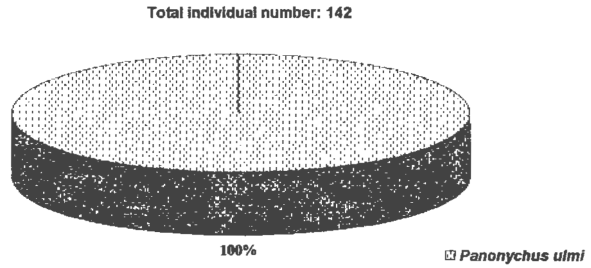

Fig. 2. Composition of Acarina in a commercial apple orchard (Újfehértó, 1987)

In the back-yard apple orchard, where the regular applications of the broad-spectrum insecticides was followed by the use of selective insecticides (diflubenzuron) the population density of *Z. mali* suddenly increased and soon it become the dominant species, suppressing the population of the fruit trees red spider mite to the minimum level. (Fig. 3).

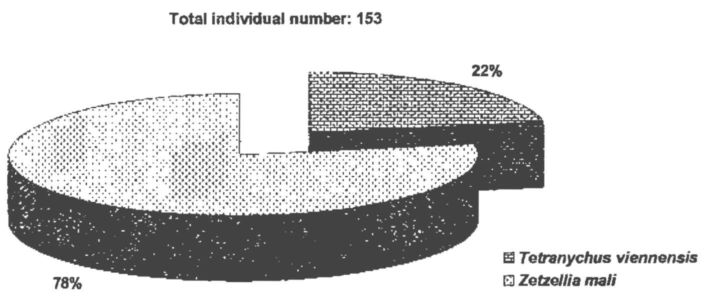

Fig. 3. Composition of Acarina populations in a back-yard apple orchard (Nyíregyháza – Füzes bokor, 1987)

In the IPM experimental apple orchard in the plot treated with conventional pesticides, mainly organo-phosphorus insecticides the fruit red spider mite (*Panonychus ulmi*) was the dominant Acarina species (Fig. 4). In the plot treated with selective insecticides (IGR) (diflubenuron, fenoxycarb, lufenuron) the specimens of *Z. mali* appeared in the first year. In the second year of the experiment it became the dominant Acarina species and considerably suppressed the population density of the phytophagous mites. Meanwhile the specimens of *A. finlandicus* appeared.(Fig. 5). At the second half of vegetation period the population density of phytoseiid mites increased, and the individual number of *Z. mali* gradually decreased.

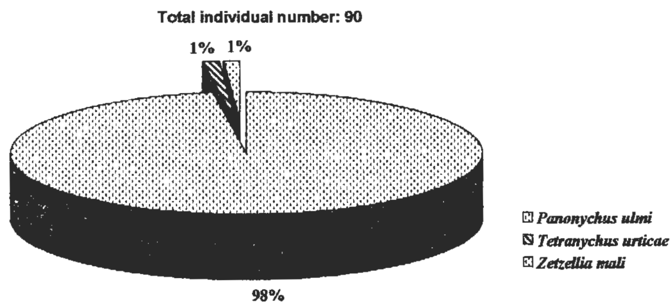

Fig. 4. Composition of Acarina populations in the plot treated with broad-spectrum insecticides of the experimental apple orchard (Kecskemét – Szakás, 1993)

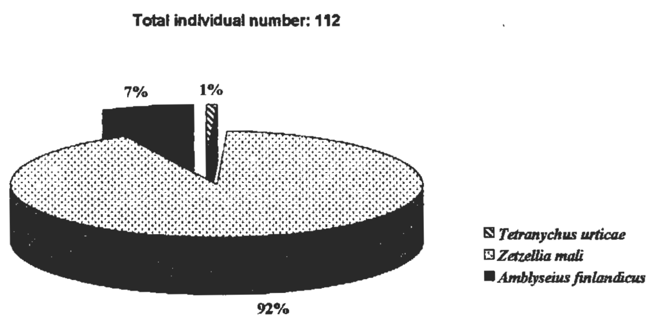

Fig. 5. Composition of Acarina in the IPM plot of the experimental apple orchard, in the second year of the experiment (Kecskemét – Szakás, 1993)

In the third year of the experiment a population community evolved which included many phytophagous and predacious mite species in low population density, similar to the Acarina species community occurring in the abandoned orchard. In this situation *Z. mali* was one member of this community, in which effective interdependence functions. In this population community *Z. mali* seems to be a weak predaceous mite, indeed. (Fig. 6).

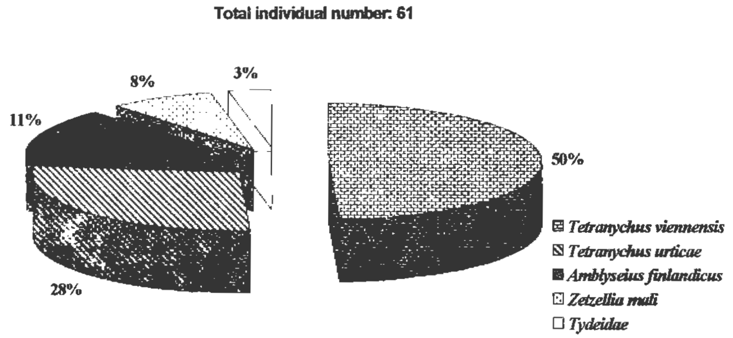

Fig. 6. Composition of Acarina populations in the IPM plot of the apple orchard, in the third year of the experiment (Kecskemét – Szakás, 1994)

The populations of *Z. mali* have a significant role

– in the regulation of the phytophagous mite populations in the orchards where the application of broad-spectrum insecticides were ceased,

– in the maintenance of the Acarina population community in which the population density of the species is low, their population dynamics are well balanced and the number of the phytophagous species cannot increase to a high individual number in a short time.

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Effects of modern bait formulated pesticides on larvae and adults of *Chrysoperla carnea* under extended-laboratory conditions

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Abstract: *Chrysoperla carnea* (Stephens) is a widespread polyphagous and naturally occuring predator, very common in olive groves where broad spectrum pesticides are commonly used. These insecticides have caused many undesirable effects to humans and environment, so at present, pest control is undertaken under IPM strategies, being the joint use of natural enemies and selective pesticides, one the most interesting tools.

Treatments against pests as olive fruit fly are made as bait sprays, so in this work, several commercial insecticides registered in Spain (natural pyrethrins+PBO and fipronil) were applied as bait formulated compounds together with the attractant Biocebo® (30% hydrolized proteins) in extended laboratory test using olive leaves. Their effect on mortality of larvae, and mortality and reproduction of adults were determined and compared with that produced by two classical neurotoxic pesticides and a new bait formulation of spinosad, which already has the bait incorporated. Pesticides were applied at the maximum field recommended concentration registered in Spain for bait sprays and at the rate recommended for the manufacturer for spinosad. The insecticides not currently used as bait-spray on Spain (fipronil and natural pyrethrins), were applied at the maximum registered rates in Spain together with the maximum rate of hydrolized proteins.

Larvae were extremely sensitive to fipronil. Twenty four hours after treatment, mortality was 100%, compared to 78% of dimethoate and 58% of trichlorfon at 72 hours. Natural pyrethrins gave a similar mortality to 20 ppm spinosad (30% aprox.), while 10 ppm spinosad only caused 16% mortality at 72 h. Adults were also very sensitive to fipronil and dimethoate (100 and 80% mortality after 72 h); trichlorfon and natural pyrethrins were slightly more toxic than to larvae (73 and 43% mortalities, respectively), and spinosad did not cause any mortality at all. Reproduction was not affected in any case.

Key words: *Chrysoperla carnea*, bait pesticides, natural pyrethrins+PBO, fipronil, trichlorfon, dimethoate, spinosad.

Introduction

Olive groves are largely represented in Spain by more than 2 millions has. Within all the olive pests, we should stand out the olive fruit fly, *Bactrocera oleae*, as the most serious pest of olives in most of the countries around the Mediterranean basin (Alvarado *et al.*, 1997). The larvae are monophagous, and feed exclusively on olive fruits. Adults feed nectar, honey dew, and other opportunistic sources of liquid or semi-liquid food. The damage caused by tunnelling of the larvae in the fruit results in about 30 percent loss of the olive crop (Mazomenos, 1989). On crops intended for oil extraction there are three differents kinds of damage as premature drop of attacked fruits, direct pulp destruction, acidity increase and other secondary effects associated with larval development of pulp infections and oviposition resulting from holes in the skin (Uceda & Hermoso, 1997). The management largely depends on bait sprays, although other techniques as trapping of adult flies, harvest timing, fruit sanitation after harvest, and biological control are also useful. Bait sprays used to control olive fly can affect not only the population of *Psyttalia concolor*, one important parasitoid native to the north of Africa and inundatively released in some Mediterranean countries, or other natural enemies that helps to maintain *Bactrocera* under a certain threshold, but also to the rest of natural enemies. Within our project of evaluating the side-effects of insecticides currently used or with potential application in olive groves, one of the main points is the study of the effects on the key beneficial living in our olive groves, *Chrysoperla carnea*, that it is far very abundant.

Material and methods

Several commercial insecticides registered in Spain were applied as bait formulated compounds together with the attractant Biocebo® (30% hydrolized proteins), and compared with a new bait formulation of spinosad, which already incorporated the bait. The studied pesticides were: Pelitre Hort® (4% natural pyrethrins + 16% PBO, C.Q. Massó), Regente® (80% fipronil, Basf), and Spinosad GF120® (0.2 g/l spinosad, DowAgrosciences), using Dimafon® (80% trichlorfon, Agrodán) and Sistematon® (40% dimethoate, Agrodán) as positive standard insecticides. Pesticides were applied at the maximum field recommended concentration registered in Spain for bait sprays (trichlorfon 4000 ppm a.i. + 1800 ppm a.i. hydrolized proteins; dimethoate 3000 ppm a.i. + 2500 ppm a.i. hydrolized proteins) and at the rate recommended for the manufacturer for spinosad (10 and 20 ppm a.i.). The insecticides not currently used as bait-spray in Spain, were applied at the maximum registered rates in Spain together with the maximum rate of hydrolized proteins: fipronil (30 ppm a.i. + 4500 ppm a.i. hydrolized proteins) and natural pyrethrins (80 ppm a.i.+ 4.500 ppm a.i. hydrolized proteins).

All pesticides were tested in an extended laboratory assay using olive leaves, following a methodology developed by Medina *et al.* (2004). Small branches of olive, with 5-6 leaves approximately, were collected from small trees and taken to the laboratory. One droplet of 5 µl of the corresponding compound were placed at random over one leave with a micropippete. Treated branches were transferred to ventilated plastic cages (10 cm in diameter, 5 cm high) after being previously introduced to eppendorf® tubes containing a nutritive solution to keep the turgidity leaves during the experiments. Eppendorf® tubes were fixed to the bottom of the cage with Plasticine®. Once the droplet was dry, insects were introduced. Two experiments were performed, using adults and third instar larvae.

Water was always offered *ad libitum* to adults in small glass vials covered with "Parafilm®" and a piece of "Spontex®" wiper and artificial diet as well. A gauze was used to collect the eggs. Three couples of adults less than 48 h old per cage and five replicates were used. Percentage of mortality at 24, 48 and 72 h after the bait application were scored. Fecundity, measured as mean number of eggs per female and day laid during a week and fertility, as percentages of eggs hatched from the laying of 5th day were also recorded to study possible sublethal effects on reproduction.

Results and discussion

Larvae were extremely sensitive to fipronil killing 100% larvae in less than 24 h. Three days later, 78% larvae were killed by dimethoate and 58% by trichlorfon. Natural pyrethrins gave a similar mortality to 20 ppm spinosad (30%) aprox., while 10 ppm spinosad only caused 16% mortality.

Table 1. Cumulative mortality scored on *Chrysoperla carnea* third instar larvae exposed to a droplet of a bait formulated insecticide, on olive leaves.

Insecticide	Conc. (mg a.i./l)	24 h[1]	48 h[2]	72 h[3]	IOBC
Control	0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	-
Bait	4,500	10.0 ± 2.0 b	10.0 ± 0.0 b	10.0 ± 0.0 b	1
Dimethoate	3,000	38.0 ± 4.9 d	68.0 ± 3.7 e	78.0 ± 2.0 g	2
Trichlorfon	1,800	22.0 ± 2.0 c	34.0 ± 2.4 d	58.0 ± 2.0 f	2
Fipronil	30	100.0 ± 0.0 e	100.0 ± 0.0 f	100.0 ± 0.0 h	4
Pyrethrins+PBO	80	24.0 ± 2.4 c	32.0 ± 2.0 d	32.0 ± 2.0 d	2
Spinosad	10	10.0 ± 0.0 a	12.0 ± 2.0 b	16.0 ± 2.4 c	1
	20	20.0 ± 0.0 c	26.0 ± 2.4 c	30.0 ± 0.0 d	2

[1]K=36,13; P<0.001. [2]F=261.6; df=7,32; P<0.001. [3]F=546.4; df=7,32; P<0.001. Data in the same column followed by a different letter are significantly different (P<0.05) (ANOVA and LSD, Kruskal-Wallis test). Data are means ± standard error of five replicates of 10 larvae per treatment.

Adults were also very sensitive to fipronil and dimethoate (100 and about 80% mortality after 72 h); trichlorfon and natural pyrethrins were slightly more toxic than to larvae (73 and 43% mortality, respectively), and spinosad did not caused any mortality at all. Reproduction was not affected in any case.

Table 2. Cumulative mortality scored on *Chrysoperla carnea* adults exposed to a droplet of a bait formulated insecticide, on olive leaves,

Insecticide	Conc. (mg a.i./l)	24 h[1]	48 h[2]	72 h[3]	IOBC
Control	0	0.0 ± 0.0	0.0 ± 0.0 a	0.0 ± 0.0 a	-
Bait	4,500	0.0 ± 0.0	0.0 ± 0.0 a	0.0 ± 0.0 a	1
Dimethoate	3,000	0.0 ± 0.0	9.9 ± 6.6 a	79.9 ± 3.3 c	2
Trichlorfon	1,800	0.0 ± 0.0	0.0 ± 0.0 a	73.3 ± 6.6 c	2
Fipronil	30	9.1 ± 4.1	53.3 ± 6.2 b	100.0 ± 0.0 d	4
Pyrethrins+PBO	80	0.0 ± 0.0	9.9 ± 4.01a	43.3 ± 4.1 b	2
Spinosad	10	0.0 ± 0.0	0.0 ± 0.0 a	0.0 ± 0.0 a	1
	20	0.0 ± 0.0	0.0 ± 0.0 a	0.0 ± 0.0 a	1

[1]Anova could not be run. [2]F=27.1; df=7,32; P<0.001. [3]K=38.4; P<0.001. Data in the same column followed by a different letter are significantly different (P<0.05) (ANOVA and LSD, Kruskal-Wallis test). Data are means ± standard error of five replicates of three couples per treatment.

From these results, it might be concluded that both, larvae and adults are attracted by bait droplets and its ingestion kill them when the insecticide is toxic. Therefore, bait formulated insecticides can be potentially as toxic as spray applications, even though they are supposed to be less harmful to natural enemies. Both neurotoxics currently employed to control olive fly in bait sprays, dimethoate and trichlorfon are toxic to adults and larvae of the predator, although trichlorfon is slightly less toxic. Within the alternatives, spinosad seemed to be the best insecticide and fipronil the worst, which is in correspondence with the studies performed by Medina *et al.* (2003; 2004), when these insecticides were applied without a bait. Natural pyrethrins are toxic in the lab, although its toxicity is more arguable in the field due to its quick degradation by the light.

Table 3. Fecundity and fertility of *Chrysoperla carnea* adults exposed to a droplet of a bait formulated insecticide.

Insecticide	Concentration (mg a.i./l)	Fecundity (eggs per female and day)[1]	Fertility (% egg hatch)[2]
Control	0	33.0 ± 1.5 a	84.2 ± 0.6 a
Bait	4,500	32.4 ± 0.7 a	84.2 ± 0.4 a
Dimethoate	3,000	32.0 ± 1.0 a	81.0 ± 0.9 b
Trichlorfon	1,800	32.4 ± 0.5 a	82.6 ± 0.5 ab
Fipronil	30	-	-
Natural pyrethrins+PBO	80	31.6 ± 0.6 a	83.0 ± 0.6 a
Spinosad	10	32.8 ± 1.0 a	81.2 ± 0.4 b
Spinosad	20	32.0 ± 0.8 a	81.0 ± 0.3 b

[1] F=0.26; df=6,28; P=0.95. [2] F=6.54; df=6,28; P<0.0002. Data in the same column followed by a different letter are significantly different (P<0.05) (ANOVA and LSD test.. Data are means ± standard error of five replicates of three couples per treatment.

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Reduced application rates of imidacloprid on apple: effect on leafhoppers, aphids and aphid predators

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Abstract: Field treatment scenarios using full, one-quarter and a combination of full and one-quarter label rates of imidacloprid were compared during mid-season. Efficacy against leafhoppers (white apple leafhopper and rose leafhopper) was assessed 2d after the final application by counting numbers of nymphs/leaf, and by rating foliar symptoms of damage by potato leafhopper (PLH). All rates and application timings provided very good control of all leafhopper pests. Against unusually high populations of PLH nymphs, multiple applications generally provided superior control, regardless of rate. Assessment of foliar damage (chlorosis and curled leaves) by PLH revealed that multiple applications of reduced rates were generally effective in the reduction of both symptoms. Because established apple trees can tolerate a good deal of damage from the indirect feeding of leafhoppers, multiple applications of imidacloprid at one-quarter label rate would suffice in most instances.

Subsequently, we examined the effects of reduced rates of imidacloprid against green apple aphid (GAA) and two of its most important natural enemies. Full, one-half, one-quarter, and one-eighth label rates were applied once during mid-summer. Evaluations (rating of aphid numbers/leaf) were made at 3d, 7d and 23d post application. Treatment effects on predators were assessed by counting the number of larvae/5 apical terminal leaves. Decreasing rates of imidacloprid provided decreasing, but effective, efficacy against GAA. Against both predators, all treatments between full and one-quarter label rates significantly reduced numbers. The one-eighth label rate however, allowed the predators to dramatically rebound by 7d post treatment. The results suggest that the one-eighth label rate provides adequate suppression of aphids, while preserving predators.

Key words: leafhopper management, aphid management, reduced application rates, aphid predators

Introduction

Two leafhopper species, the white apple leafhopper (WALH), *Typhlocyba pomaria* McAtee and the potato leafhopper (PLH) *Empoasca fabae* (Harris), comprise a complex that annually damages apple foliage in New York State. In the eastern NY region moreover, the rose leafhopper (RLH), *Edwardsiana rosae* (L.) is an annual pest. Damage to apple foliage by WALH and RLH appears as stippling or leaf chlorosis. The migratory PLH produces symptoms ranging from chlorosis to the typical 'hopper burn', resulting in reduced growth rate of terminals. Accumulated excrement from the pest complex reduces the marketability of fresh market fruit, while high adult presence at harvest causes considerable annoyance to harvesters. There are no significant natural enemies of the leafhopper complex.

The green apple aphid (GAA), *Aphis pomi* De Geer, is generally considered to be the most damaging terminal-feeding aphid in NY State. Feeding on new growth by GAA nymphs causes stunted and misshapen leaves. Populations of GAA will often be dramatically affected by a duo of natural enemies (larvae of Cecidomyiidae and Coccinellidae) if detrimental insecticides are withheld. Nonetheless, if populations remain excessive for an extended time period, growers will commonly apply pesticides against this pest.

Imidacloprid (Provado 1.6F®, Bayer Corp., Kansas City MO, USA) is generally recognized for it's high degree of efficacy against sucking insects, particularly leafhoppers and aphids. Because this insecticide is costly relative to older standards however, we performed field trials using applications of reduced rates and various application timings to examine efficacy against leafhoppers and aphids, and to assess the effects of reduced rates on aphid natural enemies.

Materials and Methods

Experimental trees were M.7/Golden Delicious, approximately 10 yr-old and 15 ft high. Treatments were applied to single-tree plots (buffered by two guard trees) replicated four to five times in a RCB design. Treatments were applied from one to three times, depending upon individual protocols. Applications were made dilute to runoff using a high-pressure handgun sprayer operated at 300 PSI, delivering 60 gal/acre. Treatment effects were evaluated using ANOVA and means were compared using Fisher's Protected LSD (P=0.05).

2001 Trial, Leafhopper Nymphs

We previously demonstrated that greatly reduced rates were effective (Straub & Jentsch, 2003), and therefore sought to examine various application scenarios. Treatments were replicated four times in a randomized complete block design. Treatment scenarios using full, one-quarter and a combination of full and one-quarter label rates of imidacloprid were compared during mid-season (3rd to 5th cover spray period). Efficacy was assessed, one day after the final application, by [a] counting the number of nymphs per 5 leaf sample, [b] assessment of adult leafhopper numbers by sweeping the tree perimeter for 4 min. with a vacuum sampling machine (Straub & Jentsch, 1994), and [c]. estimation of percent leaves damaged or curled by PLH by sampling 10 distal leaves on 10 terminals.

2002 Trial, Green Apple Aphid and Predators

Because imicacloprid is often used during early season to manage GAA infestations, an experiment was designed examine the effects of reduced rates against this pest and two of its most important natural enemies. Single applications were replicated five times. Similar to prior leafhopper studies, full, one-half, one-quarter, and one-eighth label rates of imicacloprid were applied once during mid-summer. Fifteen aphid-infested terminals/replicate were tagged for pretreatment counts and subsequent evaluation. Post treatment aphid counts were made at 3d, 7d and 23d. Aphid numbers per terminal were estimated by a rating where: 0 = no aphids; 1 = 1-10 aphids/t leaf; 2 = 11-100 aphids/leaf; and 3 = >100 aphids/leaf. Treatment effects on predators were assessed 7d post treatment by counting the number of larvae/5 apical terminal leaves.

Results and Discussion

2001 Trial, Leafhopper Nymphs

During the 3rd to 5th cover periods, indigenous WALH and RLH, and migratory PLH are usually present in eastern NY apple orchards. In general, 3rd cover applications impact immigrating adult PLH, 4th cover applications coincide with nymphal emergence of all three species, while 5th cover applications affect early instar nymphs of all species.

All rates and application timings provided very good control of WALH and RLH (Table 1). Against high populations of PLH nymphs, multiple applications generally provided superior control, regardless of rate – PLH continually re-infest new leaves not exposed to residues from precious applications. Assessment of foliar damage (chlorosis and curled leaves) by PLH revealed that multiple applications of reduced rates were generally effective in the reduction of both symptoms, particularly curled leaves. Because established apple trees can tolerate considerable damage from the indirect feeding of leafhoppers, multiple applications of imicacloprid at one-quarter label rate is a logical program.

Table 1. Efficacy of reduced rates of imidacloprid against leafhopper nymphs and damage, 2001.

			No./leaf		Percentage shoot lvs.	
Treatment	Amt./ acre	Timing[1] (no. apps.)	WALH, RLH[2]	PLH[2]	chlorosis by PLH[2]	curled by PLH[2]
Imidacloprid	8.0 oz	3C (1)	<0.1a	13.0c	66.0b	43.0b
Imidacloprid	8.0 oz	3, 4C (2)	0.0a	1.6ab	19.0a	4.0a
Imidacloprid	2.0 oz	4,5C (2)	0.0a	0.2a	56.0b	1.0a
Imidacloprid	2.0 oz	3-5C (3)	0.0a	0.7a	37.0ab	6.0a
Untreated	-	-	5.1b	11.0bc	97.0c	77.5c

Means followed by the same letter are not significantly different (*P*=0.05; Fisher's protected LSD).

[1]Third cover spray period, fourth cover spray period, etc.

[2]White apple leafhopper (WALH), rose leafhopper (RLH) and potato leafhopper (PLH).

2002 Trial, Green Apple Aphid and Predators

Table 2. Efficacy of reduced rates of imidacloprid against green apple aphid, 2002.

		3d post treat. 27 June		7d post treat. 1 July		23d post treat. 17 July	
Treatment	Amt. acre	Aphid rating[1]	Percent redn.[2]	Aphid rating	Percent redn.	Aphid rating	Percent redn.
Imidacloprid	8.0 oz	0.70 a	76.7	0.14 a	95.3	0.06 a	97.9
Imidacloprid	4.0 oz	1.05 b	65.0	0.37 b	87.7	0.05 a	98.4
Imidacloprid	2.0 oz	1.38 c	54.0	0.53 b	82.3	0.20 a	93.3
Imidacloprid	1.0 oz	1.49 c	50.3	0.89 c	70.3	0.08 a	97.5
Untreated	-	2.85 c	5.0	2.71 d	9.7	0.15 a	95.0

Means followed by the same letter are not significantly different (*P*=0.05; Fisher's protected LSD).

[1]Rating (0-3) of aphid numbers/terminal; see text for details.

[2]Based on precounts taken 24 June.

At the 3d and 7d assessment dates, GAA reductions followed a dose-response relationship (i.e., full rate > one-half rate, etc.) (Table 2). At 7d, all treatment rates reduced GAA numbers by at least 70 percent. Aphid numbers in all treatments, including untreated, decreased >90 percent 27d after application – as July wanes, aphid populations naturally decline due to lack of succulent tissue as terminal buds set, and/or because of natural enemies. The results indicate that decreasing rates of imidacloprid provide decreasing efficacy against GAA – however, all rates provided acceptable efficacy.

A single application of imidacloprid was generally detrimental to larvae of Coccinellidae and Cecidomyiidae (Table 3). All treatments between full and one-quarter label rates significantly reduced numbers of both predators. The one-eighth label rate however, allowed both predator species to increase dramatically at 7d after application – such increases may have contributed to the 70 percent reduction in GAA populations provided by this treatment (previous Table). The results suggest that the one-eighth label rate provides a high degree of aphid suppression, with little detriment to predators.

Table 3. Efficacy of reduced rates of imidacloprid against two key aphid predators, 2002.

		7d post treat. (1 July)[1]		Percent reduction[2]	
Treatment	Amt/ acre	Cocc.[3] larvae	Cecid.[3] larvae	Cocc. larvae	Cecid. larvae
Imidacloprid	8.0 oz	0.01 a	0.03 a	95.7	87.8
Imidacloprid	4.0 oz	0.02 a	0.04 a	84.3	93.8
Imidacloprid	2.0 oz	0.07 ab	0.04 a	87.8	93.2
Imidacloprid	1.0 oz	0.20 b	0.19 b	⇑ 329.2	⇑ 53.6
Untreated	-	1.19 c	0.21 b	⇑ 487.2	⇑ 22.0

Means followed by the same letter are not significantly different (*P*=0.05; Fisher's protected LSD).

[1]Average number of larvae/aphid infested terminal.

[2]Based on precounts taken 24 June.

[3]Cocc. = Coccinellidae ; Cecid. = Cecidomyiidae; ⇑ = population increase.

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Trials on the efficacy of natural products against Oriental Fruit Moth in organic peach orchard.

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Abstract: Field trials were carried out to investigate on the efficacy of products obtained from *Ryania speciosa* against the Oriental Fruit Moth (OFM), *Cydia molesta* (Busck) (Lepidoptera: Tortricidae), in organic peach orchards.
Trials took place in three farms located in Piedmont (Northern Italy).
The infestation level was surveyed weekly by recording the number of damaged shoots. Fruit infestation was checked at the end of the trials.
Results obtained show a good efficacy of the products used, especially if compared with the efficacy of *Bacillus thurigiensis* used as reference product.
At the same time, some bioassays were carried out in the laboratory, and at present investigation on the efficacy of different formulation of products obtained from *Ryania speciosa* are underway.

Key words: *Anarsia lineatella, Ryania speciosa,* natural products.

Introduction

Pest control in orchard managed according to organic farming principles is done with different natural products (EC Reg. 2092/91). Until now there is a lack in the research on the efficacy and side effects of this products (Serges *et al.*, 1998; Pasqualini *et al.*, 1999).
In 2003 trials have been set up in order to evaluate the efficacy of one of these natural products, extract from *Ryania speciosa* Vahl, against *Cydia molesta* (Busck)(OFM).
R. speciosa is a tropical shrub of the family Flacourtiaceae from Southern America, and in the past was well known for its phytoiatric properties (Pepper *et al.*, 1945). A powder with insecticidal properties is obtained from the stems and the roots, and the major active component is called ryanodine, an alkaloid highly soluble in water and in some organic solvent. Ryanodine compounds act on insects like a slow stomach poison but toxicity on mammals needs to be further investigated.
In 2002 some previous trials were carried out (Molinari *et al.*, 2004); in some cases the results were noticeable but showed a certain inconsistency of data.
This new trials were set up to better asses the effectiveness of ryania extract against Cydia molesta in the laboratory and in the field.

Materials and methods

The work was done during 2003, part in the laboratory and part in the field.

Laboratory activity

In order to evaluate the ovicidal activity (contact) and the larvicidal activity (ingestion and contact combined) laboratory trials were set up, using insect from the colony of *C. molesta* established in the laboratory of the Institute of Entomology in Piacenza.

A base mix was prepared with 3 g powder of *Ryania* in 300 ml distilled water, stirring for 1 hour at room temperature. Preparation allowed to settle 30 minutes, then the upper portion was used to prepare two different treatments:

Treatment A: no centrifugation

Treatment B: 10 min centrifugation (13000 g)

Centifugation was applied in order to assess whether the particles could improve efficacy compared to the solution alone. Both treatments were diluted as follows:

1) base (3 g of powder of *Ryania* in 300 ml water)
2) 1/2 base
3) 1/4 base
4) 1/8 base
5) 1/16 base

Eggs were dipped into different dilutions of treatment A.

Neonate larvae were put on a substrate where different dilutions of treatments A and B were sprayed; when adult emergence was complete, larval mortality was assessed.

Field activity

Efficacy trials have been carried out in organic peach orchards in Piedmont in the year 2003 as reported in table 1.

Tab. 1 – 2003. Locations of the field trials.

Year	Trial	Location	Farm
2003	1	Castellinaldo (CN)	Costa
2003	2	San Damiano (AT)	Rizzo
2003	3	Falicetto (CN)	Robasto

Two different treatments of *Ryania* against 2nd generation larvae (several application at 7-8 day intervals):

Ryania A : Sprayed ½ an hour after mixing

Ryania B : Sprayed 2 hours after mixing

An untreated control was always considered and also *Bacillus thuringiensis*, as standard product, was applied. The plots consisted of 6-8 trees and each treatment was replicated 3 times.

The flight was checked weekly with pheromone traps and the sprays on 2nd generation larvae repeated till harvest time. Damage on shoot were checked in different period during the season; damage on fruits only once before harvest.

Results

Laboratory

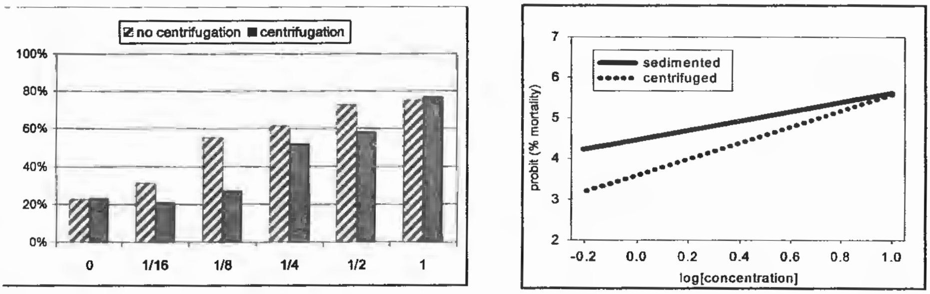

Figure 1 – Laboratory trial. Larval mortality at different dilutions of *Ryania* extract preparation (left); Probit analysis (right).

Laboratory results are expressed as a percentage of larval mortality, and with a probit analysis. *Ryania* preparation showed no ovicidal effect and an interesting activity against OFM larvae.Larvicidal activity seems to be well related with the dose, although no precise data on the ryanodine actual rate applied was available (Fig. 1).

Field

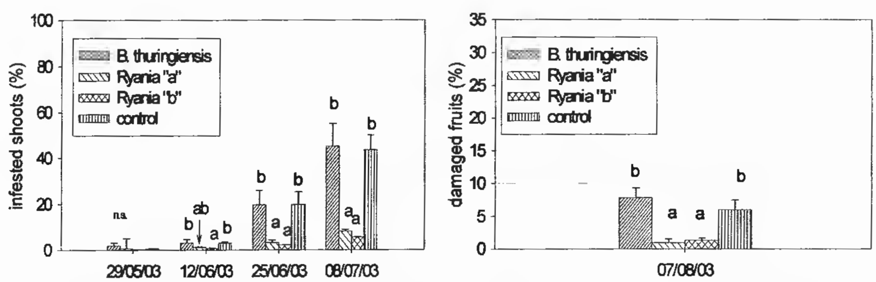

Figure 2 – Castellinaldo. Percentage of infested shoots (left) and damaged fruits (right)

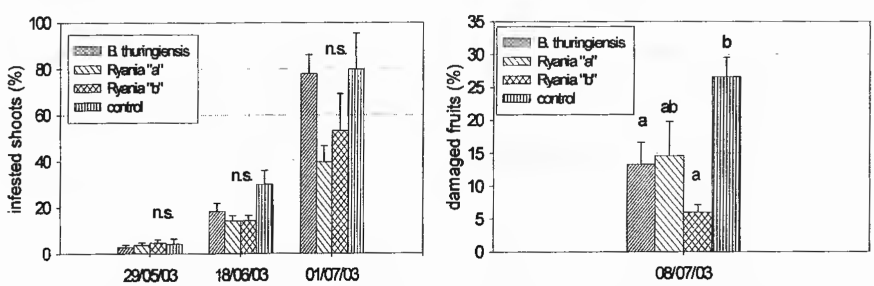

Figure 3 – S. Damiano. Percentage of infested shoots (left) and damaged fruits (right)

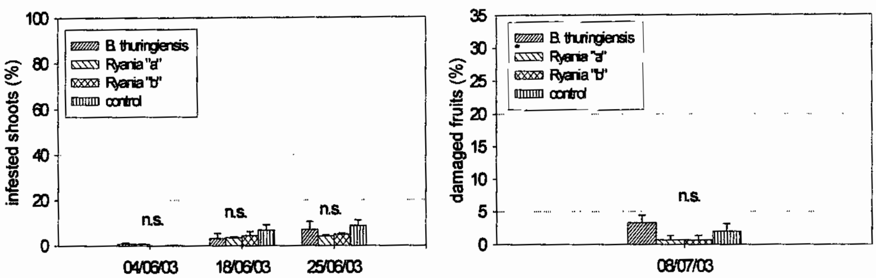

Figure 4 – Falicetto. Percentage of infested shoots (left) and damaged fruits (right)

The effects of treatments against OFM was expressed as the percentage of infested shoots and damaged fruits. Statistically significant differences have been observed in Castellinaldo (Fig. 2), and only on damaged fruits in S. Damiano (Fig. 3). Analysis of the results shows the good effect of the two formulation of *Ryania* in the three trials. Surprising is the damage on the *B.t.* thesis, used as standard, that seems to be comparable to the control (untreated). There was an extensive damage on the control in trials 1, 2 (Castellinaldo and S. Damiano), while in trial 3 (Falicetto) infestation level was low (Fig. 4).
Apart from the direct results of the experimentation, the evaluation of the efficacy of natural products must take into account that the standardization of the product (qualitative and quantitative characterization of active ingredients) is extremely important to assure the consistency of results.

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Control of Codling moth *Cydia pomonella* L. using insecticides: relationship between susceptibility and efficacy in the field.

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Abstract: Field trials carried out with different insecticides showed a generalised reduced efficacy in the control of codling moth (*Cydia pomonella*), even though the orchards were highly infested and the trials were carried out under favourable climatic conditions. A reduction in the susceptibility of the codling moth populations was therefore hypothesized.

The susceptibility of *C. pomonella* populations was assessed using two laboratory bioassays. Larvae were collected from orchards where reduced efficacy was observed. Young larvae, less than 10 mg in weight, were collected from infested fruits and tested by feeding them with apples coated with a discriminating dose of insecticide. A large difference between the expected mortality and that obtained in the dipping test were recorded for three out of 8 tested insecticides: tebufenozide -8,7%, metoxyfenozide -23,2% and chlorpyrifos-e -41,8%.

In the second bioassay, over-wintering larvae collected in the cardboard bands were treated topically with a discriminating dose of insecticide. A significant reduction in the mortality was recorded for 8 out of 13 tested insecticides when the average of two years data were considered. Two other insecticides showed a significant reduction only in the second year, while only deltametrine, spinosad, emamectine did not show significant loss in their activity.

The topical application technique was used to monitor the susceptibility of 18 populations. Over-wintering larvae collected in different apple growing districts of Trentino province were treated with the discriminating dose of tebufenozide. The survey confirmed that *C. pomonella* from orchards that received a large number of applications of tebufenozide are no longer susceptible to this insecticide.

Key words: *Cydia pomonella*, insecticide resistance, field efficacy, dipping test, topical application, apple.

Introduction

Codling moth (*Cydia pomonella*) is a key pest affecting Italian apple and pear orchards. Insecticide resistant populations have been detected in two of the main apple growing regions (Ioriatti *et al.*, 2003). The early detection of insecticide-resistant populations would greatly facilitate integrated resistance management programs. The aim of this work described herein was to compare two techniques for their ability to detect resistant populations in the field.

Materials and methods

Field trials

Field trails were carried out during the first codling moth generation in of 2003 and 2004 in order to evaluate the efficacy of six different insecticides (tab. 1). The apple orchard was located in S. Michele a/A (Trento) and contained 3-3.5 m high Golden Delicious trees. The insecticides were applied with an airblast sprayer using 15 hl/ha of water volume. The experimental plots included 20 trees in each of two adjacent rows. To avoid insecticide drift, an untreated row of the variety Florina divided the parallel plots. Each treatments was repeated four times.

The efficacy of the insecticides was evaluated in a strategy that started with a first treatment with teflubenzuron (Nomolt) when the first eggs were found in the orchard (250 °D). Lavicidal insecticides (spinosad, clorpyrifos-ethyl, thiacloprid, tebufenozide, metoxyfenozide) were applied at a later date, as soon as fresh penetrations in the apples were detected during weekly scouting. The efficacy of the larvicidal insecticides lasted two (chlorpirifos-ethyl) to three weeks (spinosad and thiacloprid), in 2003, but only one week for those tested in year 2004 (thiacloprid, chlorpirifos-e, tebufenozide and metoxyfenozide). The percentage of fruit damage at the end of the first generation was used to evaluate the efficacy of the treatments (tab. 1).

In 2003, an additional trial was set up. The efficacy of the MAC compound tebufenozide was evaluated in two different strategies. In the first, the initial treatment was applied as soon as the first eggs were laid (250 °D). Then the insecticide application was repeated 20 days later and again after 13 days. In the second strategy, the first application occurred when the first larval penetration of fruit was predicted (330 °D). Successive treatments were applied using the same intervals as in the previous strategy. Chlorpyrifos-ethyl was applied at the end of June, a week before the end of the first generation, because of high levels of fruit damage in both strategies. Fruit damage at the end of first generation was evaluated on July 1th 2003 and July 19th 2004, by sampling 250 apples in each replication (25 fruits/tree). Efficacy of the insecticide was calculated in relation to the fruit damage present in the untreated control.

Dipping test

The apple-dipping test has been used to detect resistance in larvae collected from infested fruits. According to the dose-response curve established for different insecticides on newly hatched larvae of a susceptible laboratory strain (Charmillot *et al.*, in the present volume), a discriminating dose was chosen for diflubenzuron, flufenoxuron, indoxacarb, spinosad, chlorpyrifos-methyl, thiacloprid, tebufenozide and metoxyfenozide (tab. 2). Larvae were divided into four classes according to their weight. The results obtained have shown that the mortality caused by the discriminating concentration decreases with increased larval weight. Only the first class of larvae, less than 10 mg in weight, was considered for the evaluation of the efficacy of the insecticides

Topical test

The dose-response reference curves for different insecticides topically applied on diapausing larvae of a susceptible laboratory strain have been established by Pasquier and Charmillot (2003). A discriminating doses has been chosen for each of the insecticides. The diapausing larvae collected in the apple orchard of S.Michele in the autumns of 2002 and 2003 were treated with the discriminating dose of 13 and 9 insecticides, respectively (tab. 3).

Regional survey

In the autumn of 2003, diapausing larvae were also collected in 18 orchards located in different districts of the province of Trentino. The larvae were treated topically with the discriminating dose of tebufenozide. Some of the samples (7) were treated at the Agroscope RAC Changins while others (11) at the Agricultural Institute of S.Michele (IASMA) (tab. 4). The population of S. Michele orchard has been tested both at RAC and at IASMA.

Results and discussion

Field tests

In 2003, the efficacy of the tested strategies ranged from 81,9% to 87,5% when the larvicidal insecticides followed a starting treatment with the IGR (tab. 1). In the same year the efficacy of tebufenozide varied between 76% and 59,9%; the worst result was obtained when the first treatment with tebufenozide was applied on the earliest eggs and the second treatment was repeated after three weeks, although the difference was not significantly different.

Table. 1. Field trials: efficacy of the applied strategies.

	Treatments	n° of treatments	average fruit damage				efficacy
2003	Untreated control		39,3	a	39,3	a	
	teflubenzuron + thiacloprid	1+2	4,9	b			87,5
	teflubenzuron + chlorpirifos-e	1+2	7,1	b			81,9
	teflubenzuron + spinosad	1+2	6,7	b			83
	tebufenozide (ovo-larvicidal treatment)	3			15,7	b	59,9
	tebufenozide (larvicidal treatment)	3			9,4	b	76
2004	untreated control		55,2	a			
	teflubenuzuron + thiacloprid	1+4	13,4	b			75,7
	teflubenuzuron + chlorpirifos-e	1+4	14,9	b			73
	teflubenzuron + tebufenozide	1+4	18,7	b			66,1
	teflubenuzuron + metoxyfenozide	1+4	14,1	b			68,7

A cool spring delayed development in 2004 and the first insecticide application with the IGR teflubenzuron was carried out late in May. When the fresh penetrations appeared after three weeks, the four larvicidal insecticides were sprayed four times at weekly intervals untill the end of the first generation, according to the results of the scouting activity. The efficacy of the tested strategies did not differ significantly and ranged from 66,1% to 75,7%.

Dipping test

The results of the dipping test are shown in table 2. The difference in mortality between the susceptible- and S. Michele-strain ranged from 1,7% to 3,5% for 5 out of 8 tested insecticides (diflubenzuron, flufenoxuron, indoxacarb, spinosad and thiacloprid). The larger size of the collected larvae compared to the newborn larvae used to calculate the dose-mortality line could account for the small discrepancy between the two series of data. The differences were larger when the apples where treated with tebufenozide (-8,7%), metoxyfenozide (-23,2%) and chlorpirifos-e (-41,8%).

Table. 2– Insecticides used in the dipping test: discriminating dose and mortality determined in the susceptible and tested strain.

a.i	trade mark	field dosage (ppm)	discr. dose (ppm)	% mortality (Susceptible strain)	%mortality (S.Michele strain)
Diflubenzuron	Dimilin	200	5000	90,8	87,3
Flufenoxuron	Cascade	70,5	2500	99,1	96,9
Indoxacarb	Steward	49,5	300	99,1	96,9
Thiacloprid	Calipso	101	100	96,1	93,5
Spinosad	Laser	144	450	99,0	97,3
Metoxifenozide	Runner	96	40	98,9	75,7
Tebufenozide	Mimic	192	1000	96,7	88,0
Chlorpyrifos-m	Reldan	446	18	98,4	57,6

Table 3 – Effectiveness of insecticides applied topically on diapausing larvae of both the S.Michele and the Changins susceptible strains; the mortality rate was compared using the chi-square test. (one, two or three stars for P = 0.05, 0,01 and 0,001 respectively)

	2002-2003		2002		2003	
insecticide	S.Michele	CH susceptible	S.Michele	CH susceptible	S.Michele	CH susceptible
diflubenzuron	75,9	73,9	58,4	92,6 *	93,33	55,38 *
chlorpirifos-e	23,2	91,3 ***	32,4	85,2 **	0	100 ***
chlorpirifos-m	71,9	100 ***	74,4	100 *	63,6	100 **
phosalone	58,4	100 **	58,4	100 **		
indoxacarb	54,46	82,6 *	32,4	70,4 *	73,33	100 *
imidacloprid				**		
	11,6	77,8 ***	11,6	77,8 *		
thiacloprid	71,3	100 ***	58,4	100 **	74,1	100 *
deltametrine	72,6	85,2	72,6	85,2		
spinosad	74	92,6	74	92,6		
emamectine	100	100	100	100	100	100
fenoxycarb	69,7	100 ***	84,4	100	51,2	100 ***
tebufenozide	63,68	82,6	68,8	70,4	52,1	100 ***
methoxyfenozide	56,04	92,59 *	37,6	92,6 **	71,9	

Topical test

The results of the topical test carried out on the diapausing larvae demonstrated that *C. pomonella* is resistant to a large number of insecticides. The data of 2002 and 2003 show that a significant reduction in the mortality was evident for the 3 tested organophosphorus insecticides, for the two neonicotinic insecticides and for indoxacarb, fenoxycarb and metoxyfenozide (tab. 3).

insecticides, for the two neonicotinic insecticides and for indoxacarb, fenoxycarb and metoxyfenozide (tab. 3).

The reduction in mortality was not significant for deltametrine, spinosad, emamectine and tebufenozide. For all of the tested insecticides but fenoxycarb and tebufenozide the results obtained in the two year study were consistent. Both fenoxycarb and tebufenozide caused a larval mortality not different from the susceptible strain in the first year, while it was reduced significantly in 2003.

Tab. 4 – Effectiveness of tebufenozide applied on different codling moth populations in comparison to a susceptible strain (chi-square test; one, two or three stars for P = 0.05, 0,01 and 0,001 respectively)

Location	Laborator y	Mortality abbott		Pest pressure	Pest control
CH susceptible	RAC	100			
S.Michele	RAC	52,1	***	high	conventional
Mezzocorona	RAC	70,8	*	medium	conventional
Gardolo 1	RAC	73,9	*	high	Untreated since 1999
Gardolo 2	RAC	53,3	**	high	Conventional
Gardolo 3	RAC	45,8	***	high	Organic since 1999
Mattarello	RAC	86,1		medium	Mating disruption
Borgo	RAC	93		low	Conventional
Zambana	IASMA	84,6		high	Mating disruption
Volano (reselé)	IASMA	100		low	conventional
Volano (curve)	IASMA	100		low	conventional
Dro	IASMA	100		low	conventional
Bleggio (noce)	IASMA	74,7		low	untreated
Strigno	IASMA	100		low	conventional
Tuenno	IASMA	89,5		low	organic
Revò	IASMA	100		low	Mating disruption
Bolentina	IASMA	100		low	organic
Pietramurata	IASMA	100		low	conventional
Pergine	IASMA	90		low	conventional
S.Michele	IASMA	34,5	***	high	conventional

Regional survey

In the area wide monitoring program tebufenozide was used as the reference insecticide for the detection of resistance. The S. Michele population was confirmed as significantly less susceptible than the laboratory strain (tab. 4). A significant reduction in the mortality rate was also reported for the Gardolo and Mezzocorona regions. All three populations collected in the Gardolo area exhibited a similar response to the test even though they differed in the number of insecticide treatments that they had received in the last three years: one orchard was abandoned and had not beem sprayed with insecticide, another was "organic" and had not been sprayed with conventional insecticides, by contrast, the third orchard received a conventional insecticide-control program. The area is highly urbanised with scattered

orchards. The codling moth population is large and numerous applications of insecticides have been applied in the past.
It is more difficult to explain the results obtained with the population from Mezzocorona; in this case the monitored orchards did not usually receive many insecticides applications and most of them are located in a rural area largely planted with wine grape. All the other monitored areas (13) did not show any significant reduction of activity of tebufenozide. These susceptible orchards are located either in the upper part of the region where codling moth populations are normally smaller or in the homogeneous apple district where the codling moth populations have been well controlled using mating disruption for 10 years.

Conclusions

The field trials demonstrated a reduction in the effectiveness of the insecticides that were tested. The dipping test did not detect any significant loss of activity for diflubenzuron, flufenoxuron, indoxacarb, spinosad or thiacloprid. A larger reduction in the effectiveness was detected only for the two MAC and for chlorpyrifos-m. The possible explanation is that the first step in the development of resistance is not a suddenly reduction of the insecticide effectiveness but frequently a reduction in the persistence of insecticide activity (Charmillot and Pasquier, 2002) as a consequence of a slow increase in the proportion of resistant individuals in the population. Then there will be a phase of rapid increase in the proportion of R individuals and at this time control failure will be observed.
The topical bioassay seems to be more sensitive for detecting resistance. Compared to the susceptible strain, the S. Michele population appeared significantly less susceptible to the majority of the tested insecticides. Only emamectine, spinosad and deltametrine seem not to be affected by the cross-resistance. The results of the regional survey carried out using tebufenozide as reference confirm that the suspected resistance is present in a very restricted area of Trentino where insecticides are used more frequently.

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Detection of codling moth (*Cydia pomonella*) resistance by topical application of insecticides and validation on a laboratory resistant strain by dipping of apples and incorporating products into artificial diets

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Abstract: Dose-mortality reference curves were established for 13 insecticides administered by topical application to diapausing larvae from a susceptible codling moth strain. Discriminating doses, determined for the detection of resistance in field-collected diapausing larvae, were between 1 and 10'000 ppm, depending on the product.

A resistance survey was carried out by topical application of discriminating concentrations on diapausing larvae of codling moth collected in Swiss and Italian orchards. In the worst cases, cross-resistance concerned 12 out of the 13 insecticides tested. In some other orchards, the pronounced loss of effectiveness seems to concern only some products, but the other insecticides also showed undeniable signs of "fatigue".

To validate the topical application technique as a detection tool, different products were tested in the laboratory with two other techniques on a codling moth susceptible strain (SS) as well as on a resistant strain (RS). Insecticides were applied either by dipping apples in solutions or by incorporating them into an artificial diet.

Key words: *Cydia pomonella*, insecticide, resistance, topical application, dipping of apples, artificial diet

Introduction

Ideally, detection of resistance would be based on codling moth larvae from damaged fruits. However, those larvae are at different larval stages and have different susceptibilities to insecticides. Adults cannot be used for testing IGIs and IGRs. Diapausing larvae remain the only stage susceptible to these insecticides which are available in sufficient numbers and are of a standardized age. These advantages outweigh efforts required in collecting diapausing larvae and limiting resistance studies to the spring.

To validate the topical application technique as a detection tool, different products were tested in the laboratory with other techniques on a codling moth susceptible strain (SS) as well as on a resistant strain (RS). Insecticides were applied either by dipping apples in solutions or by incorporating them into an artificial diet.

Materials and methods

Codling moth strains

A susceptible strain of the codling moth (SS strain) has been maintained at the Agroscope RAC Changins since 1997 in permanent breeding on an artificial diet or immature apples. The

larvae which supplied the stock were trapped over a period of a few years in corrugated paper bands in untreated traditional high stem orchards of the Lake Geneva region.
The resistant strain (RS) results from individuals surviving the tests of topical application carried out in the springs of 2002 and 2003. It has been maintained under pressure of selection per rearing on apples dipped in a solution of 5000 ppm of diflubenzuron in 2002 and 1000 ppm of tebufenozide from 2003 on.

Topical application on diapausing larvae
On the day of treatment, diapausing larvae were removed from cold storage and extracted from the corrugated cardboard. Using a micropipette, 1 µl of insecticide solution was applied to the dorsum of each larva. A few minutes after treatment, larvae began to spin a new cocoon in the corrugated cardboard. Larvae were placed in environmental chambers under controlled conditions (25°C, 70% RH, and L: D=16:8) until adults emerged. Experiments were conducted using at least 20 larvae per concentration. The untreated control consisted of larvae treated only with solvent. Reference curves elaborated from 13 insecticides on a laboratory susceptible strain (SS) allowed discriminant dosages to be chosen and applied for resistance detection on diapausing larvae collected in different suspected orchards or on our laboratory resistant strain (RS).

Dipping of apples
Apples were picked from an untreated plot. In the laboratory, the apples were dipped in 1 litre solutions of insecticide at different concentrations. For the larvicidal test, 60 newborn larvae (L_1) were distributed on the treated apples and the survival rate was determined after two weeks rearing. Reference curves were elaborated from a large number of insecticides on the SS strain; fewer insecticides were tested on the RS strain. A similar procedure was adopted for ovicidal tests. Codling moth adults were deposited for one or two nights on treated apples for egg laying and 8 days later egg hatching rates were determined.

Incorporation of insecticides into artificial diets
C. pomonella larvae were reared on an artificial diet into which the insecticides to be tested were incorporated. The survival rate was determined after 14 days rearing. Reference curves were elaborated using a large number of insecticides on the SS strain and some products also on the RS strain.

Analysis of data
The larvicidal or ovicidal effectiveness of the products was calculated in relation to the corresponding control experiment. The POLO-PC programme was used to determine dose-effect parameters (LeOra, 1987).

Results and discussion

Topical application
Dose-mortality curves for the 13 insecticides administered by topical application to diapausing larvae from the SS strain varied largely. LC_{50} values ranged from 0.1 ppm (mg.kg^{1}) for fenoxycarb to over 2'800 ppm for diflubenzuron and indoxacarb. Discriminating doses were determined from dose-mortality reference curves for the detection of resistance in field-collected diapausing larvae. They were chosen between 1 and 10'000 ppm depending on the product (Pasquier and Charmillot, 2003).

A resistance survey was carried out by topical application of discriminating doses on diapausing larvae collected in Swiss and Italian orchards. In the worst cases, cross-resistance concerned 12 out of 13 insecticides tested, i.e. diflubenzuron, fenoxycarb, tebufenozide, methoxyfenozide, indoxacarb, spinosad, phosalone, chlorpyrifos-methyl, chlorpyrifos-ethyl, deltamethrin, imidacloprid and thiacloprid. Emamectin, a new insecticide in development, was the only product not affected by cross-resistance. In some other orchards, the pronounced loss of effectiveness seems to concern only a few products, but the other insecticides also showed undeniable signs of "fatigue" (Charmillot *et al.*, 2003, 2004).
Discriminating doses applied on the RS strain revealed it was resistant to 7 of the 13 tested insecticides, i.e. diflubenzuron, fenoxycarb, tebufenozide, methoxyfenozide, deltamethrin, imidacloprid and thiacloprid (Figure 1).

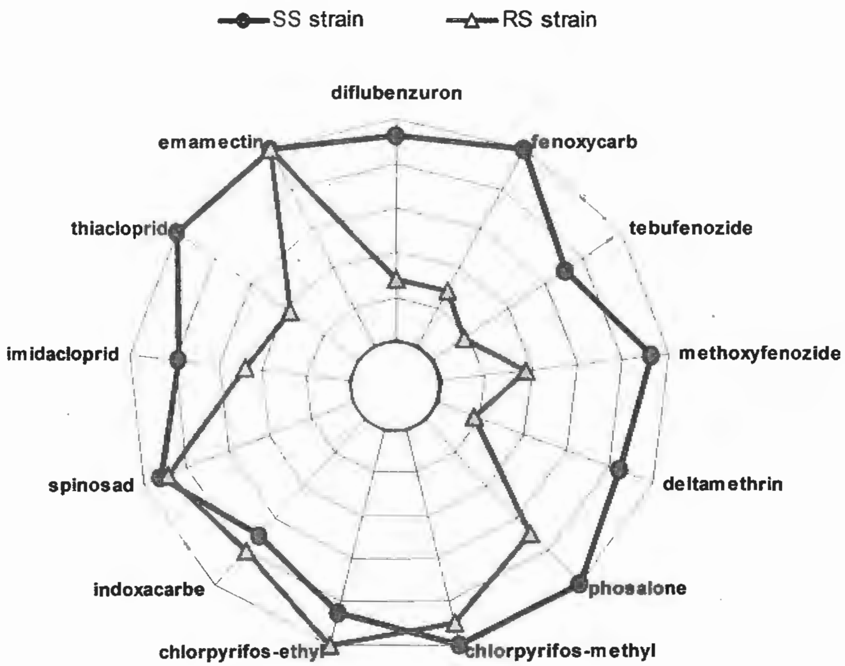

Figure 1. Effectiveness of products applied topically on diapausing larvae of susceptible SS and resistant RS strains of *C. pomonella*.

Comparison of SS and RS strains on an artificial diet
Compared to the susceptible SS strain, the RS strain presented a resistant factor, calculated at the LC_{50} level, which varied appreciably according to the tested product. In the case of diflubenzuron, the resistance factor reached 28 fold (Figure 2).

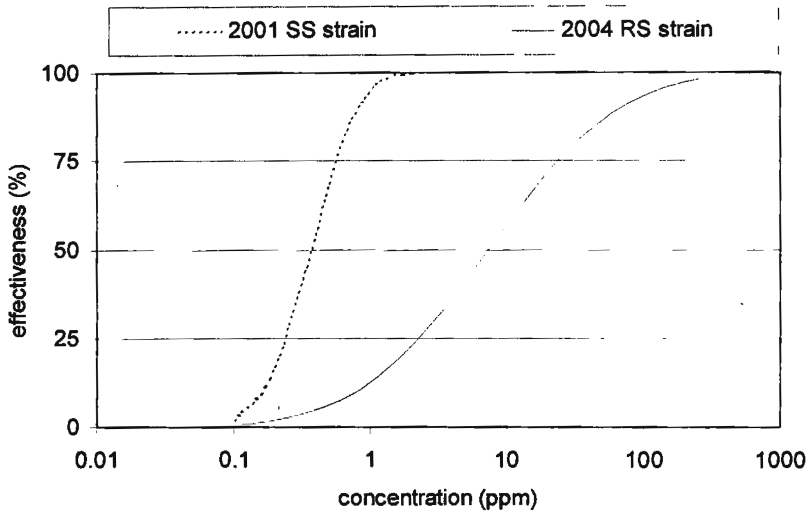

Figure 2. Effectiveness of diflubenzuron incorporated into an artificial diet on *C. pomonella* larvae of the susceptible SS and resistant RS strains.

Comparison of SS and RS strain by dipping of apples - larvicidal test

Compared to the susceptible SS strain, the RS strain seemed resistant to many insecticides. In the case of thiaclopride, the resistance factor at the LC_{50} level reached 6 fold. However, at concentrations higher than 10 ppm, effectiveness was almost the same on both strains (Figure 3). Tested by dipping of apples, the effectiveness of emamectin did not decline on the RS strain. Tests are in progress to compare the larvicidal effectiveness of other insecticides on both strains.

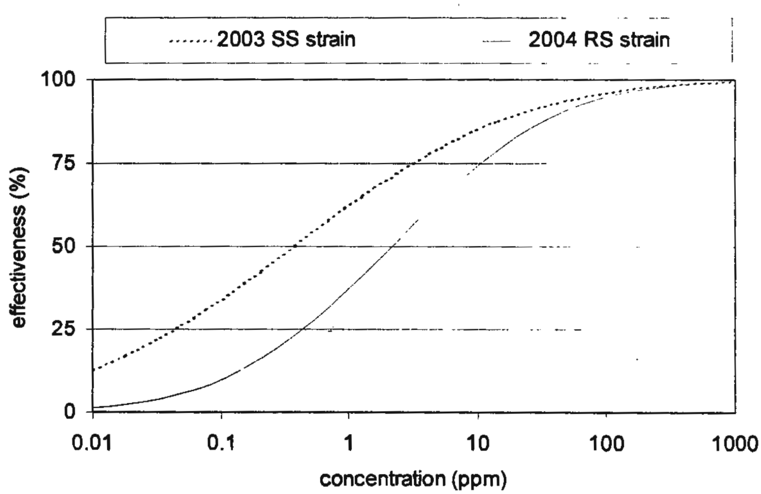

Figure 3. Effectiveness of thiaclopride applied by dipping of apples on *C. pomonella* larvae of susceptible SS and resistant RS strains.

Comparison of SS and RS strain by dipping of apples - ovicidal test

Compared to the susceptible SS strain, the RS strain seemed to be resistant on many insecticides, such as diflubenzuron, flufenoxuron and fenoxycarb. Tests are in progress to compare ovicidal effectiveness of other insecticides on both strains.

Conclusion

Topical application of discriminating concentrations of insecticides on the diapausing larvae collected in corrugated band traps in the previous season seems to be a sensitive technique for resistance detection in codling moth populations. That test has already been successfully used for resistance surveys in Switzerland, Italy and France (Charmillot *et al.*, 2003, 2004, Ioriatti *et al.*, 2000, Sauphanor *et al.* 2000).

In order to validate the topical application technique as a detection tool, trials are currently in progress of applying insecticides on both a codling moth susceptible strain (SS) and on a resistant strain (RS), either by dipping apples in products or by incorporating them into an artificial diet. For the majority of the products tested so far, the resistance of the RS strain detected by topical application seems to be confirmed by tests of dipping apples or by incorporation of the products into the artificial diet. However, the factors of resistance obtained vary according to the detection technique. The tests in progress must still be repeated to obtain statistically significant results.

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Biological control of *Metcalfa pruinosa* with *Neodrynus typhlocybae*: establishment and diffusion of the parasitoid in Trentino Alto Adige (Italy)

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Abstract: Since its recent and accidental introduction in Italy, *Metcalfa pruinosa* has become an important pest on several crops and wild plants also in the Trentino-Alto-Adige region. A release program of the parasitoid *Neodrynus typhlocybae* in six cultivated areas of the region was carried out from 1998 on. Monitoring trials carried out in 2003 and 2004, showed that *N. typhlocybae* is able to reproduce on *M. pruinosa* and to establish in the environmental conditions of this alpine region. Our observations confirmed the considerable ability of the parasitoid to spread in the environment (up to 27 km from the first release point after six years), even though the percentage of parasitization of *M. pruinosa* was below 10%. These results, together with some data on the biological activity of *N. typhlocybe*, allow us to conclude that inoculative releases of *N. typhlocybae* could be considered a good biological strategy to control *M. pruinosa* infestations over a medium-long period.

Key words: *Metcalfa pruinosa, Neodrynus typhlocybae*, biological control, release program, grapevine, apple orchards

Introduction

The introduction of *Metcalfa pruinosa* (Say) (Rhynchota:Flatidae) in Italy was documented for the first time in 1979 (Zangheri & Donadini, 1980) in the Veneto region. In 1992, *M. pruinosa* was recorded for the first time in Trentino Alto Adige (TAA), and in the subsequent years, with no natural enemies present, it reproduced became a very common pest of many cultivated (i.e. apple, grapevine), ornamental (i.e. *Cornus* sp., *Pittosporum* sp.) and wild plant species (i.e. *Cornus sanguinea, Viburnum major, Fraxinus ornus, Sambucus nigra, Ailanthus altissima*). The most severe damage on apple and grapevine crops by *M. pruinosa* is caused by the secretion of honeydew on fruits, which is the substrate for the growth of microfungi that cause sooty mould reducing the aesthetic appearance of the production.

In many areas of TAA this pest is controlled by chemical treatments, mainly organophosphorus insecticides. However, since *M. pruinosa* is polyphagous, widely distributed also in natural habitats, and has a high ability of migration, chemical control is not completely effective.

One of the main natural enemies of Flatid planthoppers is *Neodrynus typhlocybae* (Ashmead) (Hymenoptera, Dryinidae); this Dryinidae wasp, like its host, is native to the North American region and was first introduced in Italy in 1987 (Girolami & Camporese, 1994), to be used in classical biological control programs. *N. typhlocybae* searches the L3-5 instar nymphs of *M.*

pruinosa and lays one single egg on the body of the nymphs. The wasp larva develops and can be distinguished as a kind of cyst outside on the host. Finally, the larva spins a cocoon below the empty host body; adult emergence occurs in the summer, just a few months after the parasitization, or can be delayed from after the winter to the following spring, depending on the climatic conditions. The adult *N. typhlocybae* female can also prey neanids of the planthopper (Girolami & Mazzon, 1999) with a typical host-feeding behaviour that contributes to biocontrol. Herewith, we present some information on the establishment, diffusion and parasitization of the parasitoid in TAA.

Materials and Methods

Hymenoptera starving and origin

A strain of *N. typhlocybae* was collected on different wild plants (*Acer campestris*, *Ailanthus altissima*, *Sambucus nigra*, *Ulmus minor*) in the Colli Euganei area (Veneto, Italy) at the end of the summer 1997-2000, and introduced in TAA. The strain had been originally collected near Fairfield (USA) (Girolami & Camporese, 1994).

N. typhlocybae cocoons were stored inside a closed nest similar to the one described by Girolami & Mazzon (1999), and maintained in the open field till the following May.

Neodrynus typhlocybae *release*

A release program of *N. typhlocybae* pupae in six infested areas (with cultivated and wild plants) of the Trentino district (Dro, Arco, Avio, Rovereto, Marco, Trento) was conducted in May 1999, 2000, and 2001, by releasing in each area and year 100±20 overwintering pupae (sex ratio 1:1), which had been collected in the previous autumn; two release points per area, 200±50 m apart, were selected. *N. typhlocybae* pupae were hung on wild plants next to the grapevine and apple areas.

The aim was to evaluate the possibility of obtaining the establishment of the parasitoid and its progressive spread in order to obtain a permanent control of *M. pruinosa*.

Establishment, diffusion and parasitization activity of **N. typhlocybae**

The establishment, diffusion, and parasitization activity of the parasitoid were evaluated in 2004, 4-6 years after the first releases. The percentage of parasitization of *M. pruinosa* by *N. typhlocybae* was expressed as:

$$P\% = 100 \times (Np + Cn)/(Nv + E + Np + Cn)$$

Where:
P% = percentage of parasitization
Np = parasitized *M. pruinosa* nymphs
Cn = *N. typhlocybae* cysts (full + emerged)
Nv = : live *M. pruinosa* nymphs
E = L5 *M. pruinosa* ecdysis

Results and discussion

The survey showed that *N. typhlocybae* was able to reproduce on the L3-5 instar nymphs of *M. pruinosa*, that it established in at least one release point of each of the 6 areas under investigation (table 1). The wasp colonised the host preferentially on wild species, such as

Ailanthus altissima, Fraxinus ornus, and *Viburnum major*. The parasitoid spread out over a distance of up to 27 km.

The P% was generally higher in the immediate surroundings of the release point (<0.5 km), and reached a maximum of 9.1% in the Marco di Rovereto, where the wasp was firstly released (table 1).

Table 1. Establishment, diffusion and parasitization of *N. typhlocybae* in the six release areas at the end of 2004.

Release areas	**Release years**	**Reproduction in the release points**	**Max. No. of parasitoids/leaf**	**Spread distance (km)**	**P%**	
					<0.5 km	**>0.5 km**
Arco	2000-2001	2	3	> 2	4.2	1.3
Avio	2000-2001	1	2	> 1.5	2.6	0.9
Dro	2000-2001	2	6	> 4	4.3	1.5
Marco di Rovereto	1998-1999	2	9	> 27	9.1	5.5
Mezzolombardo	2000-2001	1	1	<1	0.5	0.8
Trento	1999-2000	1	4	>1	3.6	1.0

Conclusions

Due to its biological and ecological characteristics, such as its high reproductive potential, the specialized parasitoid-predator *N. typhlocybae* is one of the most frequent dominant natural enemies of the planthopper *M. pruinosa* in its native areas.

The release of *N. typhlocybae* for the biological control of *M. pruinosa* in natural and cultivated ecosystems was started in some Italian regions in the last years (Girolami & Camporese, 1994; Girolami *et al.*, 1996; Angeli *et al.*, 1997; Stefanelli & Villani, 1998; Tommasini *et al.*, 1998; Angeli *et al.*, 2001). The preliminary results discussed in this paper show that *N. typhlocybae* is able to reproduce on *M. pruinosa* and to establish also in an Alpine region like TAA. After six years, the success of the release program is stated by the wide spread of the wasp in the released areas. The percentage of parasitization was below 10 %, but this value does not account for the predator activity of *N. typhlocibae* females, and the biocontrol efficacy of the Dryinidae species may therefore be underestimated.

Previous Italian experiences have demonstrated that the establishment of the wasp is characterised by two phases: the wasp first spreads out, colonising the release area, and then an increase in the parasitization level is recorded, which can reach a peak of more than 80% after 8-10 years (Girolami, unpublished data). The rapid colonisation of the territory seems to be confirmed by the present survey.

We therefore conclude that inoculative releases of *N. typhlocybae* should be considered as a valid strategy for the biological control of *M. pruinosa* over a medium-long period.

Acknowledgements

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PHEROMONES

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Constant Monitoring Enhances the Success of Pheromones in IFP

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Abstract: In 1993 the Advisory Service decided to propose to its members to put the mating disruption method for the control of codling moth, *Cydia pomonella,* into practice. In 1993 232 ha of the South Tyrolean apple growing area were equipped with pheromone dispensers for codling moth. Until 2004 this area has risen to 14,000 ha, which amounts to 78 % of the total acreage in South Tyrol. In the past 12 years the technicians of the South Tyrolean Extension Service have therefore had ample opportunity to study the possibilities and limits of this environment-friendly plant protection technique and to increase and improve their experience in monitoring orchards with mating disruption.

Key words: mating disruption, codling moth, *Cydia pomonella*, monitoring

Introduction

From 1988 to 1992 the Research Station Laimburg and the South Tyrolean Extension Service for Fruit and Wine Growing tested the efficiency of mating disruption of codling moth, *Cydia pomonella,* on approximately 4 ha. In 1993 this technique was put into practice for the first time on a larger scale on 232 ha. The initial experiences with this new technology were so promising that the acreage increased to 2,500 ha in the following year. The ready acceptance of the mating disruption technique can also be attributed to the fact that codling moth became resistant to chitin synthesis inhibitors in South Tyrol in the early 1990s and it became imperative to find an environment-friendly replacement for them in our IP-programme. In 2004 pheromone dispensers were used on 14,000 ha apple orchard area, corresponding to 78 % of the total acreage. On the remaining 22 % of the South Tyrolean apple acreage (4,000 ha) there is virtually no codling moth or the orchards are too small and isolated to implement mating disruption effectively. Furthermore, there is still a small minority of growers who believe that they can resolve the problem only with the chemical club. In this report I would like to present some of our experiences.

Mating disruption of codling moth has always taken priority

In South Tyrol the mating disruption technique has always been focused on the control of codling moth. However, there have been several emergencies and attempts to use pheromones also for the control of other pests in apple growing. In the mid-1990s we used combined dispensers for codling moth and various leafroller species (e.g. *Adoxophyes orana, Pandemis heparana, Archips podana).* Since the spectrum of leafrollers varies depending on the season and the location this method did not assert itself against the highly effective traditional pesticides for leafrollers. At present one treatment per season is sufficient to control leafroller in most orchards whereas for codling moth up to 6 sprays would be necessary without mating disruption. Today combined dispensers for codling moth and leafrollers are used mostly in organically managed orchards.

In 1999 we discovered infestation with oriental fruit moth, *Cydia molesta,* in some areas where mating disruption of codling moth was practiced. Fortunately in the following year it turned out that oriental fruit moth can be controlled very effectively through mating disruption, too. In orchards where this is necessary (on approximately 2,000 ha) we use this technology also for oriental fruit moth.

Monitoring of areas with mating disruption of codling moth

Growers and technicians have two tools at their disposal for monitoring the infestation level: high-load lures and checking the fruits for entry holes.

1. High-load lures

We still recommend using pheromone traps with 10 mg-lures for monitoring moth flight in areas with mating disruption. We have learned, however, that you must not rely on the trap catches when assessing the infestation risk. The infestation may rise even if no codling moth males have been caught. Nevertheless, we still consider trap catches in orchards with mating disruption as a danger signal that there are either gaps in the pheromone cloud or that the population density is too high. Pheromone traps can supply additional information at best, but they can never replace fruit checks.

2. Fruit checks

2.1. Checking for wormy fruits at harvest

Establishing the number of wormy fruits at harvest plays an important role. The proportion of wormy fruits in autumn supplies information about the success or failure of codling moth control at the end of the season but it is also the basis for the control strategies in the following season.

- In orchards where harvest damage was below 1 % wormy fruits without additional sprays it is very likely that no additional treatments will be necessary in the following season, either.
- If harvest damage was between 1 and 3 % we recommend applying the first additional treatment against the larvae of the first generation as soon as the first entry holes are found.
- In orchards where in spite of mating disruption and several pesticide applications more than 3 % fruits are wormy in autumn you have to try to reduce the codling moth population drastically with the help of additional sprays. In those cases we recommend starting the additional sprays at the previously calculated time of egg hatch.

2.2. Fruit checks in the course of the vegetative period

- In orchards with a record of low codling moth infestation two checks per year are sufficient, one at the end of the egg hatch of the first generation and one shortly after half of the larvae of the second generation must theoretically have emerged. In our area these checks usually take place in late June/early July and at the end of July. Since the apples are hand thinned in June anyway, you can look out for codling moth infestation at the same time so that no separate fruit checks are necessary during that period. In this case the time required for checking 1,000 fruits amounts to not more than two times 30 – 40 minutes per orchard and season (in late July and at harvest).
- In areas with a high risk of codling moth infestation, on the other hand, fruit checks in intervals of 1 – 3 weeks are absolutely vital. The technicians can contribute by making sure that the date for the first checks is not set too early or too late. The South Tyrolean Extension Service has placed pheromone traps in all locations, also outside the orchards with mating disruption. As soon as male codling moths are caught continuously, Biofix 0 is set and a preliminary date is established when the first larvae

are likely to emerge in a certain location. Moreover, during this time we also keep in touch with the entomologists at the Research Stations Laimburg and San Michele so as to exchange information about egg deposition. Finally we check orchards with a high codling moth infestation in the preceding year particularly carefully in the days before egg hatch is expected to start. As soon as we find the first larvae we inform the growers via e-mail or circulars that the time for the first fruit checks has come.

- In orchards with a high infestation in the preceding year (> 3 %) it is absolutely indispensable to check for entry holes every week until the action threshold for an additional treatment is reached. Only in this way is it possible to determine the exact date for this first additional spray and, on the other hand, to save sprays.

The intervals between the checks after the first spray depend on the long-term effect of the pesticide used. A further treatment is required if the action threshold is exceeded again. (June 0.3, July 0.5 and August 0.8 entries per 1,000 checked fruits).

Codling moth infestation and additional sprays in apple orchards with mating disruption from 1994 – 2003

From the district Burggrafenamt, that is the area around Meran, we have a complete record of the harvest damage over 10 years. Thereby 1,000 fruits in each orchard are checked at random for codling moth damage. As can be seen from Table 1, in orchards with mating disruption the average proportion of wormy fruits has always been below 1 %. except in the year 2003.

Table 1. Codling moth harvest damage in apple orchards with mating disruption, Burggrafenamt, S. Tyrol

year	checked orchards	% harvest damage (Ø)	% orchards < 1% damage	additional sprays (Ø)
1994	421	0.5	86.7	1.5
1995	631	0.8	80.0	0.5
1996	91	0.5	92.3	0.6
1997	66	0.4	89.4	0.3
1998	156	0.9	76.9	0.5
1999	279	0.6	81.7	1.6
2000	187	0.3	92.0	0.7
2001	154	0.3	94.8	0.4
2002	184	0.7	85.8	0.6
2003	223	1.1	77.6	0.9

Even in 2003 the proportion of orchards with less than 1 % damage was relatively large (77.6 %).

As far as the average number of additional sprays is concerned, 3 years were unusual: In 1994 we had relatively little experience with this technique, which explains the relatively high number of additional treatments. In 1999 we had to use pesticides against oriental fruit moth in some areas. In 2003 codling moths found particularly favourable conditions for their development due to the high temperatures, which also required additional sprays in more orchards than usual.

Urban locations have a different rate of infestation

As has already been mentioned, codling moth found particularly favourable conditions in 2003, due to the long and hot summer. In this year the differences in codling moth damage between the orchards outside the villages and those near urbanized areas were more noticeable than usual. The average codling moth damage was 0.3 % in orchards situated farther away from settlements in contrast to 2.6 % in urbanized areas. Therefore the difference in the number of additional treatments was equally great: 0.4 in orchards outside villages and 1.8 in the built-up areas.

Can codling moth in urbanized areas be controlled better than so far through mating disruption and with fewer additional sprays?

In South Tyrol an estimated 10 % of orchards is situated near buildings or directly in built-up zones. In such locations the conditions are particularly favourable for codling moth because there it is warmer on average than in orchards which are far away from the villages. Furthermore, orchards situated in villages are often smaller than 1 ha. Both circumstances are not ideal for a successful implementation of the mating disruption technique. On the other hand, the proprietors of such orchards should use pesticides particularly carefully and sparingly so as to avoid conflicts with their neighbours. Therefore we established weekly the development of the infestation rate through fruit checks (1,000 fruits per check) in a number of orchards situated inside villages in order to keep the infestation rate as well as the number of additional sprays as low as possible. Following I would like to present two of these orchards:

Both areas are situated in Lana, South Tyrol, and are planted with Golden Delicious on seedling rootstocks. They are only 100 m apart. In both orchards mating disruption has been employed for 10 years (1,000 Isomate C+ dispensers/ha). Without mating disruption 4 – 6 sprays would have been needed to control codling moth, depending on the year and the pesticide used. In 2003 the harvest damage in orchard A was 13.0 % (1 additional treatment in mid-July), in orchard B 1.6 % (3 additional sprays). In 2004 we checked both orchards weekly.

In orchard A we decided in late May to use thiacloprid at the beginning of egg hatch. During the season the action threshold was exceeded and so an additional chlorpyriphos application became necessary. In this orchard we could achieve a harvest damage of only 1 % with merely two additional treatments through regular fruit checks.

In orchard B the first larvae were discovered in mid-June and treated with microencapsulated chlorpyriphos. In this orchard the harvest damage was 2 %, 3 additional pesticide applications were necessary.

These examples show that with constant monitoring the efficiency of the mating disruption technique can be improved even under difficult circumstances and sprays can be saved.

Conclusion

- Today mating disruption is the basis for codling moth control on three quarters of the apple acreage in South Tyrol.
- On about 50 % of the area with mating disruption codling moth populations can be kept at a low level without additional treatments.

- On about 40 % of the acreage with mating disruption 1 – 2 additional sprays are needed to keep harvest damage below 1 %.
- On about 10 % of the orchard area with mating disruption intensive monitoring is indispensable for limiting the number of additional treatments and preventing an increase of the population density.

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Mating disruption to control grapevine moth, *Lobesia botrana* (Den. and Schiff.) in Porto Wine Region : a three-year study

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Abstract: To evaluate the potential of mating disruption to control *Lobesia botrana* (Den. and Schiff.) in Porto Wine Region, a study was conducted during three consecutive growing seasons (2001 to 2003), over a surface of 3.0 ha in the first year, 25.0 ha in the second and 15.0 ha in the third. Performance of the pheromone treatment was assessed by comparing captures of moths in pheromone-baited traps and levels of infestation in the pheromone-treated plots with those in an adjacent untreated plot. The average percentage of male disorientation for the 3-year period ranged from 98 to 100%, being 100% in 63% of the 27 sampling periods studied, which suggests that treatment with pheromone almost completely prevented male moths from locating sources of synthetic pheromone. However, infestation on grape clusters at the pheromone-treated area was not reduced to a level where mating disruption could be used alone to control the pest, and insecticides had to be applied.

Key words: *Lobesia botrana*, grapevine moth, viticulture, mating disruption, pheromone

Introduction

The grapevine moth, *Lobesia botrana* (Den. and Schiff.) is a key pest of commercial vineyards mainly in Southern Europe. As a result, it has received considerable attention by researchers attempting to develop effective and environmentally safe control strategies against it. Sex pheromone-mediated mating disruption has proven successful to control the pest namely in Germany (Kast, 2001), Switzerland (Charmillot and Pasquier, 2001) and northern Italy (Varner *et al.*, 2001). However, inconclusive results have been obtained in southern regions, such as Italy (Nucifora *et al.*, 1996; Bagnoli *et al.*, 2001). In Portugal, mating disruption has been tried against *L. botrana* in some vine-growing regions, however either in small plots or for no more than one year (Aguiar *et al.*, 1999; Frescata *et al.*, 1999; Ribeiro *et al.*, 1999), also with inconclusive results. In this paper we describe the results obtained during three consecutive growing seasons at one commercial vineyard in Porto Wine Region.

Material and methods

The study was carried out at one wine-farm, Quinta de S. Luiz (Barros, Almeida & C^{a}. - Vinhos S.A.), in Porto Wine Region, over the three year period, 2001 to 2003. During 2001, the pheromone-treated plot was a 3.0-ha block of cv. Touriga Franca, a variety highly susceptible to *L. botrana.* A 2.7-ha section of vines of the same variety, in same vineyard, located 100 m south of the pheromone-treated plot was used as control. In 2002, pheromone dispensers were applied to a 25-ha block, which included on its north-west border, the plot treated in 2001. The block was composed by 16 plots with a surface ranging from 0.66 to

3.02-ha, bordered by stone walls and laneways, with dispersed trees, mainly olives and almonds. About 55 % of the surface was of cv. Touriga Franca, while the remaining surface contained several varieties (Tinta Roriz, Touriga Nacional, Rufete, Tinta da Barca and Tinta Barroca). The control was the same as in 2001. In 2003, pheromone dispensers were applied to a 15-ha section of the vineyard used in 2002. This section was composed by eight plots, including that which was pheromone-treated since 2001. The control was the same as in the two previous years.

Wire-type dispensers, developed by the Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan) were deployed at the recommended rate of 500 per hectare. In the border areas of 15-20 m, their density was increased by 20%. Dispensers were applied, on 12 March 2001, on 19 March 2002 and on 21 March 2003, prior to the emergence of the moths that give rise to the first generation.

Efficacy of the pheromone treatment was assessed by comparing pheromone-baited trap catches and grape infestation levels in the pheromone-treated (MD) and control plots. One pheromone delta trap (Agrisense BCS Ltd.) was placed in the control plot, at about its centre, in each of the three years of study. Two traps were placed in the pheromone-treated plot, in 2001 - one in its interior zone and the other in the border zone, six traps were used in 2002, two in the interior zone and four in the border zone and one trap was used in 2003, in the interior zone of the plot. One hundred randomly chosen grape clusters (i.e. an inspection station) were inspected for damage in three periods for each of the three generations of the pest and at harvest. Grape clusters were examined 21, 28 and 35 days after the peak of captures for the 1st generation and 7, 14 and 21 days after it, for the 2nd and 3rd generations except in 2003, when one only inspection was done for each generation. The percentage of male disorientation was calculated for each weekly trapping interval and the infestation by the grape moth as well as the percentage reduction of that infestation were calculated for each generation of the pest and for harvest time.

Table 1- Mean (±SD) male disorientation (%) for *Lobesia botrana,* during the flight period, 2001 to 2003. In parentheses, the number of trapping intervals (weeks).

Year	Station/location	First flight	Second flight	Third flight
2001	1 /border	100.0 (12)	99.9 ± 0.4 (7)	99.1 ± 3.1 (12)
	2 /interior	100.0 (12)	100.0 (7)	100.0 (12)
2002	1 /border	100.0 (11)	100.0 (7)	100.0 (8)
	2 /border	99.8 ± 0.7 (11)	100.0 (7)	100.0 (8)
	3 /border	98.0 ± 3.0 (11)	99.2 ± 1.7 (7)	98.8 ± 3.7 (8)
	4 /border	99.5 ± 1.8 (11)	99.5 ± 0.9 (7)	100.0 (8)
	5 /interior	100.0 (11)	100.0 (7)	100.0 (8)
	6 /interior	100.0 (11)	100.0 (7)	100.0 (8)
2003	1 /interior	99.8 ±0.7 (12)	100 (6)	99.1 ± 2.0 (11)

Results and discussion

The average percentage of male disorientation for the 3-year period ranged from 98 to 100%, being of 100% in 63% of the 27 sampling periods studied (Table 1), which suggests that treatment with pheromone almost completely prevented male moths from locating sources of synthetic sex pheromone. Except for 2003, all the captures obtained in the MD plots were done at their border zone.

In 2001, the infestation level during the first generation was low, with 28 nests per 100 flower clusters in the control plot (Table 2), for a recommended economic threshold (ET) of 100 to 200 nests (Ribeiro and Gonçalves, 2000). The reduction of the infestation level in the MD plots ranged from 83.2 to 92.9%, suggesting a good control of the pest. During the second generation the recommended ET, which is of 1 - 10% of grape clusters infested (Ribeiro and Gonçalves, 2000), was surpassed either in the control plot or at one the MD border stations, but not at the two other MD stations. The reduction of the infestation level ranged between 77.7 and 100%. A spray with flufenoxuron was done against this generation in the control and in the border of the MD plots. During the third generation the ET (1 - 10% of grape clusters infested) was exceeded in all plots and the reductions of the infestation levels were lower than in the previous generation (Table 2). The harvest level of damage was high and similar at the control (47.0%) and at the MD plots (between 31.0 and 55.0%).

Table 2. Infestation (mean ±SD)* by *Lobesia botrana* and reduction of the infestation (%) at the MD plots in relation to the control, in 2001

Station/	1st generation		2nd generation		3rd. generation		harvest	
/location	infestation	reduc.	infestation	reduc.	infestation	reduc.	infestation	reduc
MD1/inter.	3.7±3.5	86.8	0.3±0.6	97.1	16.0±9.5	21.2	55.0	**
MD2/bord.	2.0±0.0	92.9	2.3±4.0	77.7	10.0±6.0	50.7	47.0	0.0
MD3/bord.	4.7±4.6	83.2	0.0±0.0	100.0	4.7±0.6	76.8	31.0	34.0
control	28.0±18.1	-	10.3±4.7	-	20.3±7.6	-	47.0	-

* number of nests or infested flower clusters (for the 1st generation) or number of infested grape clusters (for the 2nd and 3rd generations) per 100 inspected; each value is the mean of three estimates; ** the estimated infestation was higher in the control than in the MD plot.

In 2002, the pressure of the pest was higher than in 2001, either during the first generation (36.3 nests per 100 grapes in the control), or during the second one (14.7% of grape clusters infested), and the reduction of the infestation levels was lower (between 42.1 to 69.7% during the first generation and between 16.3 to 72.8% during the second). In two MD border plots stations, the damage was even higher than in the control (Table 3). In the observed conditions a spray was applied against the first generation, with phosalone and another one against the second with flufenoxuron. During the third generation, the infestation level at the control plot was 7.0% and the reduction of the infestation at the MD plots was between 71.0 and 86.0% at the interior ones and between 14.3 and 75.7% in those of the border. A spray was carried on at the control and at the MD border plots, with lufenuron. The harvest level of damage was 5.0% at the control and between 1.0 and 3.0% at the MD plots.

In 2003, the infestation levels were similar to those of 2002, during the first and second generations (respectively 31 nests per 100 flower clusters and 13.0% of grape clusters infested, in the control). The reduction in the infestation levels was between 58.1 and 93.5% in the first generation and between 38.5 and 100.0% in the second. In spite of the relatively high levels of damage observed during this generation in the control and in most of the MD plot stations, no treatments were applied against *L. botrana*. During the third generation the infestation levels were low (only 1.0 grape cluster infested per 100 inspected in the control), which can be explained by occurrence of a period of warm spell days in the region. The harvest level of damage, although high in most of the plots, was higher in the control (52.0%) than in the MD plots (1.0 to 36.0%), suggesting some efficacy of the mating disruption to control the pest.

Table 3. Infestation (mean ±SD)* by *Lobesia botrana* and reduction of the infestation (%) at the MD plots in relation to the control, in 2002

Station/	1st generation		2nd generation		3rd generation		harvest	
/location	infestation	reduc.	infestation	reduc.	infestation	reduc.	infestation	reduc
MD1/inter.	13.3±9.1	63.4	5.0±4.6	66.0	1.0±1.0	85.7	1.0	80.0
MD2/inter.	14.3±9.3	60.6	5.3±2.1	63.9	2.0±1.0	71.4	3.0	40.0
MD3/bord	16.3±7.0	55.1	16.3±7.4	**	6.0±1.0	14.3	1.0	80.0
MD4/bord	11.0±8.2	69.7	4.0±1.0	72.8	1.7±0.6	75.7	2.0	60.0
MD5/bord	21.0±6.6	42.1	12.3±4.0	16.3	4.0±1.0	42.9	1.0	80.0
MD6/bord.	20.7±6.1	43.0	20.0±9.5	**	5.0±2.6	28.6	2.0	60.0
control	36.3±17.6	-	14.7±2.3	-	7.0±2.6	-	5.0	-

* number of nests or infested flower clusters (for the 1st generation) or number of infested grape clusters (for the 2nd and 3rd generations) per 100 inspected; ** the estimated infestation was higher in the control than in the MD plot.

Table 4. Infestation* by *Lobesia botrana* and reduction of the infestation (%) at the MD plots in relation to the control, in 2003

Station/	1st generation		2nd generation		3rd. generation		harvest	
/location	infestation	reduc.	infestation	reduc.	infestation	reduc.	infestation	reduc
MD1/inter.	7.0	77.4	0.0	100	0.0	100	19.0	63.5
MD2/bord	5.0	83.9	4.0	69.2	2.0	**	27.0	48.1
MD3/bord	2.0	93.5	5.0	61.5	0.0	100	36.0	30.8
MD4/bord	13.0	58.1	3.5	73.1	1.0	0.0	34.5	33.7
MD5/bord	3.0	90.3	8.0	38.5	0.0	100	1.0	98.1
control	31.0	-	13.0	-	1.0	-	52.0	-

* number of nests or infested flower clusters (for the 1st generation) or number of infested grape clusters (for the 2nd and 3rd generations) per 100 inspected; ** the estimated infestation was higher in the control than in the MD plot.

This three-year study suggests that treatment with pheromone almost completely prevented male moths from locating sources of synthetic pheromone. However, infestation on grape clusters at the pheromone-treated area was not reduced to a level where mating disruption could be used alone to control the pest, and insecticides had to be applied.

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Codling moth management: from I.H.E.L.P. to M.A.P.S. to A.K.I.S.S.

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Abstract: Three new approaches to manage codling moth with semiochemicals are being investigated. The I.H.E.L.P. (Internal High Emission Low Point) strategy is the use of an internal grid of clusters of sex pheromone dispensers. This approached has been tested for three years and has been as effective as the standard use of 500 hand-applied dispensers and has reduced the cost of applying dispensers 35%. M.A.P.S (Multiple Attractive Point Sources) is the use of microencapsulated sex pheromone formulations applied in an ultra low volume of water. This approach has increased the total deposition of capsules within the tree canopy and has significantly improved the performance of sprayables. A.K.I.S.S. (Attractive Killing Interception Sensory Station) is the use of the pear ester in combination with codlemone to attract and kill male and female codling moths that contact insecticide-impregnated stations. This approach is still being improved and offers a new tool to manage codling moth.

Key words: *Cydia pomonella.* sex pheromone, kairomone, pome fruit

Introduction

The use of sex pheromones in the western U.S. has been rapid since 1991 and adopted on nearly 40,000 ha in Washington State alone (Brunner et al. 2002). Various hand-applied dispensers registered for codling moth have accounted for > 90% of this treated acreage (Thomson et al. 2000). The adoption of sex pheromones was the cornerstone of an area wide program in the western U.S. that was highly successful in managing codling moth and allowed dramatic reductions in the use of insecticides (Calkins et al. 2000). Unfortunately, many growers using sex pheromones in Washington today also apply a full-season insecticide spray program.

New approaches are needed to improve the effectiveness of sex pheromone for management of codling moth. The use of aerosol puffers developed by H. Shorey has been used successfully within an area wide program in California (Elkins 2002). Puffers placed in an internal grid within orchards at a density of 1 per hectare were also successful when the orchards perimeter was treated with individually applied dispensers (Knight 2002). Unfortunately, puffers have been unreliable mechanically and have not been widely adopted. An alternative approach may be to substitute puffers with clusters of dispensers. Results from studies evaluating this approach from 2001-03 are reported here.

Sprayables for codling moth have been widely tested in the United States but have not been adopted by growers (Knight 2000). Their poor performance has been blamed on their short residual activity in the field due to instability of their formulations (Eng. et al. 2003). Alternatively, my studies have focused on improving their efficacy by increasing the deposition and retention of capsules within orchards' canopies (Knight et al. 2004). Preliminary studies are reported that compare an ultra low volume (ULV) application versus the standard air blast application.

The discovery of the potent attraction of the pear ester for female codling moth has offered a new and potentially more effective attract and kill approach (Knight et al. 2001). An array of traps baited with a codlemone and pear ester and treated with an insecticide were highly successful in reducing fruit injury through midseason. The efficacy of this program dropped sharply from July – September due to a reduced efficiency of moth catch. Studies found a clear interception trap baited with a pear ester lure caught 9X more females than a delta trap. A killing station (A.K.I.S.S.) based on this design was developed and tested in 2004.

Materials and methods

I.H.E.L.P.

Studies were conducted in 21 apple orchards (250 ha) from 2001-03. Orchards were treated with clusters of either Isomate-C PLUS or -C TT dispensers spaced in an internal 50 x 50 m grid beginning 25 m from the edge of the orchard (4 per ha). The perimeter was also treated with a 10-20 m wide band of hand-applied Isomate-C PLUS dispensers at a rate of 1,000 per ha. Comparison orchards were selected based on similarity in size, proximity, cultivar, ownership, spray practices and pest pressure and were treated with Isomate-C PLUS dispensers applied at a rate of 500 dispensers per ha. Mean moth catch per trap, number of insecticide sprays, and percent fruit injury in orchards were recorded and transformed data were analyzed with a paired t-test.

M.A.P.S.

Studies were conducted in replicated apple blocks (1-2 ha) to compare the efficacy of applications of Checkmate® CM-F with either an air blast (926 liters per ha) or an ULV sprayer (12 liters per ha). Codlemone was applied in all plots at a rate of 49.0 g a.i. per ha. Two applications were made timed 4 weeks apart. Untreated blocks were included in the study and all plots received one insecticide application. Blocks were monitored with traps and fruit injury was sampled at mid-season. Transformed injury data were analyzed with ANOVA. The density and distribution of microcapsules were estimated by spraying a similar formulation with 0.50% fluorescent dye added. The density of fluorescent microcapsules per leaf were counted on ten leaves from ten shoots collected from the lower and upper canopy. Capsule density as a function of canopy height and between the top and bottom of leaves were analyzed with a paired t-test for each spray method separately.

A.K.I.S.S.

Clear plastic sheets (0.1 m^2) were coated with clear, long-lasting grease mixed with 6.0% esfenvalerate. Eight apple plots were established (0.5 – 1.2 ha) in both Orondo and Moxee during 2004 and treated with 60 killing stations per ha. All plots in Orondo were treated with 1-2 insecticide applications. All plots in Moxee were treated with two ultra low volume applications of sex pheromone. Fruit injury was assessed at mid-season and transformed data were analyzed with a paired t-test.

Results

I.H.E.L.P.

Orchards treated with clusters of dispensers had similar cumulative moth catches (6.9 versus 6.5) and supplemental insecticide sprays applied (1.5 versus 1.8) as orchards treated with individual dispensers. Similarly, fruit injury by codling moth was not significantly different between orchards treated with clusters versus hand-applied dispensers (0.11 versus 0.10%). None of these differences were significant, P's > 0.80 in all tests.

M.A.P.S.

Moth counts were reduced 68% and 92% in the air blast and ULV-treated plots versus the untreated plots. Significant differences occurred in the levels of fruit injury among treatments (P = 0.01) with the ULV treatment having significantly less injury than the untreated plots.. Mean percent fruit injury was 8.1, 4.9, and 1.2% in the untreated, air blast, and ULV treatment, respectively. The mean density of capsules in the air blast treatment was 2.9 capsules per leaf, and the highest density was 14 capsules per leaf. The ULV application deposited an average of 19.1 capsules per leaf and a maximum of 157 microcapsules per leaf. The air blast application deposited a uniform distribution of capsules within the canopy while significantly more capsules were deposited in the upper than lower canopy with the ULV application (P < 0.01). Both application methods deposited nearly three times more capsules on the underside than on the top of leaves (P's < 0.01).

A.K.I.S.S.

Significant and near significant differences in fruit injury occurred with the addition of A.K.I.S.S. Plots treated with A.K.I.S.S. averaged 9.4% versus 16.6% fruit injury in the untreated plots in Orondo (P = 0.06) and 0.8% versus 2.1% in Moxee (P = 0.02), respectively. A number of problems were noted with this approach and the test was terminated at midseason. These included a loss in the toxicity of the panes due to removal of grease from abrasion with foliage and the cumulative effects of rain and wind; and the breakage of stations due to wind. Repellency of the killing stations and placement of the lure above the station were later found to have reduced moth contact with the treated surface of the station.

Discussion

New approaches using the sex pheromone and the pear ester kairomone are being developed to improve the management of codling moth. The use of the I.H.E.L.P. design may allow the development of a lower cost, effective management program for codling moth to be developed. Clusters of dispensers were as effective as the current program of hand-applied dispensers and reduced the cost of application by 35%. This approach was adopted on 1,300 ha during 2004 in Washington State. However, the use of aerosol puffers may offer greater flexibility in further lowering the cost of mating disruption by reducing their emission rate from 7.5 to 5.0 mg A.I. per puff and their density to $\leq$ 1 per ha. In addition, the proportion of virgin female moths has been significantly higher in puffer-treated orchards than with hand-applied dispensers.

Sprayable formulations of codlemone have not been effective in my grower's trials when applied with an air blast sprayer over the past three years. Results reported here suggest that reducing the spray volume can enhance the performance of these microencapsulated formulations by increasing the density of capsules deposited on foliage. Creation of hundreds of attractive point sources (leaves with $\geq$ 80 capsules) per tree with this approach may improve mating disruption by enhancing the role of false-trail following. Mixing concentrated insecticides with this low volume of water is being investigated as a new paradigm for IPM. The addition of the pear ester with codlemone with and without insecticide has also been suggested (Light et al. 2004).

Killing stations baited with pear ester and codlemone can be an effective tool to manage codling moth. However, several operational changes still need to be made and these improvements sufficiently field-tested. Currently a new design for the killing station has been constructed and limited testing suggests that it will be much more effective than the design used in 2004. Studies are also continuing to develop a more effective kairomone lure for codling moth in cooperation with Dr. Light.

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The parasitoids of the European grapevine moth (*Lobesia botrana* Den.-Schiff.) and predators in the mating disruption-treated vineyards in Turkey

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Abstract: The study has been conducted in mating disruption (MD) vineyards against *Lobesia botrana*, the key pest, between the years of 2003 and 2004. The methods used in this study were the sampling of leaves and different developmental stages of *L. botrana*, and visual counts. The predatory species determined were *Chrysoperla carnea, Scymnus* spp., *Orius* spp., *Scolothrips* sp. and predatory mites. Number of chrysopids and coccinellids increased in Mating Disruption vineyards compared to Comparison vineyard. The parasitism rate of *L. botrana* pupae was found as 18,2 % in MD_1, 72,5 % in MD_6 and 79,7 % in MD_7 in 2003. Additionally, no parasitoid have emerged from *Lobesia botrana* eggs collected in Mating Disruption and comparison vineyards. It can be concluded that the parasitism rate of *L. botrana* may increase and support the efficacy of the technique.

Key words : Grape, *Lobesia botrana*, secondary pests, parasitoids, predators, mating disruption

Introduction

Sultana (=Sultanina, Thompson seedless) is one of the major crops cultivated in 160 thousand hectare-vineyards in the Aegean Region of Turkey. A great majority of production is dried. Annually, 250 thousand tons-raisin is exported from the Aegean Region. Turkey is the second largest and the biggest in terms of raisin production and export in the world (Anonymous, 2003). The European grapevine moth (*Lobesia botrana* Den.-Schiff.) is considered to be the most significant pest of the grapevine in Turkey. It produces 3-4 generations per year. Damage caused by larvae of third generation is the most serious because the larvae destroy the berries directly. Forecasting system has been used for the timing of applications against the pest in the vineyards since 1984. Mating disruption technique has been used in commercial vineyards against the pest in Turkey since its registration in 2002 (Altindisli et al., 2002). It is preferred by organic grape growers in Turkey. The number of studies on the parasitoids and predators of pests in organic vineyards is very limited (Altindisli *et al.*, 2002, Koclu *et al.*, 2002). Through the study, it is aimed that the state of parasitoids and predators, their relations and contribution to the efficacy of mating disruption would be revealed in the vineyards of the Aegean Region.

Material and methods

The study has been conducted in mating disruption-treated vineyards since 2002, in the centre of Manisa Province, which is important for viticulture in the Aegean Region. Three sampling vineyards (MD_1, MD_2 and MD_3), in which mating disruption was applied, and one Comparison vineyard (C_1) have been chosen for the counts and assessment in 2003. When the first adult was captured in MD vineyards, Isonet-L dispensers had been installed at 8 m intervals on each row (1 dispenser/24 m^2) and 2 m intervals on the borders. Timing of chemical applications against *L. botrana* in C_1 vineyard has been decided by means of Forecasting System. Organophosphorous insecticides were used in C_1 vineyard four or five times against the pest. MD_3 was excluded from the study in 2004.

Sampling

Sampling was only conducted in MD_1 and C_1 as the representatives for application types to observe the fluctuation of predatory population. 25 leaves from 4^{th} or 5^{th} leaves of shoot tips have been brought, fortnightly. Two surfaces of leaves were observed under the microscope.

Collecting

Individuals at different stages such as egg, larva and pupa of *L. botrana* have been collected from vineyards. They have been maintained individually in the incubator to determine parasitism. Eggs of the pest were dipped into the fungicide solution (% 0,5) to prevent the berries from molding.

Corrugated cardboard collars were bandaged around the trunks and young shoots with clusters to find the overwintering pupae of *L. botrana* in trial vineyards on 1 October 2003. They were collected on 23 October 2003. These pupae have been maintained in the incubator to determine parasitism (Schirra and Louis, 1998). In addition, predators on 100 flower-grape clusters have been controlled and recorded throughout the study.

Results and Discussion

The predators on 25 leaves are shown in Tables 1 and 2. Number of predatory mites was very low in 2003 and 2004. It was concluded that low population level of predatory mites could be resourced from low population density of secondary pests on the leaves and inevitable sulphur applications in organic grape growing against Powdery mildew (*Uncinula necator*), the main disease, and Grape Erineum Mite (*Eriophyes vitis*).

The results obtained from 100 flower-grape clusters from MD_1 and C_1 in 2003 and 2004 have been shown in Tables 3 and 4. No predatory species was found in C_1 vineyard throughout the season in 2004. For that reason, any result related C_1 is included in Table 4.

It is clear that the population density of predators has increased at the end of vegetation period because of an increase in pest population density (Tables 1 and 2). It should be considered that predator population is higher in MD vineyards than comparison vineyard since any insecticide application has been made against the pests (Tables 1, 2, 3 and 4).

Table 1. Pests and predatory species found on the leaves from MD_I and C_I vineyards in 2003 (number/25 leaves)

Date	Vineyard	GT*	GLH*	GEM*	SP*	Tyd.*	Chry.*
02.06.	MD_I	0	0	0	0	0	0
	C_I	0	0	2	0	2	0
24.06.	MD_I	0	0	5	0	0	0
	C_I	0	1	7	0	0	0
18.07.	MD_I	0	0	0	1	0	0
	C_I	0	0	0	0	0	0
25.07.	MD_I	9	0	13	0	0	0
	C_I	3	0	0	0	0	0
29.07.	MD_I	3	0	0	0	0	1
	C_I	0	1	0	0	0	0
11.08.	MD_I	0	0	0	0	0	0
	C_I	0	0	0	3	2	0
20.08.	MD_I	0	0	0	0	0	0
	C_I	1	0	0	6	0	0

*: GT: Grape Thrips, GLH: Grape Leafhoppers, GEM: Grape Erineum Mite, SP: Two spotted spidermites, Tyd: Tydeid mites, Chry.: *Chrysoperla carnea*

Table 2. Pests and predatory species found on the leaves from MD_I and C_I vineyards in 2004 (number/25 leaves)

Date	Vineyard	GT*	GLH*	GEM*	Tyd.*	Chry.*	Any.*
14.05.	MD_I	0	0	2	0	0	0
	C_I	0	0	1	2	0	0
26.05.	MD_I	0	0	0	0	1	0
	C_I	0	0	3	0	0	0
04.06.	MD_I	1	1	2	0	3	0
	C_I	4	1	1	0	0	0
22.06.	MD_I	0	0	4	1	1	0
	C_I	0	0	1	0	0	0
06.07.	MD_I	3	0	8	0	0	0
	C_I	2	0	8	0	0	0
20.07.	MD_I	3	0	32	0	0	0
	C_I	0	0	20	0	0	0
03.08.	MD_I	93	0	93	0	0	0
	C_I	2	1	22	1	0	0
24.08.	MD_I	35	0	76	7	0	1
	C_I	3	0	43	0	0	0

*: GT: Grape Thrips, GLH: Grape Leafhoppers, GEM: Grape Erineum Mite, Tyd: Tydeid mites, Chry.: *Chrysoperla carnea,* Any.: *Anystis baccarum*

Table 3. Predatory species on 100 flower-grape clusters in MD_1 and C_1 vineyards in 2003

Vineyard	MD_1				C_1			
Species	*Chrysoperla carnea*			Coccinellid	*Chrysoperla carnea*			Coccinellid
Date	Egg	Larva	Adult		Egg	Larva	Adult	
10.06.	1	0	0	1	0	0	0	0
16.06.	2	0	1	3	1	0	1	0
17.07.	14	0	11	0	3	0	0	0
28.07.	20	3	7	2	6	0	2	0
11.08.	21	1	2	1	2	0	0	0
20.08.	15	0	4	4	4	0	0	4

Table 4. Predatory species on 100 flower-grape clusters in MD_1 and MD_2 vineyards in 2004

Species		*Chrysoperla carnea*				*Cocc.*	*Orius*	*Phytos.*	*Anystis*	*Scolothr.*
Date	Vineyard	Egg	Larva	Pupa	Adult					
04.05.	MD_1	0	0	0	0	0	1	9	0	0
10.06.	MD_1	17	1	0	1	0	0	0	0	0
	MD_2	2	0	2	2	1	0	0	0	1
17.06.	MD_1	6	0	0	0	0	0	0	0	0
	MD_2	8	0	0	0	2	0	0	0	0
14.07.	MD_1	16	0	0	0	0	0	0	0	0
20.07.	MD_1	7	0	0	1	1	0	2	0	0
27.07.	MD_1	24	0	0	0	2	0	0	0	0
03.08.	MD_1	10	0	0	0	0	0	0	0	0
	MD_2	10	0	0	0	0	0	18	1	0
25.08.	MD_1	9	0	0	0	0	0	0	0	0
	MD_2	4	0	0	0	0	0	0	3	0

Table 5. Parasitism rates of *Lobesia botrana* larvae and pupae in 2003

Date	MD_1			MD_2			MD_3			C_1		
	# of L*	# of PL*	Paras.* (%)	# of L*	# of PL*	Paras.* (%)	# of L*	# of PL*	Paras.* (%)	# of L*	# of PL*	Paras.* (%)
21.05	19	1	5,3	0	0	0	0	0	0	3	0	0
16.06	5	0	0	0	0	0	0	0	0	0	0	0
24.07	2	2	100	0	0	0	0	0	0	0	0	0
11.08	2	0	0	0	0	0	0	0	0	0	0	0
13.10	1	0	0	13	3	23	2	2	100	2	0	0
23.10	0	0	0	46	44	96	38	27	71	1	0	0
04.11	4	3	75	0	0	0	0	0	0	0	0	0
Total	33	6	18,2	59	47	79,7	40	29	72,5	6	0	0

*: L: Larvae, PL : Parasitised Larvae, Paras.: Parasitism Rate (%)

Parasitoids found in MD vineyards in 2003 were identified as *Campoplex capitator* Aub., *Venturia* sp. and *Chirotica* sp. (Hym.: Ichneumonidae). It is first record on grape for Turkey.

Samples of Braconidae Family is in identification process. No parasitoid has emerged from 55 and 23 collected eggs in MD vineyards in 2003 and 2004, respectively. In addition, 5 larvae from MD vineyards and 8 larvae from C1 were collected in 2004. No parasitoid was found from these larvae. As shown in Table 5, the parasitism rate of the last generation of *L. botrana* was very high in MD_1, MD_2 and MD_3 vineyards in 2003. For that reason, population density of the pest was very low at the first generation with the infestation rates of 0% in MD_1 and 1,5% in MD_2 in the following year, 2004. It can be concluded that biological control is an effecting factor which support the efficiency of mating disruption technique.

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Pheromone release by individual females of *Lobesia botrana* (Den. *et* Schiff.) (Lepidoptera Tortricidae) and their competition with pheromone dispensers

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Abstract: The role of aged dispensers in improving Mating Disruption (MD) efficacy against the Grapevine Moth, *Lobesia botrana* (Den. *et* Schiff.) (Lepidoptera Tortricidae), was evaluated in relation to natural pheromone sources. The release rate of the main pheromone component of calling females and of 12-month field aged MD dispensers (Isonet® L+E) was quantified by using solid-phase microextraction (SPME). The quantity of pheromone released by aged dispensers was 46 times higher in 2002 and 13,000 times higher in 2003 than that of a calling female. In wind tunnel bioassays males responded to pheromone rubber septa as well as to aged dispensers. Field trapping tests confirmed that aged dispensers still attract *L. botrana* males. The results demonstrate that field aged pheromone dispensers could contribute to the success of MD programs.

Keywords: (E,Z)-7,9-dodecadienyl acetate, mating disruption, SPME, wind tunnel, field trapping.

Introduction

The Grapevine Moth (GM), *Lobesia botrana* (Den. *et* Schiff.) (Lepidoptera Tortricidae), is the most important pest in European vineyards. The use of pheromone based control methods has recently increased and Mating Disruption (MD) has been adopted successfully in the majority of the vineyards of Trento Province (Northern Italy). However, the efficacy of this method is not satisfactory when the treated area is limited in size. Moreover, the cost of the application is not always comparable to that of chemical control. A reduction in the number of MD dispensers per hectare seems to be a realistic approach to keep the cost at a competitive level. According to Varner *et al.* (2001), MD is still effective provided that the number of dispensers is reduced after previous pheromone treatments, and that the target pest occurs at low population density levels. In this study we investigated whether 12-month field aged MD pheromone dispensers (Isonet® E+L, Shin Etsu, Japan) can release pheromone in large enough quantities to compete with the natural pheromone released by GM females, and thus have the potential to improve the efficacy of the method.

Material and methods

Insects

Insects were collected in the vineyards of the Agricultural Institute of San Michele all'Adige (IASMA) and reared on a semi-artificial diet. Rearing cages and experiments were housed in climatic chambers (22±2°C, 70±10% R.H., 17L:1T:6D photoperiod).

Dispensers

Isonet® L+E dispensers loaded with 210 mg of E7,Z9-12:Ac, the main pheromone component of *L. botrana*, and 210 mg of Z9-12:Ac, the main pheromone component of *Eupoecilia ambiguella* (Hübner), were used. In both 2002 and 2003, dispensers were placed in the vineyards of the IASMA on April 15, and retrieved after 12 months. Distance between rows was 3 m, and distance between plants within rows was 1 m (Pergola trailing system).

Release rate of pheromone

SPME in static air was used for effluvia collections (Rotundo *et al.*, 2001). Volatiles were adsorbed from the headspace using a fiber coated with a 100 μm of polydimethylsiloxane (PDMS) (SUPELCO, Bellefonte, PA), first conditioned for 5 minutes in a GC injection port at 220°C. The SPME fiber was directly inserted into the GC for thermal desorption (5 min) and analysis. Chemical analyses were performed on a HP 5890 GC, equipped with a INNOWAX fused silica capillary column (30 m x 0.32 mm i.d., f.t. 0.5 μm) programmed from 60°C (hold 3 min) at 8°C/min to 220°C (hold 7 min), interfaced with an electroantennogram apparatus (GC-EAD). Identity confirmation of the compound was obtained by comparing its GC retention time with that of the standard synthetic chemical E7,Z9-12:Ac (Sigma-Aldrich; purity 97%), and by verifying the EAG response of a GM virgin male antenna. The amount of pheromone collected in the headspace was determined comparing the GC areas (GC-EAD2000; Syntech, Hilversum, NL) obtained with direct injections of synthetic E7,Z9-12:Ac solutions, and corrected according to the efficacy of the fibre in recovering the synthetic compound, which has been previously evaluated.

Calling females: unmated females in the 3rd scotophase were transferred individually in Petri dishes and supplied with sucrose solution. Thirty minutes before light-off, each female was placed into a 2-ml vial for the collection; the needle of the SPME was inserted into the vial by piercing the vial's Teflon® septum, and exposure started 10 min after the onset of calling. Based on recovery data, collection lasted 1 h, and only the data of females calling continuously for 1 h were considered (N=10); females were checked every 15 minutes.

Dispensers: each of the two 20-cm plastic tubes forming the dispenser was cut into 10 2-cm long pieces at -20°C, and the ends were sealed with paraffin. Two pieces (one from each tube) from five different dispensers were placed in a 2-ml vial. For each dispenser, collections were made from the distal (n=2) and from the internal part (n=2). The extraction lasted 60 min and was preceded by 10 min of equilibration. The overall release of the dispenser was calculated considering the total length of the dispenser.

Wind tunnel (WT)

Charcoal filtered air was pulled through the upwind end of a wind tunnel (200x55x50 cm) at a speed of 15 cm/s. Flight tunnel conditions were 22±3°C, 45±10% R.H. and 6 lux. Each wind tunnel session started 1 h after the onset of the scotophase and continued for 2 h. Groups (n=10) of 2-3-day old males were tested. Males were placed into glass cages (10x10x12.5 cm) 15 min prior to being tested. The following treatments were compared: 2002 field aged MD dispenser; 2003 field aged MD dispenser; sprayer with an E7,Z9-12:Ac release rate of 0.6, 6 and 60 ng/h. A monitoring rubber septum baited with 10μg of E7,Z9-12:Ac was used as reference. Dispensers were hung vertically onto a metal holder in the center of the tunnel section. To obtain the sprayers with the different E7,Z9-12:Ac release rates, solutions of E7,Z9-12:Ac at 1, 10, and 100 pg/μl, respectively, were diffused through a sprayer-device assuring complete volatilization of each compound. The solutions were delivered at a rate of 10 μl/min by a microinjection syringe pump (El-Sayed *et al.*, 1999). Each treatment was tested at least four times (n=40 males) on 4 different days. The males had 15 min to respond. For each treatment, two behavioural responses were recorded: percent males flying upwind over 100 cm, and percent males landing at the pheromone source. Data were log(x+1)-transformed and compared across treatments by means of one-way ANOVAs followed by Tukey test for the posthoc comparison of means.

Field trapping

Tetra traps (n=5) were baited with aged 2002 and 2003 Isonet® L+E dispensers, and placed into vineyards located in S. Severo (Foggia, Southern Italy) during the second flight period of GM. Tetra traps (n=5) baited with monitoring rubber septa loaded with 10 μg of E7,Z9-12:Ac were used as reference. Unbaited tetra traps (n=5) placed in the same vineyards were used as blank. Traps were checked weekly. Field tests were carried out from May 15 to June 20, 2003, and from May 20 to June 25, 2004. The number of males caught was log(x+1)-transformed, and compared across treatments by means of a one-way ANOVA, followed by the Tukey test for the posthoc comparison of means.

Results

Release rate of pheromone by SPME

L. botrana females released 0.283±0.137 ng E7,Z9-12:Ac/h; the estimated turnover rate of the gland is 0.28 times/h (Arn *et al.*, 1988; El Sayed *et al.*, 1999). The release rates of the aged dispensers strongly differed between years. The particularly dry and hot summer that occurred in 2003, may explain why the release was faster in 2003 than in 2002. At the end of the 2002 season, the release rate of the dispensers was about 46 times higher than that calculated for a calling GM female. In 2003, the dispensers released the pheromone at a rate about 13,000 times higher than that of a calling female. No differences were recorded between distal and internal pieces of dispenser (*t* test; P>0.05).

Table 1. E7,Z9-12:Ac release rates of virgin GM females and field aged MD dispensers (Isonet® E+L).

	Calling female	MD dispenser 2002	MD dispenser 2003
E7,Z9-12:Ac emission rate (ng/h)	0.283±0.103	6319.5±1191.1	13.2±1.5

Wind tunnel

Significant differences among treatments emerged for the percentage of oriented flights, but not for the percentage of males landing at the pheromone source. The proportion of males landing at the pheromone source did not differ significantly among sprayers with different release rates and 2003 dispensers (Table 2). The mean percentage of males touching the source was 20% with 2002 dispensers, and 4% with 2003 dispensers; no statistically significant differences with the standard monitoring lure were recorded.

Table 2. Attraction of virgin GM males to E7,Z9-12:Ac released from different sources. Different letters within the same column indicate significant differences (Tukey's multiple range test; P<0.05).

	% oriented flight	% landing
Sprayer 0.6 ng/h	24 a	15 a
6 ng/h	37 a	23 a
60 ng/h	32 a	23 a
Rubber septa	60 b	20 a
MD dispenser 2002	60 b	20 a
MD dispenser 2003	24 a	4 a

Field trapping

Both in 2003 and 2004, the number of males captured differed significantly among traps. The dispensers used for MD in 2002 and 2003 caught GM males in the field tests in 2003 and 2004, respectively. The number of males captured in the lured traps statistically differed from that in blank traps. Traps lured with 2002 dispensers caught significantly more males than standard traps baited with rubber septa loaded with 10 μg of E7,Z9-12:Ac.

Table 3. GM males captured in Tetra traps (n=5). Different letters within the same column indicate significant differences (Tukey's multiple range test: P<0.05).

	Trap captures 2003	Trap captures 2004
Rubber septa	7.7±5.6 b	15.2±8.7 b
MD dispenser 2002	44.0±13.5 c	
MD dispenser 2003		26.0±23.1 b
blank	0 a	0 a

Discussion

Both laboratory and field data confirm that 12-month field aged Isonet® E+L dispensers can release a pheromone quantity sufficient to produce a false trail, which affects GM males. A clear difference in both pheromone content and release may explain why 2002 dispensers were more attractive than 2003 ones in the behavioural bioassays. We conclude that aged dispensers have the potential to improve the efficacy of MD programs. As a consequence, a reduction in the application rate of dispensers where MD has been previously applied, could be possible and may allow to reduce the costs of this safe GM control technique.

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New biodegradable controlled-release pheromone dispenser for mating disruption of European grapevine moth Lobesia botrana Denis and Schiffermüller (Lepidoptera:Tortricidae)

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Abstract: Pheromone dispensers play an important role in the success of the mating disruption technique. The possibility of using sepiolite, a natural mineral type-clay with excellent adsorption properties, as support for pheromones was studied. With this purpose sex pheromone dispensers of *L. botrana* were elaborated by impregnating into sepiolite, either the semiochemical as several additives in order to stabilize them. A comparative study between new dispensers and commercial Isonet-L was carried out by ageing them under controlled conditions. Residual pheromone remaining in dispensers along ageing was quantified by gas-liquid chromatography. Release patterns of all dispensers were determined and analysed.

Two of the five types of dispensers, containing 20% and 10% of additives, shown a better performance than Isonet-L. As a consequence of their slow rate of emission at the beginning of ageing, their half-lives were 75,3 and 68,6 days, respectively, while Isonet-L's was only 23,7 days. Preliminary field assays showed that new biodegradable dispensers were at least as effective as Isonet-L producing confusion. Thus, they could be an ecological alternative to those currently used.

Key words: *L. botrana*, mating disruption, controlled-release, emission kinetics, hand-applied dispenser, biodegradable pheromone dispenser, sepiolite.

Introduction

The European grapevine moth, *Lobesia botrana* Denis and Schiffermüller (Lepidoptera: Tortricidae) is a serious pest of grape culture in Europe that generally requires some measure of control (Karg and Sauer, 1997). One promising method of pest control is the application of sex pheromones in mating disruption (Charmillot and Vickers, 1991). One of the main factors contributing to the success of this technique is the availability of adequate controlled-release pheromone dispensers. Prolonged emission, kinetics close to zero-order, release rates above the optimal and performance independent on changing agroclimatic conditions are requirements for effective dispensers. In addition, they must not constitute residues in field when the pheromone is over, apart from being low-cost and easy to apply.

Although there are a large variety of dispensers, many of them do not assemble all the mentioned requests. These limitations have inspired our research group to study natural and biodegradable materials which could be used as support for pheromones. Among possible materials, sepiolite, a natural mineral type-clay derived from talc, seems to have interesting possibilities. Currently, sepiolite has a wide range of applications, mainly derived of its excellent adsorption capacity, low cost and availability. It is used mainly as cat litters, in fertilizers, moisture control and animal feed.

In this paper we report the development of controlled-release dispensers, with natural sepiolite as a physical support for the *L. botrana* sex pheromone. A comparative between the new dispensers and commercial Isonet-L, was carried out Results obtained under laboratory conditions and preliminary field assays are presented.

Material and methods

Commercial dispensers Isonet-L (Shin-Etsu Chemical Company Ltd., Tokyo, Japan) were selected as control, as they are the most extensively used. Their expected initial load was 172 mg of E,Z-7,9-dodecadienyl acetate (EZ-7,9-12:OAc). Synthetic pheromone (86,5% purity) provided by Shin-Etsu, was used to elaborate new dispensers.

Natural sepiolite was used as support for the pheromone. Sepiolite is a hydrated magnesium-silicate type clay ($Mg_4Si_6O_{15}(OH)_2{\cdot}6H_2O$). It is a naturally occurring mineral deriving from talc. Structurally, ribbons are connected to the next through an inverted Si-O-Si bond and Mg^{2+}. As a result, a staggered talc layer with a continuous tetrahedral sheet and a discontinuous octahedral sheet (T-O-T) is formed. The discontinuous nature of the octahedral sheet allows for the formation of rectangular, channel-like nanopores, which run parallel to the fibber axis The channels account in large part for the high specific surface (>300m^2/g) and its excellent adsorbent capacity.

Additives included to formulations were: Waxoline OBP (Avecia Pigments & Additives, Manchester, U.K.), with 30% Solvent Yellow 14 and Black Carbon, tert-butylhydroquinone (Aldrich Chemical, Milwaukee, WI., USA) at a purity of 98%, and compacters.

Five types of dispensers with different proportions of pheromone and additives were elaborated. Semiochemical and additives corresponding to each formulation were impregnated into the sepiolite. Individual emitters (1,27 cm diameter) loaded with 172 mg of *EZ*-7,9-12:OAc approximately, were formed by compressing mixtures with a manual hydraulic press (Graseby-Specac, Smyrna, GA., USA).

New dispensers and Isonet-L were aged under laboratory conditions during 92 days, in a ventilated oven (30°C, wind speed 0,35 m/s). Samples corresponding to each dispenser type were randomly removed at different times. Residual pheromone from dispensers was obtained by solid-liquid extraction. Quantification was performed by gas-liquid chromatography in a Perkin Elmer Clarus 500-GC with FID detector and column Zebron ZB-5. The oven temperature program was: 170°C for 10 min, ramp 30°C/min to 260°C. Dodecanol (Aldrich, Milwaukee, WI., USA), at a purity of 96,5%, was used as internal standard.

We assumed that the difference in the amount of pheromone remaining in dispensers at each time period is a measure of the amount of pheromone released. Values corresponding to residual pheromone were graphically represented versus time and then adjusted to first-order kinetic rate equations. Following parameters were then calculated: kinetic constant (K), half-life [using $t_{1/2}$=ln (2/K)] and correlation coefficient (r^2). Pheromone release rates from dispensers were obtained when deriving first order equations for each point, resulting $C= C_0 \cdot e^{-kt}$, after integration and association, where r: release rates (mg/dispenser and day); C: load (mg pheromone/g dispenser); C_0: initial load (mg pheromone/g dispenser); K: first-order kinetic constant (days^{-1}) and t: elapsed time (days). At the end of aging, half-lives of dispensers, total amount of pheromone emitted and release rates, were compared.

Results and discussion

In order to evaluate the ability of sepiolite as a pheromone controlled-release support, five types of dispensers were developed. The analysis of their emission parameters has demonstrated that two of them (Formulations 2 and 4, containing 20% and 10% compacters, respectively) exhibited better results than the dispensers most extensively used, Isonet-L. Thus, only results concerning to these two formulations are presented.

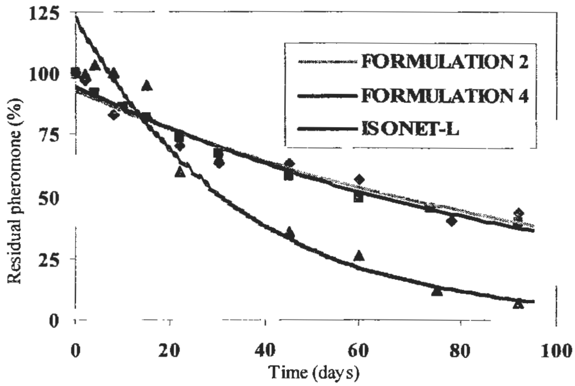

Figure 1. Pheromone release patterns under laboratory conditions.

Dispensers from Shin-Etsu released pheromone rapidly, with a half-life of 23,7 days. Dispensers from Formulations 2 and 4, however, showed a more restrained emission with less than 50% remaining after 75,3 and 68,6 days, respectively (Table 1). After 92 days, Isonet-L emitted 185,98 mg, what represents the 92,96% of their initial load. Dispensers from Formulation 2 and 4 emitted 97,49 mg and 107,478 mg, in that order (43,55% and 39,40% of their respective initial loads).

Table 1. Emission parameters from dispensers under laboratory conditions.

	*K**	r^{2**}	*Half-life (days)*
FORMULATION 2	0,0092	0,9370	75,3
FORMULATION 4	0,0101	0,9858	68,6
ISONET-L	0,0292	0,9704	23,7

*K: Kinetic constant; r^2: Correlation coefficient.

Formulations 2 and 4 showed almost the same release patterns as demonstrated by their similar kinetic constant, contrary to Isonet-L, with a higher K. As a consequence, our dispensers had more adequate first-order kinetics than commercial, being closer to zero-order. This fact suggests that sepiolite has a high efficiency retaining pheromone.

On the other hand, compacters incorporated to formulations in order to avoid emitters fragmentation when exposed to field conditions, seemed to act as release modulators. Dispensers with 20% of compacters presented slightly superior half-life than those with only 10%. This could be presumably due to the fact that compacters reduced the size of channels of sepiolite, what difficult pheromone emission.

Initial release rates from Formulations 2 and 4 were 2,44 and 2,37 mg/dispenser and day, while that exhibited by Isonet-L was around 40% higher (4 mg/dispenser and day). This fact probably supposes, a priori, an excessive and unnecessary emission of pheromone, although this fact will be corroborated in the field.

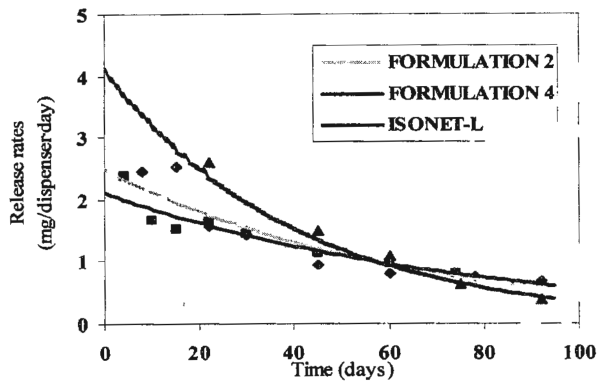

Figure 2. Release rates of dispensers under controlled conditions

At the end of aging, however, release rate from Isonet-L, with 0,392 mg/dispenser and day, was lower than values showed by our dispensers (0,67 and 0,77 mg/dispenser and day for Formulations 1 and 2 in that order).

On the basis of the above results, dispensers from Formulations 2 and 4 showed more regular emission than Isonet-L (Figure 2). If we assume that Isonet-L is effective under real field conditions and our dispensers have improved their emission parameters, it would be reasonable to expect similar or better behaviour of our dispensers under the same conditions.

With this aim, a preliminary 10-Ha field assay was conducted, in Valencia, Spain. Dispensers corresponding to Formulation 2 were placed at a density of 500 dispensers/Ha. A parallel experience was carried out in a close vineyard with Isonet-L, at the same density. Similar results were achieved in both cases. Mating disruption was successful as no captures were registered on monitoring traps and damage levels were below established thresholds. These results seems to corroborate that the new dispensers were at least as effective as Isonet-L, and they could be, in brief, an adequate tool to be used in the mating disruption technique.

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Mechanisms of the Exosect Auto-Confusion Technique

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Abstract: The Exosex Auto-confusion system differs from all other mating disruption systems in contaminating the target pest with electrostatically charged powder formulated with pheromone. The technique has now been successfully evaluated in the field against *Cydia pomonella, Lobesia botrana,* and other Lepidoptera. Research into the mechanisms of auto-confusion shows that habituation, inhibition of courtship and delay in mating are important mechanisms in "confusion" and population control.

Keywords: lepidopteran pests, mating disruption, pheromone, electrostatic powder, auto-confusion.

Introduction

The Exosex Auto-confusion technology was conceived by P.E.Howse in 1992, (UK Patent application No. EP0650322). Preliminary trials were carried out against *Cydia pomonella* (Howse & Underwood 2000). Trials were begun on control of various Lepidoptera using female sex pheromone as the sole active ingredient. Having proven the efficacy of the technique, work has begun to identify and evaluate the various mechanisms that make it so effective.

Materials and Methods

Laboratory work on *Lobesia botrana* was conducted at the University of Southampton. Cultures were obtained from Dr. F. Louis, Germany and kept in a climate controlled insectary at 20-25°C and 60-80% RH on a 16:8 h light/dark cycle. Bioassays were carried out during the first 3 h of the scotophase either in a wind tunnel (150 x 60 x 60 cm) or in 10 cm diameter glass petri dishes. Sex pheromone, (E,Z)-7,9-dodecadien-1-yl acetate, was obtained from BASF and electrophysiological analysis was carried out using a portable EAG (Syntech, Netherlands). (Z)-3-hexen-1-ol was used as the reference compound. The electrostatically chargeable powder was a commercial grade wax powder (Entostat^{TM}) produced by Exosect Ltd (Southampton).

Results and Discussion

Exosex dispensers are similar to delta traps but modified to minimize loss of powder being caused by wind action. They were deployed at 25 stations per hectare each dispenser containing 2.5 g of. Entostat powder formulated with 2.5 mg of sexual pheromone. Trials plots fulfilled as far as possible the following criteria: boundary area separating trial plots from neighbouring plots, including control and treated plots, minimum area of 5 ha, low first generation populations.

The great majority of the trials showed greater efficacy of control than pesticide-treated plots and levels of damage comparable with those resulting from conventional mating disruption treatments. Figure 1 shows a typical trial result with Lobesia botrana.

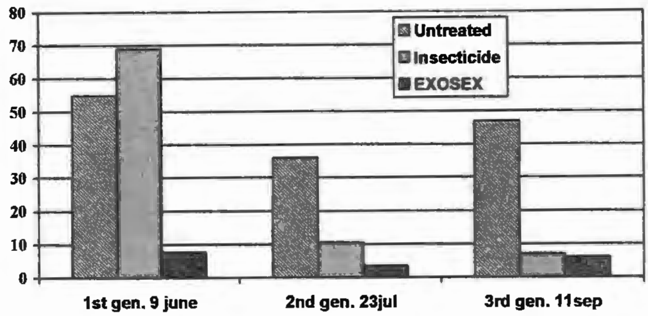

Fig 1: Lobesia trial results, Spain 2003 – Percentage grape bunches damaged

For optimisation of the system we need to know more details of the mode of action of auto-confusion. Theoretical calculations show that there are approximately 1.5 x 10^{10} particles per gram of powder. The particle size is in the range of 8-20 microns diameter. Taking into account the known threshold responsiveness of *Cydia pomonella* it can be calculated that there is theoretically sufficient pheromone in one particle resting on the surface of the antenna to produce habituation. An insect carrying approximately 1 800 particles would then be liberating sufficient pheromone in the short term to constitute an attractive source for another male moth. This means that the contents of one Exosex dispenser are theoretically capable of contaminating about one billion (10^{12}) male moths with enough pheromone to make them attractive sources to other males.

The principal factors acting in auto-confusion would therefore appear to be habituation due to responsiveness as a result of constant sensory stimulation, and males acting as false lures or mobile dispensers.

The evidence for habituation. Electroantennogram (EAG) responses of 2-3 day old virgin male *Lobesia botrana* were measured in response to a test source at 2 – 100 cm distant in the wind tunnel. Significant EAG responses to pheromone alone were found up to 100 cm, but males contaminated with powder formulated at 0.001 mg g-1 to which they were exposed by being placed in a glass vial with Entostat powder in the bottom for 30 s showed no EAG response (unpublished results, K.M.). Strong habituation thus occurs at least at a sensory level when Entostat particles are on the antennae. This appears to account for the fact that contaminated males very rarely fly in a wind tunnel.

Inhibition of courtship. Mating is strongly inhibited in male *L. botrana* contaminated with Entostat powder. When powder-contaminated males are retained with virgin females in a glass petri dish for the first 3 h of scotophase the percentage of matings drops from 80% to between 20 and 30%. The male activity is reduced, orientation towards the female does not occur and courtship display activity (including wing-fanning) is rarely seen.

A secondary effect is reduction in egg production that occurs in females kept with treated females in glass petri dishes for 24 h. The mean number of eggs produced per female is reduced by approximately one order of magnitude

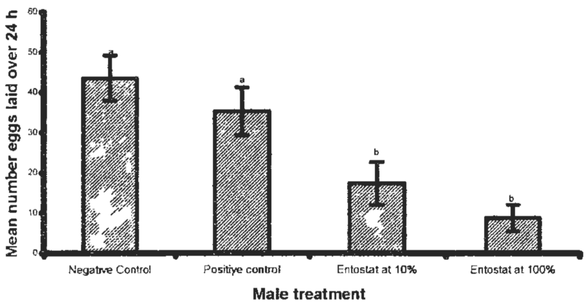

Figure 2: Mean number of eggs produced per female

Males act as dispensers. The evidence for transfer of powder between males is currently limited. However, this has been shown to occur among locust hoppers and cockroaches when a group of uncontaminated individuals is confined with a single powder-contaminated individual. In the laboratory, powder transmission has been shown to occur between male and female tsetse flies (*Glossina austeni*) during mating in the laboratory (Underwood & Jackson, unpublished), and in the field between contaminated male medfly and females (Underwood & Howse, unpublished). EAG studies on *L.botrana* show that a male antenna can detect pheromone released from three contaminated males

Effects on fecundity. Prevention of mate-location is unlikely to be the main factor in conventional mating disruption. In high population densities between 70 and 80% of codling moth females are mated in pheromone-treated orchards (Brunner 2003), while in low population densities only between 30 and 35% of females are mated, implying that the population growth is slowed in low population densities, but not stopped or reversed. Other factors must therefore come into play. One such factor in codling moth control has been shown to be increased egg predation, around 20% higher in orchards treated for mating disruption compared with orchards under conventional insecticide treatment (Knight 1996), another is the effect of delayed mating.

With conventional mating disruption techniques used against *Cydia pomonella*, mating tends to be delayed. Brunner (2003 and Knight (1996) believe this delay results from increased difficulty in mate location. The effects of delayed mating are a) a decrease in the number of eggs produced, b) a decrease in the percentage of fertile eggs, and c) a decrease in multiple matings.

Knight (1996) found a delay of two days in mating during the first flight in pheromone-treated orchards. In laboratory studies, females that did not mate until the second day produced over 40% fewer viable eggs. When mating was delayed for five days, less than 50% of the maximum number of eggs was laid and over 75% of these failed to hatch. Multiple matings in *C. pomonella* lead to females producing more fertile eggs, but multiple matings are significantly reduced in pheromone-treated orchards (Knight 2003).

In the Exosex Auto-confusion system these powerful factors acting to reduce fecundity and egg fertility are supplemented by a more efficient method of preventing mate location than occurs in conventional mating disruption techniques. The evidence suggests that powder-contaminated males are unable to locate sexual pheromone, are incompetent, and act as mobile dispensers.

Future prospects. The Exosex system is proving to be a viable method in the integrated management of many Lepidopteran crop pests. First registrations for commercial use have been achieved in the USA and the UK and others are expected to follow in the coming 12 months. The Entostat powder carrier system is also being developed as an "auto-dissemination" technique, in which minute amounts of active ingredients can be delivered to the target species as part of a lure and kill strategy. This method has been shown to be effective against codling moth and tephritid fruit flies (Howse & Underwood 2000) and research and development will now continue with industrial and institutional partners

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Attraction of four tortricid moth species to high dosage pheromone rope dispensers: Observations implicating false plume following as an important factor in mating disruption

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Abstract: Orientational responses of four species of feral tortricid moths to polyethylene-tube pheromone dispensers were observed in 0.8 ha apple orchards treated with such pheromone dispensers and in untreated 0.8 ha orchards. Male oblique-banded leafrollers, *Choristoneura rosaceana* (Walker) (mean 7.2 ± 0.4 over 21 nights), Oriental fruit moths, *Grapholita molesta* (Busck) (mean 10.5 ± 2.1 over 20 evenings), and redbanded leafrollers, *Argyrotaenia velutinana* (Walker) (mean 2.0 ± 1.1 over 14 nights) were attracted within 100 cm of their respective polyethylene-tube pheromone dispensers in an untreated orchard. Furthermore, *C. rosaceana* (mean 2.0 ± 0.7 over 17 nights), *G. molesta* (mean 1.5 ± 0.4 over 20 evenings), and *Cydia pomonella* (L.) (mean 1.1 ± 0.2 over 10 nights) came within 100 cm of their respective polyethylene-tube pheromone dispensers in pheromone-treated orchards. Most visits lasted less than 10 s, after which the majority of moths departed by flying upwind. In the untreated orchard, the number of *C. rosaceana* observed orienting to polyethylene-tube dispensers was greater than the number captured in optimized monitoring traps (1.9 ± 0.4) per night of observation. The numbers of *A. velutinana* (2.0 ± 1.1) or *G. molesta* (10.5 ± 2.1) attracted to polyethylene-tube dispensers in the untreated orchard did not differ statistically from the numbers captured in optimized monitoring traps per night of observation. In the pheromone-treated orchard, the number of *C. rosaceana* (2.0 ± 0.4) or *G. molesta* (1.2 ± 0.2) observed orienting to polyethylene-tube dispensers did not differ statistically from the numbers of male moths of these species captured in optimized monitoring traps per night of observation. At deployment densities of 350 and 3,500 Isomate-C Plus dispensers per Ha, the mean numbers of *C. pomonella* observed approaching dispensers within trees (0.7 ± 0.2 over 17 nights and 1.1 ± 0.2 over 10 nights, respesctively) were not statistically different from the mean numbers captured in monitoring traps (0.6 ± 0.1 and 1.6 ± 0.3, respectively). Attraction of male moths to polyethylene-tube dispensers occurred in each species observed. These results support false-plume-following as an important component of the mechanisms mediating communicational disruption in moths by polyethylene-tube dispensers.

Key words: *Choristoneura rosaceana, Argyrotaenia velutinana, Grapholita molesta, Cydia pomonella*, mating disruption, false-plume-following

Introduction

Pheromone-based mating disruption is an important biorational pest-management tactic for insects relying on long-distance pheromones for mate finding (Cardé and Minks, 1995). Currently, hand-applied, polyethylene "rope" dispensers at *ca.* one to four per tree are the dominant method of dispensing pheromone for mating disruption of moth pests in orchards (Knight et al., 1998). However, the mechanism(s) by which such dispensers disrupt mating remain circumstantial. Here we describe behavioural observations of four tortricid moth species attracted within close proximity of their respective rope dispensers in natural orchards settings. The data suggest false-plume-following is an important mechanism mediating mating disruption.

Material and methods

Field Observations

This study was conducted May-September of 2003 and 2004 at the Trevor Nichols Research Complex (TNRC) of Michigan State University in Fennville, MI. Visual observations were conducted on sunny and calm evenings starting at 16:30 and lasting through 24:30 in two 0.8 ha orchards of 18 year old Delicious apple trees with *ca.* 2-3 m canopy heights. Wind speeds during observations varied from *ca.* 0.13 to 2.2 m/s. Control orchards were left untreated while treated orchards received three types of polyethylene-tube pheromone dispensers at recommended label rates. A more detailed description of pheromone dispensers and experimental protocols can be found in Stelinski et al. (2004a).

Pheromone Dispensers

The polyethylene-tube pheromone dispensers used for observations and applied as mating disruption treatments were: 1) Isomate-M Rosso targeting *G. molesta*; 2) Isomate-C Plus targeting *C. pomonella*; and 3) Isomate OBLR/PLR Plus targeting both *C. rosaceana* and *A. velutinana*. All dispensers were hung in trees *ca.* 1.5 - 2 m above the ground and in the upper third of the tree canopy.

Observational Arena

In 2003, observations were conducted using an observational arena. The observational arena was a 71 cm high, vinyl-covered table measuring 86 x 86 cm. The table top was demarcated with tape in a 10 x 10 cm grid to aid estimation of the proximity of observed moths to a polyethylene-tube pheromone dispenser affixed above the table's centre. An individual pheromone dispenser was twisted onto an apple branch removed from a tree in the experimental plot. The apple branch was then affixed horizontally and approximately 32 cm above the tabletop to a steel ring-stand. The stand was positioned in the centre of the observational table. In 2004, Isomate-C Plus dispensers targeting *C. pomonella* were observed directly within tree canopies in orchards treated with such dispensers at 350 or 3,500 dispensers per Ha.

Observed events were spoken into a hand-held microcassette audio recorder by an investigator located 0.75 m from the pheromone dispenser under observation. Data recorded included: anemotactic orientations to the dispenser, closest approach to the dispenser, landings at the observation arena, time during the diel period, and duration of visits. Observations after dusk were assisted by night-vision goggles. For each species, direct observations were carried out between two and five times per week during their respective adult generations.

Concurrent with the observations, attraction of male moths to sex pheromone was monitored using pheromone traps for each species. Traps were maintained *ca.* 50 m from the observation arena and were hung *ca.* 1.5 - 2 m above ground level. Moths captured in traps were counted and removed following each observational period.

Results and Discussion

Studies utilizing observational arena. Over the course of their respective flight periods, *C. rosaceana*, *G. molesta*, and *A. velutinana* were consistently attracted within 100 cm of their respective polyethylene-tube pheromone dispensers in the untreated orchard. Likewise, *C.*

rosaceana, and *G. molesta,* came within 100 cm of their respective dispensers in pheromone-treated orchards. *C. pomonella* did not approach Isomate-C Plus dispensers at the observational arena

The mean number of *C. rosaceana* observed at the observational arena (7.2 ± 0.4 over 21 nights) was significantly ($P = 0.006$) greater than the mean number of *C. rosaceana* captured per monitoring trap (1.9 ± 0.4) per night of observation. The mean number of *A. velutinana* observed at the observational arena (2.0 ± 1.1 over 14 nights) was not statistically ($P = 0.57$) different from the mean number of *A. velutinana* captured per monitoring trap (6.3 ± 1.0) per night of observation. The mean number of *G. molesta* observed at the observational arena (10.5 ± 2.1 over 20 nights) was not statistically ($P = 0.49$) different from the mean number of *G. molesta* captured per monitoring trap (8.5 ± 1.0) per night of observation.

Duration of stay and proximity to ropes.

The majority of *C. rosaceana* left the observational arena within 10 s of initial sighting in both untreated (78%) and pheromone-treated (92 %) plots. Nearly all *C. rosaceana* were attracted within 100 cm of the rope in both untreated (99 %) and pheromone-treated (93 %) plots and 29 % approached within 10 cm of the rope. The majority of *A. velutinana* (84 %) observed in the untreated plot left the arena within 10 s of initial sighting. All *A. velutinana* approached within 70 cm of the rope in the untreated plot. No *A. velutinana* were observed approaching the dispenser in the pheromone-treated plot.

G. molesta was the only species observed alighting on the observational arena (table or branch containing rope). The majority of *G. molesta* observed at the observational arena left within 10 s in both untreated (77 %) and pheromone-treated (86 %) plots. However, only 46 % of *G. molesta* observed landing on the arena left within 10 s of initial sighting in the untreated plot and only 5% visited longer than 80 s. *G. molesta* that landed on the table wing-fanned vigorously and walked rapidly; they remained in motion for the duration of their stay on the table. All of the *G. molesta* observed approached within 100 cm of the dispenser.

All *A. velutinana* approached within 70 cm of the dispenser in the untreated orchard. The majority of *A. velutinana* observed in the untreated orchard left the observational arena within 10 s of initial sighting. No *A. velutinana* were observed approaching their dispensers in the pheromone-treated orchard. Also, no individuals of this species were observed landing at the observational arena in the untreated orchard. Upon leaving the arena, the majority of moths from all species flew upwind.

Observations of Isomate-C Plus dispensers within trees.

At deployment densities of 350 Isomate-C Plus dispensers per Ha, the mean number of *C. pomonella* observed approaching dispensers within trees (0.7 ± 0.2 over 17 nights) was not statistically ($P > 0.5$) different from the mean number captured per monitoring trap (0.6 ± 0.1) per night of observation. Likewise, at deployment densities of 3,500 Isomate-C Plus dispensers per Ha, mean number of *C. pomonella* observed approaching dispensers within trees (1.09 ± 0.2 over 10 nights) was not statistically ($P > 0.5$) different from the mean number captured per monitoring trap (1.6 ± 0.3). The majority (84%) of *C. pomonella* observed came within 150 cm of Isomate-C Plus dispensers and 89 % of those observed flew away from dispensers within 60s.

Discussion.

Attraction of male moths to polyethylene-tube dispensers occurred in all four species observed in this study. Furthermore, polyethylene-tube dispensers attracted approximately equal numbers of *A. velutinana*, *G. molesta*, and *C. rosaceana* as were captured in traps with optimally tuned monitoring lures in an untreated orchard. These results suggest that false-plume-following is an important mechanism mediating mating disruption using polyethylene-tube "rope" dispensers.

An additional effect of false-plume-following to dispensers may, in some cases, be the neurophysiological modifications induced by exposure to high dosages of pheromone (Cardé et al., 1998). Indeed, in companion flight tunnel investigations we have documented long-lasting behavioural modifications in three of the four species discussed above following seconds-long, close-proximity encounters with their respective rope dispensers. Specifically, the proportion of male *C. rosaceana* locking onto sources of pheromone and the duration of sustained flights increased by up to 4-fold following seconds-long pre-exposure to Isomate OBLR/PLR Plus dispensers (Stelinski et al., 2004b). Furthermore, the same study revealed that the behavioral responses of *A. velutinana*, after identical exposures to those imposed on *C. rosaceana*, decreased for up to 24 after the pre-exposure treatment. Finally, seconds-long pre-exposures of *G. molesta* to Isomate M-Rosso dispensers resulted in subsequent increases in the proportion of males locking onto pheromones plumes but decreases in the duration of sustained flights along such plumes (Stelinski et al., unpublished data).

Previous investigators have stressed the difficulty in conducting direct visual observations of male moths responding to pheromone sources under authentic field conditions. Our two year field investigation suggests that such an approach is indeed possible and valuable. Furthermore, the comparative approach taken here, where the behaviours of multiple species were compared both in the field and in the laboratory was valuable given the differences uncovered between species. Our data suggest that a potentially important first step in mating disruption by polyethylene-tube "rope" dispensers is attraction of male moths within close proximity (150 cm or less). Subsequent behavioural modifications due to pheromone exposure following bouts of false-plume-following may result in adaptation, habituation, or sensitization depending on the moth species in question. Further work should focus on how such post-exposure behavioural modifications influence the level of mating disruption in the field. Improved understanding of the general mechanisms underlying mating disruption among multiple pest species should help pest managers improve the efficacy and practicality of this management tactic.

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Semiochemical driven autodissemination of *Cydia pomonella* and *Adoxophyes orana* baculoviruses

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Abstract: The first year's results of a three year study aimed at developing a new method for the control of codling moth (*Cydia pomonella*) and summer fruit tortrix moth (*Adoxophyes orana*) in apple orchards are outlined. The method entails luring the adult moths with semiochemicals to autodisseminators where the adults become contaminated with baculoviruses. Once adults are contaminated, the baculoviruses spread between individuals during mating, and eggs together the surrounding area can become contaminated during oviposition. In the first year, the spread of virus from points in the centre of orchards was investigated, using laboratory produced powder formulations of the viruses. A small but significant proportion of larvae became infected within the one hectare area surrounding the central point.

Introduction

Semiochemical driven autodissemination of baculoviruses is a process that theoretically involves three stages. During the first stage (Attraction and contamination), semiochemicals are used to attract male and/or female insects to locations where their bodies can become contaminated with baculovirus. In the second stage (Horizontal transmission) insects disperse, males mate and contaminate females, females contaminate eggs and larvae become infected by consuming their egg case or by feeding on the plant in the vicinity. In the third stage (Vertical transmission) virus is passed from the female to the embryo, usually internally as a result of a sub-lethal infection.

In this paper we outline the first years results of a three year research project, started in 2003 and funded by The Department for Environment, Food and Rural Affairs (Defra), UK, to assess the feasibility of sex pheromone (and/or in the case of codling moth host volatile) driven autodissemination of codling moth (CM) (*Cydia pomonella*) and summer fruit tortrix moth (SFT) (*Adoxophyes orana*) granuloviruses by their respective adult moths. The aims of the scientific investigation are firstly to understand the processes of autodissemination, to optimise them and then ultimately to see if they can be exploited for control of the target pests in the field. The two granuloviruses and their hosts provide two contrasting models for the concept of baculovirus autodissemination. *C. pomonella* GV (CpGV) is generally regarded as a fast acting virus which kills its host rapidly, shortly after egg hatch, before the larva can bore into the core of the fruit. Historically, it was not considered to persist significantly in the codling moth populations from one generation to the next or from one year to the next. In contrast, *A. orana* GV (AdorGV) is a slow acting virus which kills its host larvae in the final instar stage, regardless of the larval stage inoculated.

Hrdý et al (1996) and Pultar et al. (2000) working in the Czech Republic investigated a sex pheromone attractant aided dissemination of a powder formulation of CpGV in orchard trials in the 1990s. Transfer of viral infection by contaminated males to females and to caterpillars was observed as well as a suppression of the codling moth population and fruit damage. In later experiments, the autodissemination method was used in conjunction with field sprays of the virus. They concluded that pheromone aided autodissemination of CpGV could be useful as one of the alternative pest control strategies, especially when used at low population densities of the target pest.

In the first year of our project, laboratory experiments were conducted to develop suitable powder and liquid formulations of each granulovirus (CpGV and AdorGV. Sensitive and accurate methods of detecting the viruses on leaves, in larvae, and in damaged plant tissue from the field were developed. Laboratory experiments to study vertical and horizontal transmission of the viruses between individual insects were also carried out. In the field, two experiments, one on CM one on SFT, were conducted to determine the best spatial orientation of the virus presenting surface for the autodisseminator devices, which were likely to result in the highest proportion of moths becoming contaminated with virus. Two further experiments used fluorescent dyes to determine the proportions of the populations of each moth that became contaminated by a powder versus a liquid formulation. Three observational field experiments, two on CM, one using the sex pheromone and one using the pear ester host volatile bisexual attractant ,respectively and one on SFT using the sex pheromone attractant (no host volatile attractant of SFT has been identified). In these experiments, the spread of virus from a point in the middle of an orchard was studied.

Formulation Development

Powders: Several solid carriers were assessed for their ability to be picked up by adult moths. Scanning electron microscopy showed that the adults picked up and retained the powdered silicon oxide on all parts of their body and appendages. Silicon oxide was selected as the carrier for powdered preparation of the virus. This carrier had been previously used as a carrier for freeze-dried virus by Jaraslav Weiser in Czechoslovakia. The virus concentration in the powders was about $5x10^{12}$ CpGV / 10g of powder (equivalent to 20 final instar larvae) and 1×10^{13} AdorGV /10g of powder (equivalent to 60 larvae). In the autodissemination field experiments, 10g of each freeze-dried powder formulation of CpGV and AdorGV were used to cover the base of a delta trap coated with sandpaper, to retain the powder. The powder was replaced continuously at weekly intervals for twelve weeks following the first and including the second flight of the moths.

Liquids: Several viscous liquids were tested as gel carriers for the virus but they trapped and killed the moths. Solid matrices were tested to hold the liquids and J-cloth was shown to be the best surface, after removal of excess liquid, compared to Mira cloth, coarse paper towel and velvet. A mixture of glycerol and water coated on J-cloth attached to the bottom of the delta trap which allowed the moths to alight, pick up the liquid and fly off. Scanning electron microscopy showed that the body and appendages were coated with a film of the liquid.

Fluorescently labelled formulations: A powder and liquid formulation, analogous to the baculovirus formulations above, but not containing live virus were prepared with different coloured, non water soluble, finely particulate fluorescent dyes for the dye transfer field experiment.

Determining the effect of storing various virus formulations/preparations at 4°C:

C. pomonella GV neonate bioassays were carried out at time zero and at 4 and 8 weeks post-storage at 4°C, for three different formulations/preparations of CpGV. These included a crude filtered virus extract, a powder formulation based on silicon oxide and a liquid based on glycerol. The results showed that the crude virus extract and gel showed no loss of efficacy after storage at 4°C for 8 weeks. However, the efficacy of the powder formulation dropped between 0 and 4 weeks and then remained at the same level for a further 4 weeks. The efficacy of the powdered formulation was less than the crude extract or gel at time zero. Therefore there was some loss of efficacy associated with the powder formulant/preparation method.

A. orana GV neonate bioassays were carried out on three preparations/formulations of AdorGV - a crude filtered virus extract, a powder formulation based on silicon oxide and a liquid/gel based on glycerol. The powder preparation showed a large reduction in efficacy of AdorGV compared to the extract and the gel. However, there was no further decline in efficacy on storage at 4°C for 9 weeks. The gel and crude virus extract retained their efficacy on storage at 4°C for the period of 9 weeks studied.

Determining batch to batch variation:
Two batches of AdorGV were compared as a crude virus extract, powder or gel. The decrease in efficacy resulting from the preparation of a powder formulation compared to the crude extract and gel was confirmed.

The effect of exposing the powder formulation in the orchard for one week periods:
The efficacy of 5 samples from one batch of CpGV and one batch of AdorGV was assessed after exposure for one week in weeks 1 to 5 in the virus spread experiments 1 and 3 (see below). The powders were bioassayed on neonate larvae by comparing the efficacy to the published LD_{50} and LD_{80} for purified virus. All 5 samples showed similar activity after exposure for one week in the field on 5 consecutive weeks throughout the season, however it required ten fold more virus to achieve a LD_{50} dose compared to unformulated virus. It was difficult to say if this was as a result of the formulation process or due to deterioration in the orchard. The powder sample exposed in week 3 did appear to be more efficacious but this could not be related to any differences in meteorological conditions.

Autodisseminator design

Two field experiments were conducted, one on CM, one on SFT, to investigate which orientation of surface would be optimum for maximising the frequency of contact of moths with an autodisseminator device. It had been suggested that only a very small proportion of the field population of adult male moths enter a sex pheromone-bated delta trap for instance. Nine trap designs with sticky surfaces to trap moths in contact with each surface were constructed out of white plastic (Correx). Each trap design was fitted with a sex pheromone lure specific for the moth.

Data analysis of the number of moths sticking to each orientation of surface showed that the upper surface of a horizontal plane was by the most effective for both species. Few moths became stuck to vertical surfaces and very few to the undersides of horizontal surfaces. The horizontal, upward facing sticky surface inside a delta trap caught slightly less moths than the equivalent surface in the open, but differences were small and not statistically significant. For practical purposes, the upward facing surface from which the baculovirus is dispensed needs to be protected from rain and sunlight. A two tray trap (double saucer or perhaps Zoecon winged design) with omni-directional access for moths and with the virus presented on the upper surface of the lower tray is likely to be a good practical design.

Efficiency of acquisition of formulated product by adult moths

The aim was to compare the proportions of natural populations of adult moths that became contaminated with powder versus liquid formulant (traced with different coloured fluorescent dye, see above) in the field. Delta traps were used as autodisseminators. Two experiments were conducted, one on CM comparing the sex pheromone and the DA2313 pear ester kairomone lure, one on SFT using sex pheromone lures only. The autodisseminators were deployed initially for an exposure period. The autodisseminators containing the dye formulants were then replaced with delta traps containing sticky bases in the same locations and with the same lures for a capture period. For the CM experiment, the first exposure and the first capture periods were each 5 days followed immediately by one further exposure and capture period of the same duration (20 days total). For SFT there were four successive 1 day exposure/1 day capture periods (8 days total). The moths in the sticky traps were viewed by fluorescent microscopy to look for fluorescently labelled moths. The different coloured tracer dyes distinguished the different formulations tested.

Numbers of moths caught were rather small as, although the orchards were heavily infested with the target species, the moth flights had passed their peak when the experiments were done. However, important clear conclusions could be drawn from the results. For CM, no tracer dye as detected on 68% of moths captured. Approximately 3x more CM were contaminated by the liquid formulation than by the powder. Six times more CM caught with sex pheromone lure than with DA lure regardless of formulation. For SFT, no tracer dye was detected on 74% caught. A total of 9 moths had tracer dye from the liquid formulation autodisseminators, only 1 moth had dye from the powder formulation.

These results are encouraging because significant proportions of moths (30%) became contaminated from single, widely separated autodisseminators. The results also indicate that the liquid formulation is likely to result in a higher proportion of moths being contaminated. This maybe because a greater proportion of moths become contaminated initially, because a higher level of initial contamination occurs or because the liquid formulation is maintained on the adult moths body more persistently, or for a combination of these reasons.

Virus spread from autodisseminators loaded with the powder formulation on a central tree

Three non-replicated observational studies were conducted in commercial apple orchards in Kent in 2003 to investigate the spread of virus from 4 delta trap autodisseminators on a single tree in the centre of a c 1ha, square apple orchard. The 3 experiments investigated one of 3 different experimental treatments

Experiment 1: CM with sex pheromone lures
Experiment 2: CM with DA2313 kairomone lures
Experiment 3: SFT with sex pheromone lures

Each orchard was divided into 25 square plots in a 5 x 5 array. Four delta trap autodisseminators were hung in the central tree of each plot. The spread of virus was assessed by sampling the damaged fruit on the central tree in each plot in July and at harvest in September. Larvae were tested for virus infection in the laboratory. Samples of larvae were taken from adjacent orchards to test the background levels of virus in the populations in the vicinity. In order to determine whether significant levels of virus had spread from the autodisseminators by abotic means, e.g. wind dispersal, leaves on the central and surrounding trees were swabbed and screened by PCR to determine the presence or absence of virus. However, this method gave non reproducible results. This problem has not been resolved.

Both viruses in a powder formulation disseminated in the orchard from the autodisseminators. Results of the three experiments are summarised as follows:

Experiment 1 (CM, CpGV, sex pheromone lure): Numbers of codling moth damaged apples on the central tree in each plot were variable but there was no obvious marked suppression of damage in the central plot or its surrounding plots compared to the other plots. No background virus was detected. 4.8% of larvae were found to be infected with virus, the infected larvae being distributed erratically throughout the whole 1 ha experimental area.

Experiment 2 (CM, CpGV, DA2313 kairomone lure): Numbers of codling moth damaged apples on the central tree in each plot were variable but there was no obvious marked suppression of damage in the central plot or its surrounding plots compared to the other plots. Unfortunately, 7.1% of larvae in adjacent orchards were found to be infected indicating possible background infection, probably due to sprays of CpGV applied by the grower two years earlier. 5.3% of larvae were found to be infected with virus in the test orchard, the infected larvae being found in a localised area in the central plot and 5 other plots to the North and West of the central plot. The occurrence of greater levels of virus in the background samples makes this experiment difficult to interpret.

Experiment 3 (SFT, AdorGV, sex pheromone lure). Numbers of SFT larvae on the central trees in each plot were variable but there was no obvious marked suppression of infestation or damage in the central plot or its surrounding plots compared to the other plots. No background AdorGV infection was found. 5.4% of larvae were found to be infected with virus, the infected larvae being distributed manly in the centre and its surrounding plots in 1 ha experimental area.

These experiments demonstrate that virus transmission occurred in both species but it was not possible to determine whether the virus was spread by the moths or by abiotic factors such as wind or rain.

Laboratory based virus transmission experiments

A recombinant CpGV expressing the green fluorescent protein behind the constitutive heat shock promoter from *Drosophila* was used as a tool to study horizontal and vertical transmission of virus from contaminated moths. Infected larvae can be identified under UV illumination. The GFP CpGV was incorporated into powder and gel preparations. A recombinant AdorGV was not available and therefore this study had to rely on direct observation and molecular techniques to determine low levels of infection.

Various mating scenarios involving virus-contaminated moths were devised to investigate horizontal transmission.

A. Transfer of virus from contaminated female via clean virgin male
B. Transfer of virus from contaminated male to ova laid by clean virgin female
C. Transfer of virus to ova following an uncontaminated mating

There were problems in carrying out these experiments since the gel and powder formulations were deposited on the walls of the mating chambers as seen from fluorescent labelling experiments. It therefore was impossible to prove whether moths had become contaminated with virus by contact with another individual or by contact with the walls of the chamber. With the powder-contaminated moths the contamination was much greater since the mating chambers were plastic and are probably charged with static. However, despite these difficulties there was clear evidence for horizontal transmission particularly where mated females are contaminated prior to egg laying.

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A reliable field test for the efficiency of mating disruption techniques

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Abstract: A field test for mating disruption treatments is described. It consists of a pair of cages (volume ca. 7 cubic meters), one of them placed in a treated area, the other in an untreated control area. Males are released in these cages, and caught in traps baited with females. Success of a mating disruption treatment is assessed by the fraction of males caught in the treated compared to the control. By removing the dispensers from the test area and placing them around the control cage, the roles of test and control cage are exchanged, and thus effects of microclimate differences can be cancelled out. Measurements of disruption success in treatments with various dispenser release rates are presented. The method can also be used to compare the attractiveness of females and synthetic lures. The advantages of this method are: results are gained with high statistical precision within a few weeks at costs comparable or lower than classical field tests.

Keywords: Lobesia botrana, *Eupoecilia ambiguella*, field test, pheromone, mating disruption, dispenser release rate, pheromone traps, pheromone lures

Introduction

Although mating disruption has achieved an increasing economic importance and success in recent years, there are still problems and open questions remaining. While a large variety of formulations are available, it remains difficult to demonstrate the degree of efficiency of such devices under field conditions. In such experiments, a large number of partially conflicting requirements have to be met: field size must be large enough to reduce edge effects, distance between experiment and control must be wide enough to avoid pheromone transfer, must have micro climate, vegetation, infestation level and abundance of beneficials comparable between experiment and control, must deal with the occurrence of small size dense clusters of infestation in fields with an average low damage level. Finally, a classical field test takes the time of a whole growing season and substantial efforts and costs for materials and damage assessment. On the other side, wind tunnel tests with all their well known advantages deliver results which are difficult to transmit to real nature and thus do not help to predict the success or failure of a given pheromone treatment.

To improve the situation, it seems worthwhile to investigate procedures capable to test the efficiency of mating disruption devices with methods that are independent of the variable influences described above and offer results at lower costs and in a shorter time. On the other hand, such a novel test method should set up conditions which are as close as possible to the actual conditions in the real field. Our test method gives a rather precise replica of the conditions under which the approach of a male towards a female moth is inhibited by the pheromone in the field under treatment.

Materials and Methods

A pheromone treated area is created in a vineyard by applying 100 dispensers in an area of ca. 48·42 m. A cage covering two wine plants is set up in the centre (Fig. 1). It consists of two half-shells permitting, in the middle, the passage of the wires which hold the wine plants in the row. The size of the cage is 2.30 m high, 2.30 m long and 1.60 m wide. Care has been taken to use construction materials which do not adsorb and subsequently desorb pheromone. In the centre of the cage, at 1.50 m height, a standard delta-trap is placed. The lure consists of two virgin female moths placed inside a ball shaped cage ca. 25 mm diameter made from stainless steel mesh. The females are replaced twice a week. A second cage of identical design is set up in an untreated plot serving as control. To start an experiment, 40 male moths, at age 1 to 2 days after emergence are released inside the cages: This release is repeated after 3 days. The traps are checked every day. Males and females (*Lobesia botrana or Eupoecilia ambiguella*) are taken from the well-established rearing facility in the Institute in Freiburg. 3 days after the second release, the dispensers are removed. The females are regularly replaced so that all males in both cages are caught within a further 3 to 4 days. In the second part of the experiment, the dispensers are placed at the site of the former control cage, and the procedure of male releases is repeated. By this scheme of exchanging control and treated area, the influence of micro climate and plant conditions can be cancelled out.

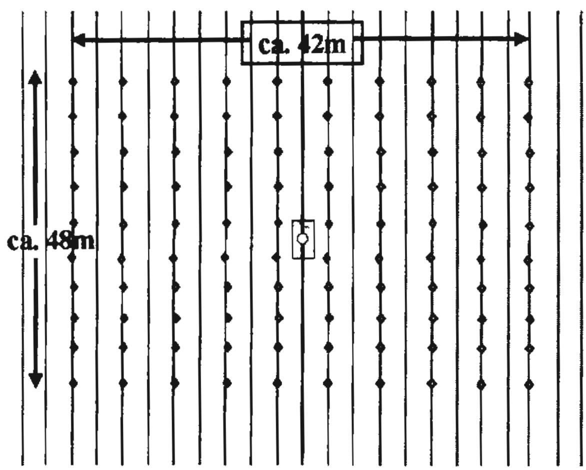

Fig. 1 The experimental cage is placed in the centre of a pheromone treated area, created by the application of 100 dispensers, indicated by the small diamonds. The vertical lines indicate the rows of wine. This layout corresponds to a density of 500 dispensers per hectare.

The cages can also be used for comparing the attractiveness of pheromone lures and calling females. To this aim, two delta traps are put in a cage, one baited with the pheromone lure under test, the other baited with 2 virgin females. The distance between both traps is about

0.4m. Then 50 males are released into the cage. After 3 to 4 days, the position of the traps is reversed and the female baited trap is loaded with 2 virgin females again. The procedure of male release (n=50) is repeated and trap catches are recorded for another 3 to 4 days.

Results

Pheromone persistence

Researchers have investigated whether after the removal of dispensers from a treated area, behaviour changing effects of pheromone to lepidopteran males persist (e.g. Schmitz et al., 1997). Several cases are reported where leaves exposed close to a dispenser were later able to attract males in a trap. Measurements of pheromone concentration in vineyards show a steady decline of the concentration with a time constant on the order of 10 to 20 minutes. From these data, we assume that concentrations relevant to mating disruption effects should be negligible within a few hours after dispenser removal. To test this concept, we removed the dispensers from a test cage by 11:00 a.m., and immediately afterwards, placed 40 males in the cage together with the 2 virgin females in the trap. When trap catches were checked the next morning, the number of males caught in this cage was not different from the number of males caught in a control cage, located in an untreated vineyard.
Thus pheromone persistence shows no effects in our system to *L. botrana* and *E. ambiguella*.

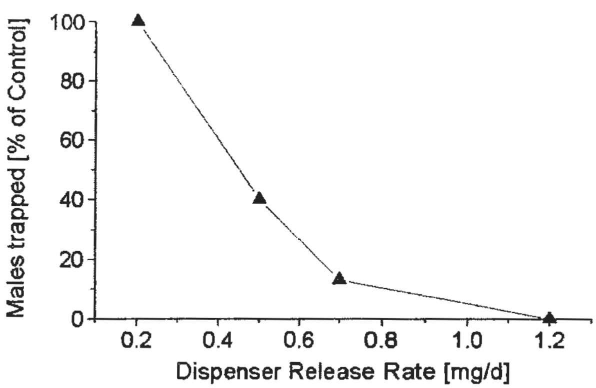

Fig.2: Relation between release rates of dispensers and disruption success against *L. botrana*. Total number of released males: 320 each trial.

Disruption success vs. dispenser release rate or is there a threshold for MD success?

Using classical methods to assess the success of mating disruption does not permit to set up a dose-response curve in which the mating disruption effect can be plotted versus the release rate of the dispensers applied. Since our system offers high reproducibility, low statistical errors and short test duration's, such measurements are now possible. To this aim, standard polyethylene dispensers were filled with special mixtures of pheromone and an inert non diffusing additive to yield dispenser types of defined, reduced release rates. These release rates were experimentally checked using weight loss measurements in a wind tunnel with temperature and wind speed control, and GC measurements of the released materials. Thus, 4 different dispenser types loaded with E7-Z9 12AC (*L. botrana* pheromone) were produced. Their release rates as measured in the wind tunnel at 25°C and 0.4m/s were 0.2, 0.5, 0.7 and 1.2 mg/day respectively. Of each release rate class, 100 dispensers were produced and their mating disruption effect tested using the procedures described above, including the reversal of the role of the test cage and the pheromone-free control cage. A total number of 320 of *L. botrana* males were released for each dispenser type. The results (Fig. 2.) show a clear dependence of mating disruption success on pheromone dosage. Only at the highest release rate (1.2 mg/d), a complete shutdown of the female baited trap can be observed.

Comparison of attractiveness

We measured the attractiveness of commercial BIOTRAP©-lures for *E. ambiguella* and *L. botrana* in comparison to virgin females of the equivalent species. The trials were performed in the way described above; a total of 100 males were used for the test in each species. The trap baited with a BIOTRAP©-lure for *L. botrana*, caught 45 males, the trap with two virgin females caught 37 males. In the experiments with *E. ambiguella,* the trap baited with the BIOTRAP©-lure for *E. ambiguella* caught 60 males, the trap with two virgin females caught no male at all. Obviously, the artificial *E. ambiguella* lure was much more attractive than the virgin females.

Discussion

Cages have been used in different set-ups for tests of mating disruption effects. In many cases, a small number of males and females were released in a cage, and mating success was assessed by dissection of the female. The new idea in our approach is to use the virgin female only as lure in a trap, competing with the pheromone atmosphere generated by the dispensers. The capacity of a trap to catch males is virtually unlimited, and thus a single calling female can generate results with high statistical relevance in a single night.

Field measurements showed that a stable pheromone atmosphere is established within a treated vineyard at a distance of ca. 10 m from the border. Thus, the small number of dispensers necessary to create a stable pheromone atmosphere (cf. Fig.1) permits to reverse the roles of control and experimental cage, cancelling effects of micro climate and vegetation.

Our results show that for a complete shutdown of a female baited trap, a minimum release rate of the dispensers is required. These findings can be used to set standard requirements for the minimum release rate of commercial mating disruption dispensers. Such minimum release rates may be species specific and should be measured now for each species.

Since pheromone traps are applied in different tasks, the requirements for the release rate of the artificial lure are quite diverse. If a trap is to be used as an indicator of sufficient pheromone coverage in a treated plot, the attractiveness of the lure should be comparable to a female. If the lure acts much stronger, trap catches occur in a treated field, leading to the assumption that the pheromone concentrations in the field might not be high enough. As long as the suppliers of pheromone lures do not specify their release rates, our comparison method may be helpful in clarifying lure properties.

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New developments in EAG techniques for field pheromone and plant odour measurements

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Abstract: The EAG system developed in Kaiserslautern for the measurement of aerial concentrations of pheromone has been upgraded into a device for the study of effects of controlled superposition of odours on the EAG. A novel concept for storage and application of up to 14 individual odour sources was implemented. This system was used to study the effect of plant volatiles on the EAG measurement of pheromone. No significant interference between plant volatiles and pheromone was found in the case of Lobesia botrana with linalool and nonanal as test odours.

Key words: EAG measurement system, superpositioning experiments, pheromone, plant volatiles

Introduction

In recent years aerial concentrations of pheromones were successfully measured in fields treated with pheromone dispensers for mating disruption using an EAG measurement system developed at the University of Kaiserslautern. Successful measurements were performed in apple orchards (Koch et al., 1997), maize fields (Koch et al., 2002), vineyards (Koch et al., 1992), cotton fields (Färbert et al., 1997), forests and cranberry bogs. Although a high degree of reproducibility has been reached, there are several open pathways for further development with high potential benefit. Plant volatiles elicit a strong response in a field EAG measurement that mixes with the signal from the pheromone sources of interest. An appropriate superposition method developed at the University of Kaiserslautern is usually capable to suppress this plant odour background. However, more experimental data is desirable to better understand the interactions between pheromone and plant odour compounds and, in general, interactions between different odours in the EAG. To this end, we need a system for the study of EAG responses to the controlled superposition of a wide range of odour stimuli.

Material and Methods

EAG measurement system

For such experiments it is essential to use stable and reproducible odour sources. In standard procedures filter papers are used, but these sources lack the required reproducibility. Instead, we use syringes containing various ratios of oil and odour material. If such syringes are to be applied in a superposition experiment, they should be mounted in a way that they all generate the same stimulation effect. This was achieved in the existing pheromone measurement system by positioning three syringes in a plane which were permanently connected to the central air channel that leads to the antenna. Since the antenna's sensitivity and threshold vary and since it is desirable to apply many different odours, the intervals between odour presentations must be kept small. Therefore many more (e.g. 12) syringes are desirable. To solve this problem, the previous construction was replaced by a system that can hold up to 14 syringes. This was accomplished by replacing one of the fixed syringes by a rotating barrel carrying 12 syringes which are brought into position as needed. All the complex movements (such as motion of syringe pistons at individual times and speeds, rotation and engagement of syringes from barrel, charcoal filter opening) are fully automated to meet the timing constraints in a reproducible way. The commands changing the experimental conditions are generated by the computer controlling the experiment and recording the EAG and other relevant variables.

Test procedure

In order to have well defined conditions in a superposition experiment, we concieved a special test procedure. First, the background odour and the superposition odour are tested sequentially at two concentrations to generate a section of the dose-response curve for each odour. During the superposition phase first the background stimulus was applied and after 1.85 s the superposition stimulus was added to the background for 1.0 s, while the background stimulus was continued for a further 1.75 s. This was repeated after a resting period of 5.0 s with the same background signal, but a tenfold stronger superposition stimulus (cf. Fig. 1).

Analysis

For an adequate evaluation, we considered the test situation as if we were measuring pheromone in the field; with the background stimulus representing the well known plateau which appears when the charcoal filter is opened. We calculated the pheromone level in the usual way, using the amplitude of the superposition pulse response and the slope of the antenna's dose resonse curve to pheromone. This yields a mimicked level of pheromone concentration in the „outside air“, caused by the presence of the background. We define the selectivity of the pheromone response of the EAG with respect to a given background stimulus as „concentration of the background stimulus divided by the 'pheromone' concentration mimicked by the background stimulus“.

Results

Superposition experiments with the grapevine moth (**Lobesia botrana*****)***

In the experiments with *Lobesia botrana* we used nonanal and linalool as background stimuli and E7Z9-12 acetate as superposition stimulus. We found selectivities of 182 for a background stimulus of $5.6 \cdot 10^{-6}$ and 1848 for $5.6 \cdot 10^{-5}$ relative units of nonanal, at a pheromone concentration of $5.6 \cdot 10^{-7}$ relative units. (As an example, 10^{-7} relative units are defined as the concentration existing in a syringe filled with a mixture of 1 part of odour material and 10^{7} parts of paraffin oil.). This means, that the amount of mimicked pheromone concentration was independent of the concentration of the background signal. The net effect of the background on pheromone detection therefore must be negligibly small. In short, no significant interactions between the pheromone and the plant volatiles nonanal and linalool were found.(see figure 1 and figure 2).

Superposition experiments with the Colorado potato beetle (**Pseudotarsus decemlineata*****)***

The colorado potato beetle has a wide range of *sensilla types* (Ma & Visser, 1978). This has stimulated efforts to study a large variety of odours in their superposition effects on the EAG. In our experiments we observed cases of strong interaction between a large number of plant volatiles. We also found a small group of volatiles that did not show interactions between them. Such „orthogonal odours sets“ could be used as test odours for the analysis of unknown odour blends in a new kind of multidimensional EAG device. As an example, the antenna of the colorado potato beetle has been used to detect volatiles released by diseased potato tubers. (Schütz et al., 1999)

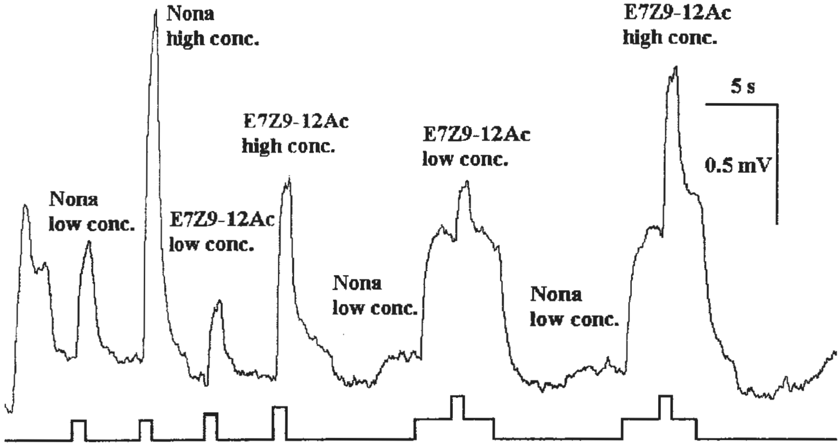

Figure 1. Superposition experiment with nonanal as background odour and E7Z9-12:Ac as superposition odour. In presence of the background odour the EAG-amplitudes in response to the pheromone stimuli show a slight decrease. Lower trace: time course of odour application.

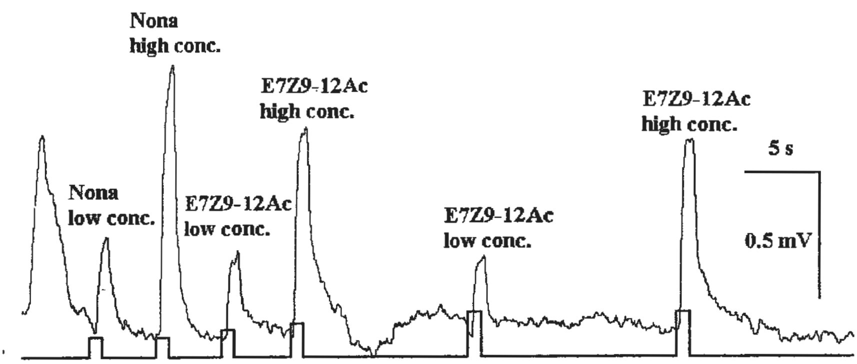

Figure 2. Control experiment without background stimulation. The decrease in EAG-amplitudes seen here is used to correct responses in the superposition experiment above.

Discussion

Are there interactions between pheromone and plant volatiles?

The experiments show that there are no significant interactions between pheromone of the grapevine moth and the plant volatiles nonanal and linalool. Thus plant volatiles don't interfere with the EAG measurement of aerial concentration of the pheromone of the grapevine moth. Rumbo *et al.* (1995) and Karg *et al.* (1997) found interactions between the pheromone of the lightbrown apple moth *(Apiphyas postvittana)* and plant volatiles. Both argued that EAG measurements therefore can not be used for a quantitative determination of of pheromone concentration in the field. We can explain the discrepancy between these findings by the fact that Rumbo & Karg used a permanent background source suspended in the inlet of their device. Every time a stimulation pulse from the pheromone source is applied, air containing only pheromone but not background odour is added to the main airstream. Since the main airstream flow rate is constant, the amount of background odour is effectively reduced, lowering the level of the EAG response voltage. The response to the superimposed pheromone pulse „sinks into the valley of reduced background" and therefore seems strongly reduced. This systematical error is overcome in our setup, since both odour stimuli are applied by the same type of odour source, and the concentrations in the main air stream don't change.

Is the distribution of the pheromone in the central air channel adequate?

In pheromone concentration calculations, one of the assumptions is that the pheromone from the outside air and the pheromone from the added stimulus are evely mixed and distributed over the cross section of the main air channel leading from the charcoal filter to the antenna. This even distribution is also necessary to guarantee a constant and reproducible response of antennae which are known to have an uneven distribution of sensilla types. The features of our system which were added to provide this even distribution have shown several drawbacks. We think that a substantial improvement of the detection performance is possible, if we can improve these mixing procedures. Therefore, we are in the process of analyzing the air flow in our apparatus using dedicated measurements and simulations by computer fluid dynamics methods.

Acknowledgements

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Survey of pheromone emission from different kinds of dispensers used for mating disruption in orchards and vineyards

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Abstract: For the purpose of checking the amount of pheromone in dispensers used for mating disruption and assessment of their emission rate during the season, three techniques are routinely used at the Agroscope RAC Changins.

Extraction: At the beginning of the season, an extraction of attractant with methanol is made for each kind of dispenser to determine the total content of pheromone mixture and its conformity with the registered amount of attractant.

Gravimetry: During the whole season dispensers exposed outside are weighed weekly to estimate the emission of the total pheromone mixture.

GC analysis: Dispensers placed in vineyards or orchards at the beginning of the flight of different pests are recovered each week for a GC analysis in order to determine the remaining amounts of pheromone and to calculate the emission rate during the season.

For the last few years, the pheromone loads of Isomate-C Plus or Isomate-CTT dispensers for codling moth control, as well as Isonet-LE, Isonet-L and Isonet-E dispensers for grape moth control were high enough to ensure a sufficient emission until the end of the flight periods of the different pests, even during the summer of 2003 with unusually high temperatures.

Until 2003, the amount of pheromone registered in Switzerland in Rak1+2, Rak2 and Bocep Viti dispensers used for the control of both grape moth species was not always sufficient to maintain a consistent emission until the end of the second flight period of the pests. However, an increase in the registered amount starting from 2004 should guarantee a better protection at the end of the season.

Key words: *Cydia pomonella, Lobesia botrana, Eupoecilia ambiguella,* mating disruption, dispensers, emission, GC-analysis

Introduction

The success of mating disruption is closely related to the characteristics and performances of the dispensers which must ensure a consistent and sufficient emission of pheromone covering all the flight periods of the pests. For the purpose of checking the amount of pheromone in dispensers used for mating disruption and assessment of their emission rate during the season, three techniques are routinely used at the Agroscope RAC Changins.

Material and methods

Extraction

For each kind of dispenser, 10 units were sampled, weighed separately, cut into small pieces and dipped in methanol. After 2 days soaking, the pieces were dried and weighed. The initial amount of pheromone mixture in the dispensers was determined by the difference between the two weights.

Weighing of dispensers

For each kind of dispenser, 2 units were placed in a vineyard or orchard in Changins at the beginning of the flight of different pests. During the whole season, they were weighed once a week to estimate the evolution of weight loss which represents the emission of the total pheromone mixture.

GC analysis

Dispensers were cut into small pieces and the pheromone was extracted over a 24-48 hour period in 20 ml methanol containing 200 mg of internal standard (12-OH or 13-OH). That mixture was analysed by GC and compared with solutions of known pheromone concentration. The GC-analyser was a Carlo-Erba HRGC 5300 mega series, doted with a capillary column Supelco OmegaWax 320 (film 025 µm), 30 m x 0.32 mm; with a pre-column and a FID detector.

Conditions: before injection, 2 minutes stabilisation at 50°C. Injection, 1 minute at 50°C, heating by 20°C/minute up to 200°C. Plate of 10 minutes at 200°C.

For each kind of dispenser, 20 units were placed in a vineyard or orchard in Changins at the beginning of the flight of different pests. Every week, one unit was recovered and stored in the refrigerator at –20°C for later GC analysis in order to determine the evolution of the remaining amount of pheromone and the emission rate during the season.

Results and discussion

Dispensers for codling moth control (*Cydia pomonella*)

Isomate-C Plus red tubing dispensers are registered in Switzerland for an application at a density of 1000 units/ha. They contain 165 mg of pheromone mixture composed of 103 mg E8E10-12:OH (codlemone, 63%), 52 mg 12:OH (31%) and 10 mg 14:OH (6%). Isomate-CTT twin dispensers are registered for an application at a density of 500 units/ha. They contain 330 mg of pheromone mixture composed of 280 mg codlemone (85%), 42 mg 12:OH (13%) and 8 mg 14:OH (2%).

Table 1. Dispensers registered in Switzerland for controlling codling moth C. pomonella, E. ambiguella and L. botrana. Amount of pheromone mixture recovered by extraction from 10 new dispensers of each kind over the last few years.

specie	Dispenser type	Attractant mixture determined by extraction (mg)				
		2000	2001	2002	2003	2004
C. pomonella	Isomate-C Plus	234.6	-	232.5	230.3	225.5
	Isomate-CTT	448.5	468.6	466.3	455.4	463.1
E. ambiguella	RAK 1+2 (1)	238.8	255.3	372.3	376.2	357.9
	Isonet-E	251.9	-	255.5	264.0	256.8
L. botrana	RAK 1+2 (2)	151.8	157.6	247.8	285.5	271.6
	RAK 2	-	-	-	164.3	141.8
	Isonet-L	259.9	-	265.8	263.6	275.2
Both *E.a.* and *L.b.*	Isonet-LE	-	496.8	475.6	485.7	465.6

Extraction from 10 new dispensers showed that Isomate-C Plus contained roughly 230 mg of attractant mixture and Isomate-CTT about 460 mg, with very small variations from year to year (Table 1).

Emission from both kinds of Isomate dispensers, determined by weekly weighing, was consistent during the whole season and practically the same for each year, except in 2003 which was an abnormally hot season (Figure 1). Average emission of the pheromone mixture, with a density per ha of 1000 Isomate-C Plus, varied between 34 to 52 mg/ha.h. It varied between 30 and 55 mg/ha.h with Isomate-CTT at 500 units /ha. GC-analysis of codlemone confirmed a consistent emission during the whole season, proportional to weighing and included sufficient reserves of pheromones (Figure 2).

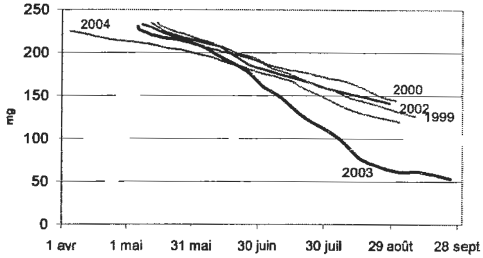

Fig. 1. Amount of pheromone mixture remaining in Isomate-C Plus dispensers determined by weighing for 5 seasons.

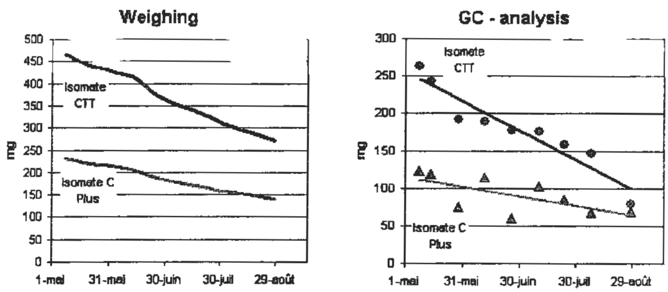

Figure 2. Evolution of remaining attractant in Isomate-C Plus and Isomate-CTT dispensers during summer 2002, determined by weighing and GC-analysis of codlemone.

***Dispensers for grape moths control* E. ambiguella *and* L. botrana**

Many kinds of dispensers are used in Switzerland for an application at 500 units/ha to control grape moths E. ambiguella with Z9-12:Ac and/or L. botrana with E7Z9-12:Ac. For E. ambiguella control Bocep Viti and Isonet-E were registered with respectively 340 mg and 220 mg of Z9-12:Ac. For L. botrana Rak2 and Isonet-L were registered with respectively 120 mg and 172 mg of E7Z9-12:Ac. Combined for both species, Rak1+2 had 230 mg and 120 mg respectively for E. ambiguella and L. botrana and Isonet-LE had 182 mg for each species. Since this year, registered amounts of pheromone in some dispensers have been increased to guarantee a better protection at the end of the season: Rak1+2 to respectively 350 mg and 240 mg for E. ambiguella and L. botrana and Rak2 to 350 mg for L. botrana.

Extraction from 10 new dispensers showed that over the 5 last years the amount of attractant in Rak1+2 dispensers varied between 238 and 376 mg for *E. ambiguella* and between 151 and 285 mg for L. botrana (Table 1). On the other hand, the amount of product

in Isonet-E, Isonet-L and Isonet-LE contained, respectively, roughly 255 mg, 260 mg and 480 mg with very little variation from year to year.
Emission from the 3 kinds of Isonet dispensers, determined by weekly weighing, was very consistent during the whole season and practically the same each year, even in 2003 (Figure 3). Emission of the pheromone mixture with a density of 500 dispensers/ha, averaged 50 mg/ha.h for Isonet-LE, varied between 24 and 29 mg/ha.h for L. botrana pheromone in Isonet-L and between 31 and 34 mg/ha.h for E. ambiguella in Isonet-E. GC-analysis confirmed a very consistent emission during the whole season, proportional to weighing and included sufficient reserves of pheromones.

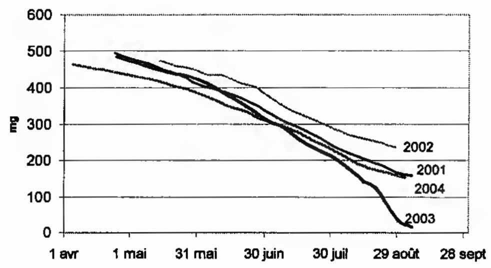

Fig. 3. Amount of pheromone mixture remaining in Isonet-LE dispensers determined by weighing for 4 seasons.

Emission of attractant from Rak1+2 and Rak2 varied more during the season and in some years when the initial load was too low, the dispensers were almost empty before the end of the second flight, especially with Rak1+2 in the ampulla 1, containing E. ambiguella pheromone (Figure 4). Depending on the year and the initial load, average emission with a density of 500 dispensers/ha varied between 30 and 55 mg/ha.h for *E. ambiguella* and between 17 and 36 mg/ha.h for *L. botrana*.

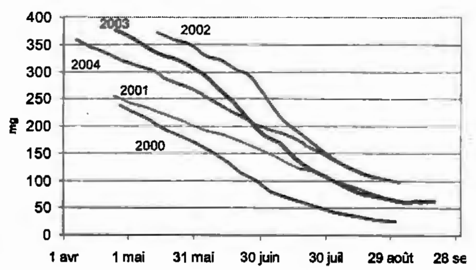

Fig. 4. Amount of pheromone *E. ambiguella* remaining in ampulla 1 of Rak1+2 dispensers determined by weighing for 5 seasons.

Conclusion

For the last few years, the pheromone loads of Isomate-C Plus or Isomate-CTT dispensers for codling moth control, as well as Isonet-LE, Isonet-L and Isonet-E dispensers for grape moth control, were high enough to ensure a sufficient emission until the end of the flight periods of the different pests, even during the summer of 2003 with unusually high temperatures.

Until 2003, the amount of pheromone registered in Switzerland in Rak1+2, Rak2 and Bocep Viti dispensers used for the control of both grape moth species was not always sufficient to maintain a consistent emission until the end of the second flight period of the pests. However, an increase in the registered amount starting from 2004 should guarantee a better protection at the end of the season.

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Kairomone-augmented mating disruption control for codling moth in Californian walnuts and apples

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Abstract: Novel control methods, using both sprayable microencapsulated formulations (MEC) and hand-applied (HA) dispensers, have demonstrated that the pear-ester (PE) attractant kairomone (ethyl (2*E*, 4*Z*)-2,4-decadienoate), termed "DA"-kairomone, improves mating disruption (MD) of male codling moths (CM) in both walnut and apple orchards of California. MD treatment blocks compared pheromone alone *vs*. pheromone + PE kairomone, using both hand applied dispensers and MEC spray tactics. Pheromone trap capture was "shut-down" (>90%) in all pheromone-treated blocks with and without PE adjuvant. Percentage of female multiple-mating was decreased by the addition of PE adjuvant to pheromone MD. For both MEC spray and HA, percent harvest damage in MD blocks was generally decreased by the inclusion of the PE adjuvant with the pheromone disruptant.

Key words: codling moth, *Cydia pomonella*, mating disruption, kairomone, pear-ester

Introduction

Mating disruption (MD) of codling moths (CM), Cydia pomonella, would benefit from improved efficacy and reduction in material and application costs. Male CM attraction to pheromone lures is enhanced by the addition of a kairomone, the pear-ester (PE), (2E, 4Z)-2,4-decadienoate, as now demonstrated worldwide. This provokes the hypothesis that a combination of the two semiochemicals might disrupt mating more effectively than pheromone alone. We report the ability of a PE kairomone adjuvant to influence/effect pheromone MD through field trials testing two application tactics, both micro-encapsulated (MEC) sprayable formulations and hand-applied (HA) dispensers.

Materials and methods

Sprayable semiochemicals

In ten central Californian walnut orchards (2003 and 2004), control activity of reduced rate Ph-MEC alone (MEC-CM, Certis, Inc. and 3M Corp.) was compared to Ph-MEC augmented with a low rate of PE-MEC (CIDETRAK DA-MEC, Trécé, Inc.) in three test blocks in each orchards: 1) Pheromone-Alone Block (25 grams/ha Ph-MEC), 2) Pheromone + PE Block (tank-mixed 25 gm/ha Ph-MEC + 5-9 gm/ha PE-MEC), 3) conventional "Grower Standard" (GS) Block, and/or 4) a distant control block/orchard. MEC treatment blocks were 4-6 ha and GS blocks were 10+ ha, with MEC applied by fan-sprayer at 946 l/ha. Timing and number of applications were based on both male monitoring with pheromone traps and female monitoring with DA traps. MEC sprays were applied during the first, or 1A (April), 1B

(May), 2nd (June), and 3rd (August) flights, timed for onset of the sustained female CM emergence.

Hand-applied dispensers
Commercial Isomate C+ and CTT pheromone dispensers (Pacific Biocontrol, Inc.) and experimental, impregnated polymeric matrix "block" with pheromone and/or PE dispensers (CIDETRAK products, Trécé, Inc.) were used in orchard tests in central California. In 2003, paired-treatment blocks in organic apple (Golden Delicious) and walnut (Hartley) orchards were hung with: 1) Isomate C+ or CTT *vs.* 2) Isomate with co-hung PE dispensers (CIDE-TRAK DA). In 2004, four blocks in conventional apple (Granny Smith) orchards were hung with treatment dispensers: 1) Isomate CTT alone, 2) Isomate CTT and PE dispensers, 3) pheromone (CIDETRAK CM-Ph) alone, and 4) combined pheromone + PE (CIDETRAK CM Ph-DA COMBO). Dispensers were hung in late April – early May in apples at 1000/ha (Isomate C+) and 500/ha (Isomate CTT), and in walnuts at either 345 or 500/ha.

Monitoring
Each block had two to seven pairs of monitoring traps, comprised of a DA-baited trap and a "1X" CM-L2 pheromone (PHEROCON, Trécé, Inc.) trap. Traps were checked weekly, lures replaced monthly, and DA captured moths sexed and female mating status determined. Degree of "shutdown" of capture of male CM in pheromone traps was used to assess adequacy of MEC and HA emission, coverage, and longevity of disruptant levels of pheromone in the orchards. Damage was assessed by monthly "canopy counts" and harvest counts of damaged apples or nuts (1,000 fruits, taken at 25 fruits/tree from 40 trees).

Results and discussion

During the 1A flight of April each year, prior to pheromone dispenser hanging or pheromone MEC sprays, all test orchards had CM populations, ranging from low to high captures of males in pheromone traps. Once the Ph-MEC and HA-Pheromone applications were sprayed/hung the capture of male CM in pheromone-baited traps rapidly and dramatically decreased to a low level of capture throughout the 1B, 2nd and 3rd flights. Pheromone trap "shut-down" ranged from 83% to 95% in pheromone alone blocks and 89% to 98% in Ph &/+ PE blocks (Table 1). Monitoring traps caught more CM in GS/control blocks than MD blocks, while percent female capture in DA traps increased in MD blocks over GS/control blocks. In all GS and MD blocks, a consistent percentage of females captured were both mated (65 – 87%) and 'single-mated' (90 – 99%) (Table 2). However, the percent "double-multiple-mated" differed significantly, with the addition of the PE adjuvant decreasing (0 – 4%) the degree of doubled-mated females captured over pheromone alone blocks (1.8 – 6%) (Table 2). For both HA and MEC apple and walnut orchards, the addition of the PE kairomone adjuvant decreased harvest damage rates (significantly in half the studies) (Table 1). Damage was less in all MD block treatments (0.5 – 1.8%) than in GS/control blocks (1.7 – 4%). Moreover, all MEC treated blocks required fewer supplemental insecticide sprays (1: 2003 and 1-3: 2004) than GS/control blocks (4-5: 2003 and 2-4: 2004). Due to high CM pressure, all HA apple blocks received three supplemental insecticide sprays.

Demonstration of the PE kairomone augmenting pheromone-based MD tactics provides a novel means of improving the control efficacy (% multiple-mated and damage) and lowering rates of required pheromone, thereby making these low-risk MD control strategies more affordable and acceptable in IPM for walnuts and apples.

Acknowledgements

We appreciate test materials supplied by Trécé, Inc., Pacific Biocontrol, Inc., and Certis, Inc. and 3M Corp.; grant support by Walnut Marketing Board and USDA-IFAFS-RAMP; cooperation of growers and PCAs; and assistance by Kathy Reynolds, Paula Bouyssounouse, Mathew Lashua, Elisa Noble, Matilda Gross, Megan DiGirolamo, and Jean Satterwhite.

Table 1. Field trials in both apple and walnut orchards comparing mating disruption efficacy of the traditional pheromone alone tactic *vs.* the tactic of pheromone combined with the pear ester kairomone (PE), using both hand-applied dispensers and sprayable micro-encapsulated formulations (MEC). Suppression efficacy was evaluated by the percent shut-down of male codling moth capture in pheromone traps and the percent of damage at harvest.

Treatments:	Number Replicate Blocks:	Percent Shut-Down Pheromone Trap Capture:	Percent Harvest Damage: Percent
Hand-Applied Dispensers:			
2003, Apples, Golden Delicious (Organic):			
Isomate C+	1	N/A	1.75
Isomate C+ & CIDETRAK PE	2	N/A	1.60 ± 0.53
2003, Walnuts, Hartley (Organic):			
Isomate CTT	2	94.8 ± 1.4	0.72 ± 0.49
Isomate CTT & CIDETRAK PE	2	98.0 ± 0.4	0.50 ± 0.20
Control Blocks	2	0	2.14 ± 0.77
2004, Apples, Granny Smith (Conventional):			
Isomate CTT	6	82.7 ± 3.3^{b}	0.78 ± 0.13^{a}
Isomate CTT & Pear Ester	5	86.4 ± 4.1^{ab}	0.45 ± 0.16^{b}
CIDETRAK CM-Pheromone	3	90.3 ± 3.1^{a}	0.47 ± 0.18^{ab}
CIDETRAK-Dual CM-Pheromone + PE	5	91.6 ± 3.1^{a}	0.48 ± 0.14^{b}
Sprayable MEC – MD, Walnuts, 2003:			
Pheromone MEC-CM Alone	15	95.3 ± 1.5^{a}	1.25 ± 0.18^{ab}
Pheromone MEC-CM & CIDETRAK PE	17	94.1 ± 1.2^{a}	0.60 ± 0.12^{c}
Grower Standard (4–5 insecticide sprays)	4	43.4 ± 5.6^{b}	0.78 ± 0.32^{bc}
Control Blocks w/o MD & Insecticides	3	0	1.70 ± 0.5^{a}
2004:			
Pheromone MEC-CM Alone	10	88.8 ± 4.2^{a}	N/A
Pheromone MEC-CM & CIDETRAK PE	10	92.9 ± 3.9^{a}	N/A
Grower Standard Conventional w/o MD	9	53.6 ± 7.2^{b}	N/A
Conventional Orchards w/o MD	6	0	N/A

Means ± SEM, with significant differences ($P \leq 0.05$) in column means distinguished by different letters, chi-square test. "N/A"– not available data for pheromone trap shut-down and uncompleted 2004 walnut crack-out assessment. "Pear ester" (PE).

Table 2. Comparison of the mating status of female codling moths caught in kairomone DA lure-baited traps hung in apple and walnut orchards with treatment blocks having mating disruption tactics of pheromone alone *vs*. pheromone combined with the pear ester kairomone (PE), using both hand-applied dispensers and sprayable micro-encapsulated formulations (MEC). Mating status evaluated by the percentage of females captured, mated, and degree of mating, either singular or double – multiple mated. Means ± SEM, with significant differences ($P \leq 0.05$) in column means distinguished by different letters, chi-square test.

Treatments:	Total CM Caught/Block:	% Females Caught:	% Females Mated:	% Single Mated:	% Multiple Mated:
<u>Hand-Applied Dispensers:</u>					
<u>2003, Walnuts</u>, (Organic):					
Isomate CTT	173.7 ± 9.1	64.9 ± 4.6	79.2 ± 4.6	98.2 ± 6.8	1.8 ± 0.7
Isomate CTT & CIDETRAK PE	24.5 ± 4.5	64.5 ± 4.5	69.2 ± 11.6	100	0
Control Blocks	258.0 ± 1.0	66.7 ± 5.3	80.0 ± 8.2	97.1 ± 7.6	2.9 ± 0.6

<u>2004, Apples</u>, Too few females were caught for analysis (combined total of 157 females in the all 4 treatment blocks in 2 orchards), though all females caught were single-mated except for one female in one of the Isomate CTT blocks.

Treatments:	Total CM Caught/Block:	% Females Caught:	% Females Mated:	% Single Mated:	% Multiple Mated:
<u>Sprayable MEC:</u>					
<u>2003, Walnuts</u>:					
MEC-CM Pheromone	334.4 ± 100.5[b]	76.7 ± 6.9[a]	75.0 ± 2.5	94.4 ± 2.6	5.6 ± 0.7[b]
MEC-CM Ph. & MEC-PE	126.1 ± 29.5[c]	75.3 ± 4.3[a]	74.8 ± 2.9	96.2 ± 2.7	3.8 ± 0.8[c]
Grower Standard, Conv.	816.6 ± 180.0[a]	45.3 ± 7.8[b]	81.6 ± 1.6	89.6 ± 1.3	10.4 ± 0.4[a]
Control Blocks w/o MD	260.2 ± 60.3[b]	52.5 ± 3.9[b]	75.1 ± 2.8	91.1 ± 2.9	8.9 ± 1.0[a]
<u>2004, Walnuts</u>:					
MEC-CM Pheromone	426.3 ± 79.9[ab]	79.4 ± 4.4[a]	87.1 ± 1.2	95.8 ± 2.2	4.2 ± 0.7[b]
MEC-CM Ph. & MEC-PE	169.3 ± 33.0[c]	82.0 ± 10.0[a]	85.2 ± 1.7	98.5 ± 1.6	1.5 ± 0.8[c]
Grower Standard, Conv.	294.5 ± 49.0[b]	66.1 ± 2.4[b]	84.1 ± 2.0	94.4 ± 2.4	5.6 ± 2.2[ab]
Conv. Orchards w/o MD	581.2 ± 194.6[a]	52.4 ± 1.2[c]	84.5 ± 2.2	93.4 ± 0.8	6.6 ± 0.8[a]

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Season-long control of Oriental fruit moth by mating disruption in apples

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Abstract: To examine alternative pheromone application schedules of hand-applied dispensers for mating disruption of Oriental fruit moth, following treatments were compared at two locations in Henderson County, NC, during 2002: Late May application of hand-applied dispensers; Late June application of hand-applied dispensers; Late May application of hand-applied dispensers supplemented with a late August application of sprayable pheromone dispensers; Late May application of hand-applied dispensers which have a longer activity period, and conventional insecticides as a control. All treatments were sprayed with an insecticide at petal fall for thinning and for control of the first generation Oriental fruit moth adults. Significantly fewer moths were caught in all mating disruption blocks compared with conventional insecticide blocks. Fruit damage was low in all treatments.

Key words: *Grapholita molesta*, mating disruption, hand-applied dispenser, sprayable pheromone, season-long control

Introduction

Oriental fruit moth (OFM), *Grapholita molesta* (Busck), has been a primary pest of peaches for many years, and recently it has also emerged as a key pest of apples in the eastern United States (Hull *et al.*, 2001). In North Carolina (NC), OFM emerged as a serious pest of apples in 1998 (Walgenbach *et al.*, 1999), when a number of fruit loads were rejected at processing plants for the presence of OFM larvae in the fruit. OFM completes four generations per year in NC, and because of overlapping of generations late in the season, continuous egg laying can occur from mid-July through October, which makes chemical control of the insect difficult (Walgenbach *et al.*, 2000).

The use of sex pheromones for mating disruption of lepidopteran pests of agriculture has, over the last decade, become an important component of integrated pest management programs in a number of crops (Casagrande & Jones, 1997). Mating disruption involves the prolonged release of large quantities of pheromone to disrupt communication between male and female insects, which results in delayed mating, fewer fertile eggs, and less damage (Kirsch, 1988, Rice & Kirsch, 1990, Cardé & Minks, 1995, Knight, 1996).

Hand-applied dispensers are the most commonly used pheromone-dispensing system for mating disruption in the US and has produced economically acceptable control (Cardé & Minks 1995). In NC, Isomate-M 100 dispensers have been applied in late May just before the emergence of second generation OFM; chemical control of the first generation is achieved by insecticides applied for control of other pests and for fruit thinning. Although Rice (1993) reported that mating disruption of several generations is necessary to achieve season-long control of OFM in California, this has not been found to be the case in North Carolina. Populations of second generation OFM adults and resultant eggs are typically very low in NC apple orchards, and it is not until the third or fourth generation that populations build to damaging levels (Borchert, 2003). When Isomate-M 100 dispensers are applied in late May in NC, they remain active for about 70 d. Consequently, one application may not provide season-long mating disruption for those cultivars harvested after August. In fact, low levels of late-season fruit damage have been observed in some orchards due to inadequate dispenser longevity (Kovanci, 2003). The purpose of this study was to investigate alternative pheromone application schedules of Isomate-M 100 dispensers, with and without supplementary sprayable pheromone applications.

Material and methods

Description of experiment

Large plot trials were conducted in two commercial apple orchards (Henderson and Staton) in Henderson County, NC, in 2002. All plots were treated with carbaryl (Sevin, Bayer Crop Science, Research Triangle Park, NC) at petal fall for thinning and for control of the first OFM generation. To investigate alternative pheromone application schedules, the following pheromone treatments were compared with conventional insecticides as specified below:

1) Late May application of Isomate-M 100. Isomate-M 100 dispensers were applied at the end of May just before the beginning of the second generation flight. Each dispenser contained 243.8 mg of synthetic sex pheromone [88.5% (Z)-8-dodecenyl acetate (Z8-12:Ac), 5.7% (E)-8-dodecenyl acetate (E8-12Ac), and 1% (Z)-8-dodenenol (Z8-12:OH) and 4.8% inert ingredients]. Dispensers were deployed in the upper canopy of trees at least 2 m above the ground. They were applied at a rate of 250 dispensers/ha.

2) Late June application of Isomate-M 100. The application of Isomate-M 100 dispensers was delayed until late June, just before emergence of third generation moths. They were applied at a rate of 250 dispensers/ha. The use of a delayed application was intended to extend the release of pheromone into September and avoid a costly second dispenser application.

3) Late May application of Isomate-M 100 supplemented with sprayable pheromone. Isomate-M 100 dispensers were applied at the end of May just before the beginning of the second generation flight. They were applied at a rate of 250 dispensers/ha. This treatment was supplemented with an application of sprayable pheromone (Phase V; 3M Canada Company, London, Ontario) at a rate of 24.7g (ai)/ha in late August. Sprayable pheromone is a water-based microencapsulated (MEC) formulation containing 18.6% Z8-12:Ac, E8-12:Ac, and Z8-12:OH and 80% inert ingredients.

4) Late May application of Isomate-M Rosso dispensers. Isomate-M Rosso (Shin-Etsu Chemical, Tokyo, Japan) was designed to extend the release of pheromone from dispensers to more than 150 days. Each dispenser contained 250.2 mg active ingredients of OFM pheromone including 88.5% Z8-12:Ac , 5.7% E8-12:Ac, and 1% Z8-12:OH and 4.8% inert ingredients. Hand-applied dispensers were tied tightly to lateral branches in upper third of the tree canopy in late May. They were applied at a rate of 400 dispensers/ha.

5) Conventional insecticide treatment. A non-pheromone treated conventional block was included at each site and sprayed with five to six applications of the organophosphate insecticides, azinphos-methyl (Guthion 50WP, Bayer, Research Triangle Park, NC) and/or phosmet (Imidan 70WP, Gowan, AZ) for OFM, *Cydia pomonella* (L.) and *Rhagoletis pomonella* (Walsh).

Assessment of treatment efficacy

Wing-style pheromone traps (Pherocon 1C Trap, Trécé, Salinas, CA) were used to monitor OFM populations in each treatment. For each treatment, traps were hung at a density of one trap per 0.4 ha, and each trap was placed in the upper third of the canopy. Rubber septa (Thomas scientific, Swedesboro, NJ) were loaded with 100 µg of OFM pheromone (Bedoukian Research Inc., Danbury, CT) containing 93% Z8-12:Ac , 6% E8-12:Ac, and 1% Z8-12:OH. Pheromone lures were changed every 4 weeks.

Fruit damage was evaluated by examining 100 apples per tree from 10 trees per treatment at harvest during mid-September. OFM injury was placed into one of three categories: "sting" represented surface blemishes caused by a complex of lepidopterous larvae, "entry" represented larval tunneling into the fruit flesh, and "live larva" was used for fruit containing a live larva. The insecticide treatment at Henderson was harvested before damage assessment was conducted, and a total of 100 fruits were examined at this treatment. All fruit were cut to check for the presence of internal lepidopterous damage.

Data analysis

The study was conducted using a randomized complete block design and pooled data were subjected to analysis of variance (ANOVA) (PROC GLM, SAS Institute, 2000). Data are presented as mean cumulative moth catches per trap, but counts were transformed to log (x + 0.5) for ANOVA. Mean percentage fruit damage was transformed using arcsine square root prior to ANOVA analysis. Protected LSD test was used for mean separation (P = 0.05).

Results and Discussion

Pheromone trap catches

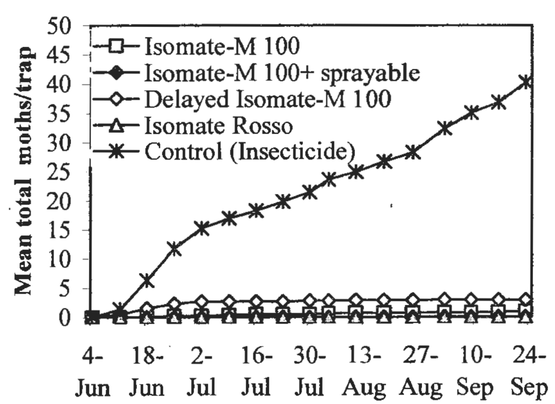

Figure 1. Mean total Oriental fruit moth pheromone trap captures in blocks treated with various mating disruption options and conventional organophosphate insecticides averaged across both orchards.

Mean seasonal total pheromone trap capture of OFM males ranged from 34 to 47 moths per trap in insecticide-treated blocks, indicating that OFM population pressure was moderate in both the Staton and Henderson orchards. Season total OFM trap captures were significantly lower in all mating disruption blocks compared with insecticide-treated blocks (Fig. 1).

Among pheromone treatments, significantly more moths were caught in the delayed Isomate-M 100 treatment at each site. This was mainly due to early season catches before application of dispensers. Following application, no moth captures occurred at this treatment. Several moths were captured in September in the Isomate-M 100 treatment applied in late May, whereas there was no late season trap capture in the Isomate-M 100 treatment supplemented with sprayable pheromone in late August. Early season application of Isomate-M 100 dispensers supplemented with a single sprayable pheromone application in the latter part of season was therefore successful in disrupting the ability of males to locate females in the orchard. This integrated approach not only achieved season-long mating disruption, but also demonstrated ease with which sprayable pheromone technology can be used in mating disruption programs. These findings suggest that sprayable pheromones can be used as a supplementary tool to prevent short-term problems occurring before harvest.

Isomate-M Rosso was highly effective in suppressing trap captures season long. OFM trap catches were impressively low throughout the season, remaining at zero in Staton and near zero in Henderson. This can be attributed to the longer residual emission of pheromone from Isomate-M Rosso dispensers (Sexton & Il'ichev, 2001). Additionally, the polyethylene used in Isomate-M Rosso may also have played an important role for higher release rates in late season because it is constructed of a red colored pigment that protects the active ingredient from degradation in sunlight.

Fruit damage

Damage in all categories was low in all treatments, except for the number of entries in the conventional insecticide blocks (Table 1). The mean percentage of infested fruits in mating disruption blocks was not significantly different than those in conventional blocks (F = 1.00; df = 4, 4; P = 0.50). The highest incidence of entries was recorded in the conventional block (1.6 %), although treatments were not significantly different (F = 1.34; df = 4, 4; P = 0.39). No entries were found in Isomate-M Rosso and Isomate-M 100 blocks supplemented with sprayable pheromone. The mean percentage of stings ranged from 0.2 to 1.0 % among treatments, and no significant differences were detected (F = 0.10; df = 4, 4; P = 0.98).

Table 1. Mean percentage (±SEM) fruit damage averaged across two locations treated with various mating disruption options and conventional organophosphate insecticides

Treatment	n	Sting	Entry	Live worm
Isomate-M 100	2	0.5 (0.3)a	0.2 (0.1)a	0.0 (0.0)a
Isomate-M 100 + sprayable	2	0.4 (0.1)a	0.0 (0.0)a	0.0 (0.0)a
Delayed Isomate-M 100	2	0.4 (0.0)a	0.1 (0.1)a	0.0 (0.0)a
Isomate Rosso	2	0.2 (0.0)a	0.0 (0.0)a	0.0 (0.0)a
Control	2	1.0 (1.0)a	1.6 (1.5)a	0.1 (0.1)a

Our results indicate that combination of mating disruption with insecticide application against first generation OFM is as effective as the insecticide treatment. Similar results have been obtained in mating disruption studies in peaches using early insecticide application to reduce the initial population density of OFM in peaches (Vickers, 1990; Pree *et al.*, 1994; Trimble *et al.*, 2001; Atanassov *et al.*, 2002). Pree *et al.*, (1994) reported that mating disruption could not be used alone if pheromone trap catches exceed 10.5 moths/trap/week. The first generation OFM has the largest moth population in NC and a single insecticide application at petal fall worked well for controlling early season populations at all treatments.

Acknowledgements

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Plant odours influence the host finding behaviour of apple psyllids (*Cacopsylla picta*; *C. melanoneura*)

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Abstract: The apple proliferation (AP) is an economically important plant disease which is caused by phytoplasmas. Because this disease is difficult to control, the management of the insect vectors responsible for the transmission of the AP phytoplasma, the phloem-feeding psyllids Cacopsylla picta and C. melanoneura (Homoptera: Psyllidae), could be the most promising strategy. The aim of this study is to find chemical components which influence the migration behaviour of apple psyllids in summer and after winter, respectively. Up to now, less is known about the chemical signals which are used by apple psyllids to distinguish between their host and non-host plants and between their summer- and winter-hosts. The responses of two psyllid species occurring on apple trees (Cacopsylla picta and C. melanoneura) to leaf odours of different plant species were investigated using a static four-chamber olfactometer. The first results are presented here: During spring, the polyphagous C. melanoneura clearly preferred its summer-host plants odours (apple, hawthorn) to a non-host plant odour (cherry), and hawthorn to apple odour. After reproduction in spring, the new generations of both psyllid species leave their hosts plants in early summer and switch to at least partially unknown host plants, maybe conifers, where they will spend the winter. During this migration period, the oligophagous C. picta prefer the odours of spruce and pine to the odour of its summer host plant apple, while the polyphagous C. melanoneura showed neither a preference for apple/hawthorn odours nor coniferous odours.

Keywords: apple proliferation, apple psyllids, host finding behaviour, olfactory orientation, plant odours, Psyllidae.

Introduction

Apple proliferation (AP) is an economically important plant disease which is caused by phytoplasmas (Seemüller 1990, Seemüller and Schneider 2004). Since this disease is difficult to control, the management of the insect vectors responsible for the transmission of the AP phytoplasma (Frisinghelli et al. 2000, Jarausch et al. 2003, Seemüller et al. 2004, Tedeschi et al. 2002), the phloem-feeding psyllids *Cacopsylla picta* and *C. melanoneura* (Homoptera: Psyllidae), could be the most promising strategy. While *C. picta* is oligophagous on apple and an unknown winter host plant, *C. melanoneura* is polyphagous (Frisinghelli et al. 2000, Hodkinson 1974, Jäger and Topp 2002, Novak and Achtziger 1995). Up to now, little is known about the chemical signals which are used by apple psyllids to distinguish between their host and non-host plants and between their summer- and winter-hosts, and a possible influence of the AP phytoplasma. Thus, we developed a method for testing the olfactory influenced behaviour of the psyllids, and investigated, if the psyllids are able to distinguish between the odours of different plants. A further aim of this project is to find (repellent or attractive) chemical components which are responsible for triggering the migration behaviour of apple psyllids from their different host plants in summer and after winter, respectively. These substances could be used for the development of new control methods in future.

Materials and methods

Insects and plants

Overwintered adult apple psyllids were collected in Dossenheim and Neustadt in early April 2004 from their summer host plants (apple tree (*Malus domestica*): *Cacopsylla picta*; hawthorn (*Crataegus monogyna*): *C. melanoneura*) in the field. They were kept in gauze cages (60 x 95 x 60 cm) in a climatisized greenhouse during the summer under natural light conditions at 20 °C. *C. picta* was kept on 3 month old apple trees, *C. melanoneura* on ca. 1 year old hawthorn bushes. After mating, the new generation was reared from the layed eggs and only young adults were used for the behavioural studies. The plants used in the experiments were bought in a local tree nursery (*Crataegus monogyna*, *Pinus silvestris*, *Picea abies*) or growed in our institute (*M. domestica*, cultivar "Gala", *Prunus avium*, cultivar "Hedelfinger").

Olfactometer

The responses of the two psyllid species to leaf odours of different plant species were investigated using a modified static four-chamber olfactometer (Steidle & Schöller 1997). The olfactometer investigations were done in a climatic chamber a 20 - 22 °C under diffuse light (6000 lux) from the top. In order to prevent side effects, the olfactometer was turned a quarter with every new run.

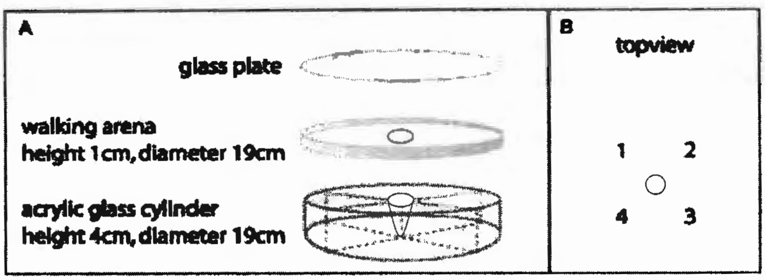

Fig. 1: Four-chamber olfactometer: **A.** Construction: bottom with four chambers, walking arena and lid. **B.** Top view and numbering of the fields: Odour sources were placed in chambers 1 and 3, while chambers 2 and 4 remained empty. Drawing: Hacker 2003, after Steidle & Schöller 1997, modified)

The olfactometer (Fig. 1) was built by an plastic cylinder (height 4 cm, diameter 19 cm) divided in four chambers. In each chamber a water filled glass vial was placed. Two different plant twigs were cut directly before the experiments and placed each in one of the vials. Two odour sources were tested simultaneously against one another and two empty control fields. A walking arena made by metal gauze (height 4 cm, diameter 19 cm) with a hole in the centre (which fits for an Eppendorf tube) was attached and closed by a glass plate.

Behavioural studies

A adult psyllid from the summer generation was collected from the rearing cage, sexed and put into an Eppendorf tube (Eppendorf AG, Hamburg). Then the tube was set into the hole located in the centre of the olfactometer, the lid was opened, and the glass plate was attached.

After having left the tube, the behaviour of the psyllid was observed for a period of 5 min. The duration spent in each of the four odour fields was recorded using the computer program "The Observer 5.0" (Noldus IT, Wageningen, NL). Psyllids which did not leave the cup after 5 min. were not longer observed.

Results and discussion

The psyllids duration of stay within the 4 fields of the olfactometer differed statistically significant between some of the offered plant odour fields and the empty fields (Fig. 2 and 3). The psyllids must have orientated by using volatile plant odours. Thus, the static 4-chamber olfactometer is appropriate for testing their olfactory orientation behaviour.

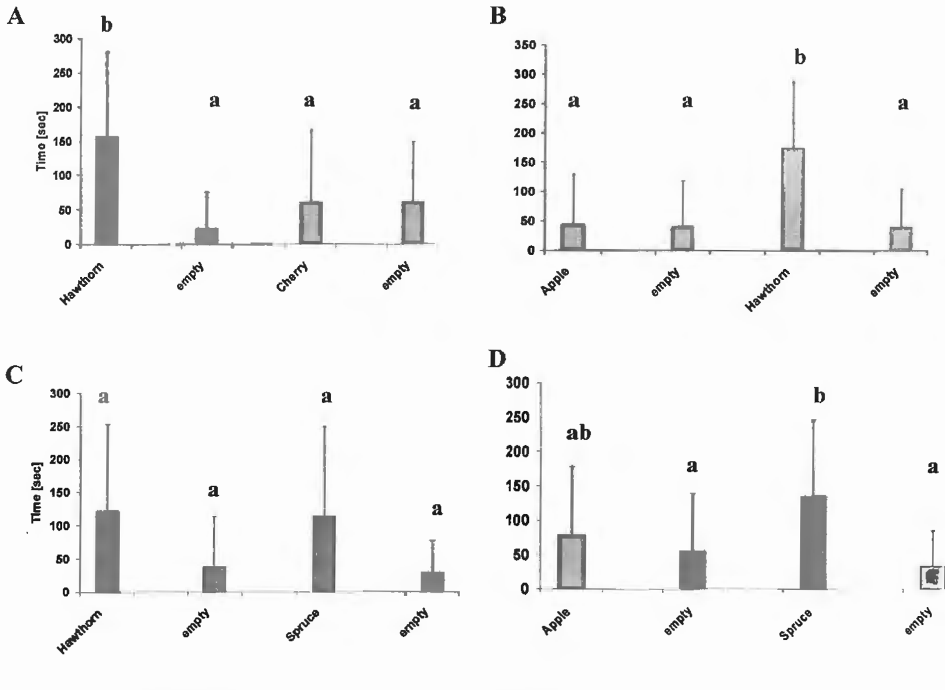

Fig. 2: Olfactory orientation of *Cacopsylla melanoneura* (experience: hawthorn): Mean walking time above each olfactometer chamber and standard deviation are shown. **A** Hawthorn (*Crataegus monogyna*) *vs.* non-host plant (cherry tree *Prunus avium*), n=20. **B** Apple tree (*Malus domestica*) *vs.* hawthorn (only female psyllids), n=34. (7.-28.05.04) **C** Hawthorn vs. spruce (*Picea abies*), n=28. **D** Apple tree vs. spruce, n=28. (24.05.-24.06.04). Different letters indicate significant differences ($p < 0.05$), Wilcox-Wilcoxon test.

During spring, the polyphagous *C. melanoneura* (reared on hawthorn) clearly preferred one of its summer-host plant odours (hawthorn) to a non-host plant odour (cherry tree, Fig. 2 A). The females preferred hawthorn, on which they were kept, rather than apple odour (Fig. 2 B), while the males did not distinguish between the odours of these summer hosts. After reproduction in spring, the new generations of both psyllid species leave their hosts plants and switch to at least partially unknown host plants, maybe conifers, where they will spend the winter. In experiments conducted during this migration period, the polyphagous *C. melanoneura* showed neither a preference for apple/hawthorn odours nor coniferous odours (Fig 2 C, D), while the oligophagous *C. picta* tended to prefer the odours of spruce and pine trees rather than the odour of its summer host plant, the apple tree (Fig. 3 A, B). The lack of statistical significance may be due to the small number of repetitions.

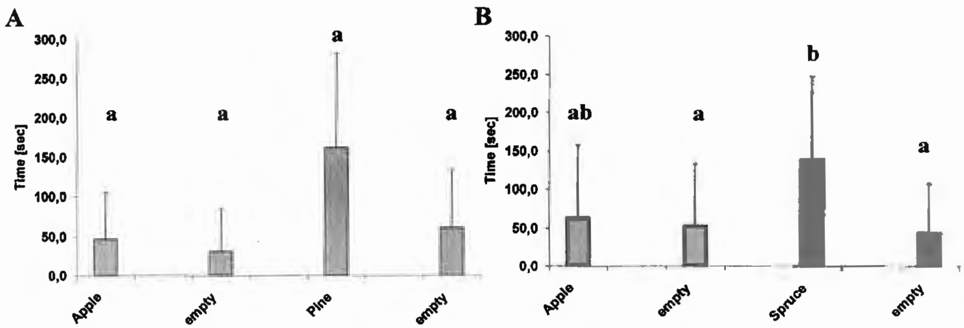

Fig. 3: Olfactory orientation of *Cacopsylla picta* (experience: apple tree): Mean walking time above each olfactometer chamber and standard deviation are shown. **A** Apple tree vs. pine *(Pinus sylvestris)*, n=9 **B** Apple tree *vs.* spruce, n=24. (4.-28.06.04). Different letters indicate significant differences ($p < 0.05$), Wilcox-Wilcoxon test.

Only the first results of the project are presented in this paper. Much more olfactory experiments from beginning to end of the vegetation period during different seasons are necessary to assess, if the psyllids are changing their olfactory preferences towards chemical components during their migration periods, or if the plant odour spectrum will change during the course of the year. Therefore, we will analyse the headspace of the different plant species in order to identify the chemical components which may influence the host-finding behaviour of the psyllids in future studies.

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Tortricid species caught by the Codling Moth kairomone ethyl (2E,4Z)-2,4-decadienoate: monitoring trials and electrophysiological responses

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Abstract: Ethyl(2E,4Z)-2,4-decadienoate (pear ester) has been reported to act as a kairomone for males and females of *Cydia pomonella* (L.) (Lepidoptera Tortricidae). Since it was shown to be attractive also for other Tortricid species, such as *C. splendana* (Hb.), *C. fagiglandana* (Zell.), and *Hedya nubiferana* Haworth, its potential for monitoring purposes and its capacity of inducing electrophysiological responses in the four above mentioned species was investigated.

Key words: kairomone, ethyl(2E,4Z)-2,4-decadienoate, Tortricid species, EAG, field trapping

Introduction

Ethyl(2E,4Z)-2,4-decadienoate was described as an attractant with pheromonal potency for males and females of Cydia pomonella (Light et al., 2001). It has been reported to act as a species specific kairomone. In codling moth field trapping experiments it seemed to attract also males and females of other Tortricid species, such as C. splendana (Hb.), C. fagiglandana (Zell.), and Hedya nubiferana Haworth (Ioriatti, pers. com.). C. fagiglandana and H. nubiferana males captures with pear ester baited traps were recorded in field screening tests carried out in apple orchards (Coracini et. al., 2004).

The oligophagous moths *C. splendana* and *C. fagiglandana* are two of the most important insect pests of chestnut fruit. Their chemical control is difficult because of the endophytic development of the larvae and the size of chestnut trees. The potential of ethyl(2E,4Z)-2,4-decadienoate for practical applications in chestnut moth control and for monitoring purposes should therefore be investigated. *H. nubiferana* instead is a secondary pest in apple orchards and the possibility of monitoring its presence with the same bait used for *C. pomonella* should be considered.

We thus decided to investigate the monitoring efficacy of the pear ester on all four species mentioned above in specially designed field trapping experiments, and to study its capacity of inducing electrophysiological activity by establishing dose-response curves.

Material and methods

Insects

Larvae of *H. nubiferana* were collected from infested apple leaves during May 2004 in Baselga di Pinè (Trentino Region). Larvae of *C. fagiglandana* were obtained from infested chestnut fruits, collected during September 2003 in Benevento (Campania Region). Pupae of *C. pomonella* reared on an artificial diet, were supplied by Catev (Faenza, Italy). Adults of *C. splendana* were directly collected from traps baited either with pheromone or with the pear ester suspended on chestnut trees in Drena (Trentino Region); only insects with intact antennae were used for EAG analyses.

Field trials

The trials were carried out in 2003 and 2004 in different trapping sites (see below). In each trapping site, Pherocon IIB (Trécé) traps were used, and baits (Trécé gray rubber septa) were replaced every 5 weeks. We tested five doses of ethyl(2E,4Z)-2,4-decadienoate (0.1, 1, 3, 10, and 40 mg, respectively), replicated three times. The traps were checked weekly, the captured moths were brought to the laboratory; the captured species were identified, and the number of males, and virgin and mated females was recorded. Three unbaited traps and 3 pheromone baited traps acted as control treatments.

All treatments were tested on four Tortricid species: *C. fagiglandana* and *C. splendana* trapping experiments were carried out in chestnut copses (in Trentino and Campania, respectively), *C. pomonella* captures were observed in an apple orchard (Molise, Southern Italy), and the *H. nubiferana* field trial was carried out in mixed apple and cherry orchards (Trentino). For each species, year, and trapping site, the total number of males, females, and specimens (males + females) captured was compared among treatments using one-way ANOVAs, followed by Duncan's Test for posthoc comparisons of means. Prior to the statistical analysis the data were transformed to $\sqrt{x+0.5}$ and submitted to Levene test for homogeneity of variance.

Electrophysiology

EAG responses were recorded as described in a previous paper (Den Otter *et. al.,* 1997). The odour stimulus was the pear ester kairomone (Et-2E,4Z-DD; > 97% purity). The attractant was supplied by Sigma-Aldrich. Aliquots of 10 µl of hexane solutions of the chemical were absorbed on a piece of filter paper (1 cm^2), which was inserted in a Pasteur pipette. Nine different stimuli, containing from 10^{-6} to 10^{2} µg kairomone/µl, were prepared. The doses were applied in increasing order at 60-s intervals. Before and after each series of stimuli a reference stimulus (10 ng/µl of Et-2E,4Z-DD) was applied to verify a possible decreasing of sensibility in the antennal response. For each species male and female (n=10) responses (mV) were recorded.

Results

Field trials

Et-*2E,4Z*-DD was attractive for all four Tortricid species, but differences among species in relation to efficacy, dose, and male-female ratio emerged.

For *C. pomonella* the total captures were significant lower than those obtained with codlemone. At the lowest dose (0.1 mg) the pear ester was attractive to both males and females. Higher doses were less attractive, especially on females.

Table 1. Mean number of males, females, and total number of moths captured per trapping site and year. Different letters within the same row indicate significant differences (Duncan's Test: P<0.05). No letter indicates Levene test p< 0.05.

Erogator content	0.1mg Da	1mg Da	3mg Da	10mg Da	40mg Da	Control	Ph
Hedya nubiferana	2004 trapping site Trento						
Males	1.00	3.33		1.67	4.00	2.33	
Females	3.00	7.33		0.67	2.00	0.67	
Total	4.00a	11.33a		3.67a	6.00a	3.00a	
Cydia fagiglandana	2003 trapping site Trento						
Males	0.33	0.33		0.00	2.33	0.00	42.67
Females	0.67	1.00		0.33	1.00	0.00	0.00
Total	1.00a	1.33a		0.33a	3.33a	0.00a	42.67b
Cydia fagiglandana	2003 trapping site Campania						
Males	0.33a	2.67a	1.00a	2.33a	7.00b	0.33a	37.00c
Females	0.33a	1.33a	1.33a	1.00a	7.33b	0.00a	0.00a
Total	0.67a	4.00a	2.33a	3.33a	14.33b	0.33a	37.00c
Cydia fagiglandana	2004 trapping site Trento						
Males	0.33	0.00		0.00	0.00	0.00	1.33
Females	0.00	0.00		0.67	1.67	0.00	0.00
Total	0.33a	0.00a		0.67a	1.67a	0.00a	1.33a
Cydia fagiglandana	2004 trapping site Campania						
Males	0.00a	1.67a	2.33a	2.67a	7.33b	0.00a	52.00c
Females	0.33a	3.00a	1.33a	1.33a	10.00b	0.33a	2.00a
Total	0.33a	4.67a	3.66a	4.00a	17.33b	0.33a	54.00c
Cydia splendana	2003 trapping site Trento						
Males	0.33	7.33		5.33	9.67	0.33	44.33
Females	1.00	7.33		6.33	12.00	0.00	0.00
Total	1.33a	14.67b		11.67b	21.67b	0.33a	44.33c
Cydia splendana	2003 trapping site Campania						
Males	0.33a	6.00b	15.00b		11.33b	0.33a	9.33b
Females	3.00b	5.33b	7.33b		13.00b	0.00a	0.00a
Total	3.33a	11.33b	22.33c		24.33c	0.33a	9.33ab
Cydia splendana	2004 trapping site Trento						
Males	0.33	0.33		0.33	1.67	0.00	12.00
Females	1.00	0.33		3.67	6.00	0.33	1.00
Total	1.33ab	0.67a		4.00ab	7.67bc	0.33a	13.00c
Cydia splendana	2004 trapping site Campania						
Males	4.00ab	6.33ab	2.33a	5.67ab	10.67b	0.33a	9.67b
Females	3.67b	6.00b	5.00b	7.33b	9.33b	0.00a	0.00a
Total	7.67b	12.33b	7.33b	13.00b	20.00c	0.33a	9.67b
Cydia pomonella	2003 trapping site Campania						
Males	21.3b	25.3b	27.7b	30.0b	11.0c	0.33d	110.7a
Females	15.7a	13.7a	13.3a	5.0b	4.7b	1.0c	1.3c
Total	37.0b	39.0b	41.0b	35.0b	15.7c	1.33d	112a
Cydia pomonella	2004 trapping site Campania						
Males	2.7c	1.3d	10.3b	5.0bc	3.0c	0.0d	27.7a
Females	1.3abc	1.3abc	2.7ab	3.3a	0.3bc	0.0c	0.3bc
Total	4.0c	2.6c	13.0b	8.3b	3.3c	0.0d	30.0a

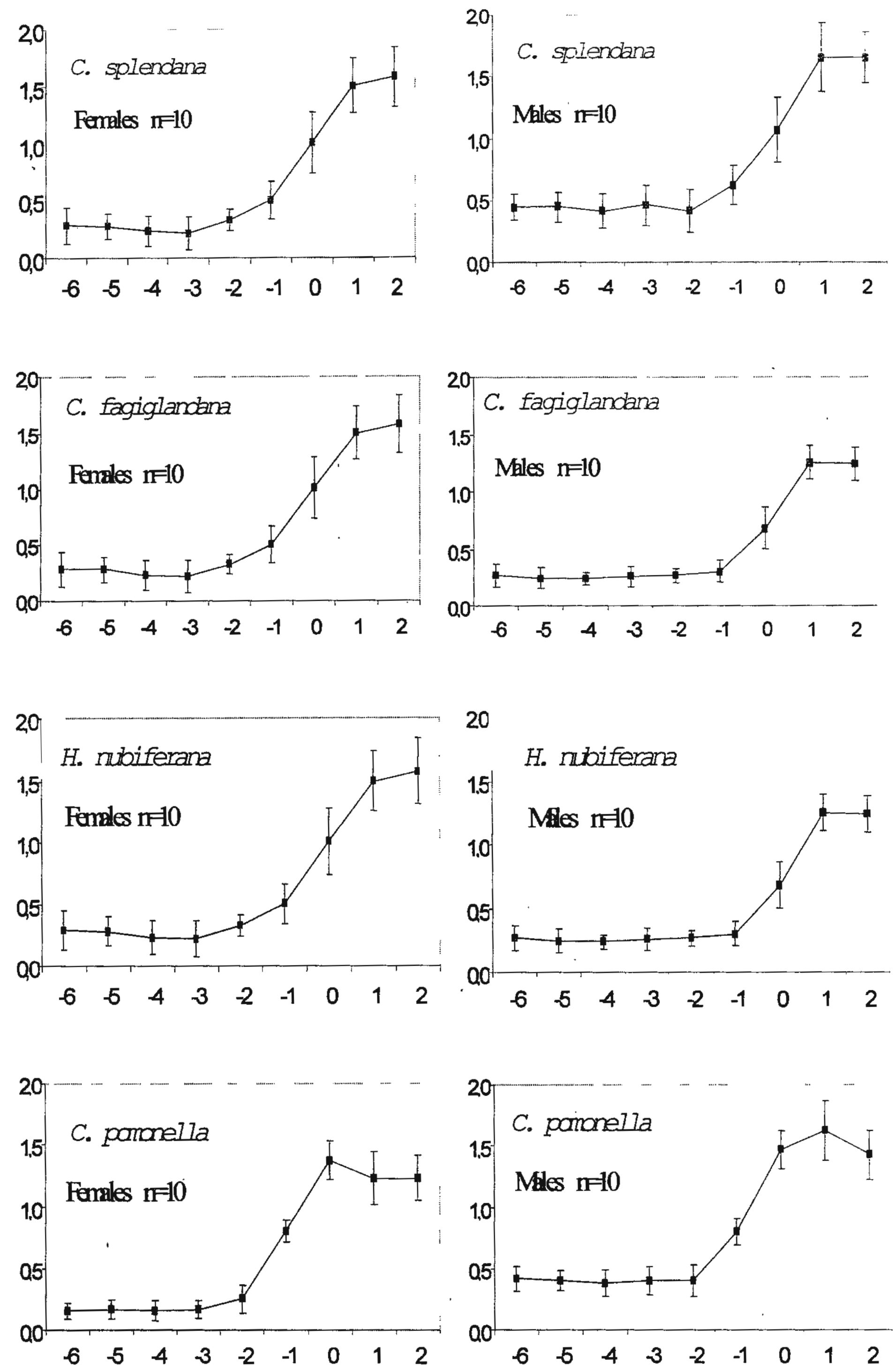

Figure1-8. Electrophysiological dose-response curves; x-axis: Dose (Lg_{10} ug/ul), y-axis: electrophysiological response (mV)

In Trentino, the pheromone lure captured more *C. splendana* moths than the pear ester lures at different doses. Et-*2E,4Z*-DD caught more females than males, and the higher doses gave better results. In Campania the highest dose of the kairomone caught more moths than the pheromone.

The adopted pear ester lures were not efficient in monitoring *H. nubiferana* and *C. fagiglandana* adults, even though the kairomone was attractive for both males and females.

Electrophysiology

All Tortricid species responded to the antennal stimulation with the higher doses of Et-*2E,4Z*-DD (1, 10, 100µg/µl, respectively). Females showed a similar response to Et-*2E,4Z*-DD than males, especially in *C. splendana*. In *H. nubiferana* and in *C. fagiglandana* the pear ester revealed slightly higher responses on females than on males, while in *C. pomonella* it was more active on males.

Discussion

Even though Et-2E,4Z-DD is not a chestnut plant odour, it was EAG-active on both chestnut Tortricid species, *C. splendana* and *C. fagiglandana*. Moreover, in field trapping experiments with *C. splendana*, the kairomone showed an attractant efficacy similar to that of the pheromone. This could be due to the fact that the pear ester acts not only as a kairomone, but also as a pheromone mimic. A pheripheral interaction in the codling moth perception of Et-*2E,4Z*-DD and *E8,E10*-12OH has already been demonstrated (De Cristofaro *et al.*, 2002), and SCR revealed the presence of olfactory cells responding to both molecules (De Cristofaro *et al.*, 2004). The similarity of the DA molecule with *E8,E10*-12OH, a pheromonal component of *C. fagiglandana* and *H. nubiferana*, could explain the activity of the pear ester on these moths. Moreover, EAG recordings on *C. splendana* revealed that this species is also sensitive to *E8,E10*-12OH (Den Otter *et al.*, 1996).

In the field Et-2E,4Z-DD was attractive also to females of the other species. The use of the pear ester in integrated production could therefore be extended to other Tortricid species.

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Potential for disruption of mate-seeking *Sesamia nonagrioides* (Lef.) males by (Z)-9-tetradecenyl acetate

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Abstract: Trapping tests showed that catches of male *Sesamia nonagrioides* (Lef.) (Lepidoptera Noctuidae) moths were strongly inhibited by incorporating (Z)-9-tetradecenyl acetate (Z9-14:Ac), even at low dose, to the synthetic sex pheromone blend. Orientation disruption tests were performed by placing an oil trap, baited with the sex attractant blend, in the centre of a small plot (144 m^2) of a maize field and distributing around it polyethylene vials or multilayer cellulose dispensers containing 10 and 50 µg of Z9-14:Ac, respectively. At the beginning of the vegetation period of second crop maize, when the adult presence was low, a significant male disruption was achieved by applying Z9-14:Ac at 2400 mg/144 m^2 dose but this activity proved to be inconsistent during three-year trials. During the physiological maturity stage of maize plant, when the adult presence was high, the disruptant activity of Z9-14:Ac was more evident and significant starting from 1200 mg/144 m^2 dose. In order to improve the effectiveness of Z9-14:Ac to disrupt orientation of mate-seeking *S. nonagrioides* males a better understanding of the factors influencing its activity is needed.

Key words: *Sesamia nonagrioides*, corn stalk borer, Lepidoptera, Noctuidae, (Z)-9-tetradecenyl acetate, attraction antagonist, field tests.

Introduction

The corn stalk borer, *Sesamia nonagrioides* (Lef.) (Lepidoptera Noctuidae), is the most serious maize pest in many Mediterranean countries. Larval feeding causes young plant death and yield losses as a direct damage and penetration in wounded plants of mycotoxinogenic agents as an indirect one.

Sex pheromone components from sympatric species could disrupt the orientation of mate-seeking males (Vakenti *et al.*, 1988; Suckling and Burnip, 1996; Ferrao *et al.*, 1998).

A previous study, focused on the interspecific activity of the sex pheromone components of *Sesamia cretica* (Lederer) (Lepidoptera Noctuidae) on the sex communication system of the sympatric and coseasonal *S. nonagrioides*, showed that (Z)-9-tetradecenyl acetate (Z9-14:Ac) is able to significantly interfere with the orientation of *S. nonagrioides* males to both virgin females and the synthetic sex pheromone blend (Rotundo *et al.*, 2001).

In southern Italy, *S. nonagrioides* adults were recorded during the entire vegetation period of second crop maize. Their population density was low at the beginning of the vegetation period till the 2nd decade of August and showed a peak during the 2nd decade of September when the maize plants reached the physiological maturity stage (Rotundo *et al.*, 1985).

In the present work, the impact of different doses of Z9-14:Ac on the attractiveness of the sex pheromone blend of *S. nonagrioides* was investigated in trapping experiments. In addition, to better understand the potential for disruption of the pheromonal communication by Z9-14:Ac, orientation disruption trials were carried out in small plots at the beginning of vegetation period (low adult presence) and during the physiological maturity stage (high adult presence) of second crop maize.

Materials and methods

Field

Tests were carried out in second crop maize fields grown in southern Italy (Battipaglia, in the province of Salerno) where in the previous years high corn stalk borer infestation levels were recorded (on the average 4 larvae/plant).

Trapping tests

Trapping experiments, aimed at evaluating the inhibitory effect of Z9-14:Ac on the attractiveness of an effective sex attractant blend [90 μg of (Z)-11-hexadecenyl acetate (Z11-16:Ac), 10 μg of (Z)-11-hexadecenol (Z11-16:OH), 10 μg of (Z)-11-hexadecenal (Z11-16:Ald), 10 μg of dodecyl acetate (12:Ac)] for *S. nonagrioides* (Germinara *et al.*, 2001) were conducted from the 3rd decade of July to the 3rd decade of September during 2000 and 2001.

Oil traps baited with rubber septa dispensers were placed along the field edges 40 m apart fixed to metallic poles approximately 1 m above ground.

Dispensers were loaded with the following lures: (a) the sex attractant blend, (b) the sex attractant blend plus 10 μg of Z9-14:Ac (year 2000); (a), (b), and (c) the sex attractant blend plus 1 μg of Z9-14:Ac (year 2001). For each lure, 3 traps were installed. Unbaited traps (n=3) were used as control.

Orientation disruption tests

A trap baited with the sex attractant blend was placed in the centre of a small plot (144 m^2) and polyethylene vials (0.35 ml) or multilayer cellulose (2.0 x 1.5 x 0.3 cm) dispensers (Novapher, Milan, Italy) containing 10 or 50 mg of Z9-14:Ac respectively, were disposed around it according to one of the following distributions:

- polyethylene vials at a distance of 2x2 m (n=48; 480 mg);
- multilayer cellulose dispensers at a distance of 3x3 m (n=24; 1200 mg);
- multilayer cellulose dispensers at a distance of 2x2 m (n=48; 2400 mg).

Dispensers were hung to the maize plants by iron wires about 1 m above ground. Traps (n=3) baited with the attractant blend were installed in untreated plots (200 m apart) and used as control. Treatments were performed from the 3rd decade of July to the 2nd decade of August (low adult presence) and from the 3rd decade of August to the 2nd decade of September (high adult presence) during three consecutive years (2000-2002).

Data analysis

Trap catches were collected at 1-week intervals and insect species determined by examining genitalia. *S. nonagrioides* male catches, expressed as number of males/trap/week, were transformed to $\sqrt{x + 0.5}$ and submitted to the analysis of variance (ANOVA).

For the trapping tests, means were ranked by Student's *t*-test or Duncan's multiple range test (P=0.01).

For the orientation disruption tests, a Disruption Index (D.I.) was calculated according to the formula: 100-[(catch in treatment/catch in control) x 100] (Suckling and Burnip, 1997). In order to determine the significance of D.I., catches in treated plots and those in the related controls were submitted to the *t*-test (P=0.05 and P=0.01).

Results

Trapping tests

During 2000, from July 28 to September 22, male catches by the sex attractant blend (936 males) were more than 12 times as high as those by the sex attractant blend plus 10 μg of Z9-14:Ac (73 males). For each week of this period, the difference in terms of male per trap between the sex attractant alone or plus 10 μg of Z9-14:Ac was significant for P=0.01 (*t*-test) (Fig. 1).

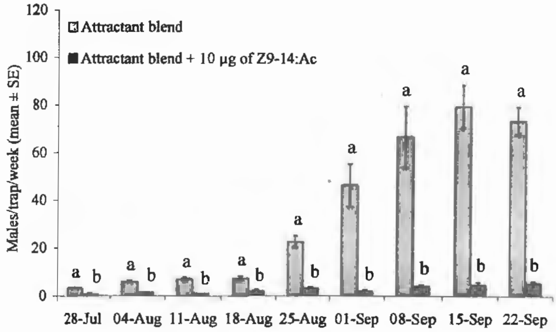

Fig. 1. Mean number of *S. nonagrioides* males captured in oil traps baited with the sex attractant blend alone or in combination with 10 μg of Z9-14:Ac during 2000. Means with different letters are significantly different for P=0.01 (*t*-test).

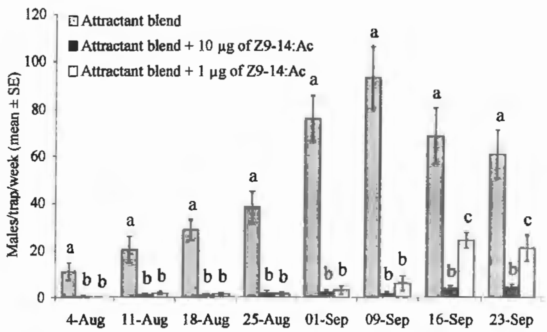

Fig. 2. Mean number of *S. nonagrioides* males captured in oil traps baited with the sex attractant blend alone or in combination with 1 or 10 μg of Z9-14:Ac during 2001. Bars with different letters are significantly different for P=0.01 (Duncan test).

During 2001, from August 4 to September 23, male catches by the sex attractant blend (1192 males) were more than 26 and 6 times as high as those by the sex attractant blend plus 10 μg (45 males) or 1 μg (176 males) of Z9-14:Ac, respectively. For each week of this period, the reduction of male catches per trap produced by incorporating 10 μg or 1 μg of Z9-14:Ac in the sex attractant blend was significant for P=0.01 (Duncan test) (Fig. 2). During the last two weeks of sampling, traps baited with the lure containing 10 μg of Z9-14:Ac captured, on the average, significantly fewer males than traps baited with the lure containing 1 μg of the same compound (Fig. 2).

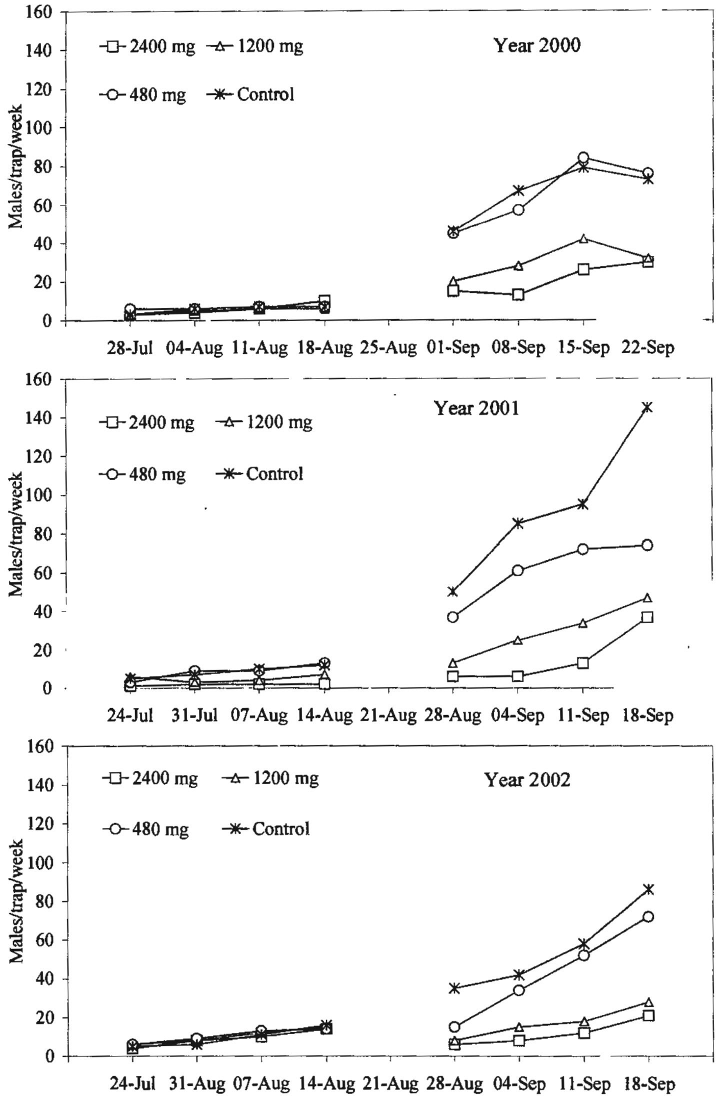

Fig. 3. Mean number of *S. nonagrioides* males captured in oil traps baited with sex attractant blend in small plots (144 m^2) treated with 480, 1200 or 2400 mg of Z9-14:Ac during 2000-2002.

Orientation disruption tests

The mean catches per trap per week of *S. nonagrioides* males by oil traps baited with sex attractant blend in small plots (144 m^2) treated with different doses of Z9-14:Ac from the 3rd decade of July to the 2nd decade of August (low adult presence) and from the 3rd decade of August to the 2nd decade of September (high adult presence) during three consecutive years (2000-2002) are presented in Fig. 3.
During 2000-2002, from the 3rd decade of July to the 2nd decade of August (low adult presence), male catches in plots treated with 480 and 1200 mg of Z9-14:Ac were similar to those in the control ones (Tab. 1). For the treatment with 2400 mg of Z9-14:Ac a significant (P=0.05; Duncan test) reduction of male catches was recorded only in 2001 (Tab. 1) and the corresponding D.I. (79) was significant for P=0.01 (*t*-test) (Tab. 1).

Tab. 1 - Mean catch per trap per week (± SE) of *S. nonagrioides* males by oil traps baited with sex attractant blend in small plots (144 m^2) treated with 480, 1200 or 2400 mg of Z9-14:Ac, disruption indices (DI) and their significance.

Year	Adult presence	mg of Z9-14:Ac/144 m^2						
		480		1200		2400		Control
		Catches Mean ± SE	D.I.	Catches Mean ± SE	D.I.	Catches Mean ± SE	D.I.	Catches Mean ± SE
2000	low	6.5±0.3 a	-25	5.0±0.7 a	11	5.8±1.5 a	1	5.8±0.9 a
2001	low	8.5±2.1 a	3	5.0±0.9 a	35	1.8±0.3 b	79 **	8.5±1.6 a
2002	low	10.5±1.8 a	-19	10.3±1.8 a	-14	9.0±2.1 a	2	9.5±2.5 a
2000	high	65.5±8.9 a	2	30.5±4.6 b	54 **	21.0±4.1 b	67 **	66.3±7.2 a
2001	high	61.0±8.5 a	32	29.8±7.2 b	69 *	15.5±7.4 b	87 **	93.8±19.6 a
2002	high	43.3±12.2 a	26	17.3±4.2 b	70 *	11.8±3.3 b	80 **	55.3±11.3 a

Means within each line followed by different letters are significantly different for P=0.05 (Duncan test).
* = significant for P=0.05 (*t*-test); ** significant for P=0.01 (*t*-test).

During 2000-2002, from the 3rd decade of August to the 2nd decade of September (high adult presence), male catches in plots treated with 1200 and 2400 mg of Z9-14:Ac were significantly (P=0.05; Duncan test) lower than those in the control ones (Tab. 1). The D.I. of male orientation ranged from 54 to 70 for the dose of 1200 mg and from 67 to 87 for that of 2400 mg (Tab. 1).

Conclusions

Z9-14:Ac showed a strong inhibitory effect on the attraction of *S. nonagrioides* male moths when it was presented, even at low dose (1 µg), on the same bait with the sex attractant blend. When Z9-14:Ac was released by physically separated dispensers in small plots its effect was variable. At the beginning of vegetation period, when the adult presence is low, a significant orientation disruption of *S. nonagrioides* males was achieved by applying Z9-14:Ac at the highest dose (2400 mg/144 m^2). However the effect of this dose was inconsistent during the three-year trials. During the physiological maturity stage of maize plant, when the adult presence is high, the disruptant activity of Z9-14:Ac on mate-seeking *S. nonagrioides* males was more evident. During the three years, a significant male orientation disruption was achieved by applying at least 1200 mg of Z9-14:Ac per 144 m^2; this activity proved to be dependent from dose.

Our results are in agreement with the situation reported for *Planotortrix octo* (Dugdale) (Lepidoptera Tortricidae) where the inhibitor (Z)-5-tetradecenyl acetate both reduced trap catch and was a partial disruptant (Suckling and Burnip, 1996). Reduced attraction to pheromone but not disruption of orientation behaviour of males was reported for other attractant antagonists (Suckling and Burnip, 1997 and references therein).

The factors that are determined the variability of the disruptant activity of Z9-14:Ac is difficult to understand. Changes in the emission of volatile compounds and in the possible adsorption and release of the attractant antagonist by host plant may play an important role.

The disruptant activity of Z9-14:Ac on mate-seeking *S. nonagrioides* males could be enhanced by using higher doses and/or optimising its release by more appropriate dispenser materials. In addition, as blends of sex pheromone and attraction antagonists can be potent mating disruptants (Bengtsson *et al.*, 1994; Suckling *et al.*, 1994), the inclusion of Z9-14:Ac in the sex pheromone formulation for mating disruption of *S. nonagrioides* (Albajes *et al.*, 2002) may amplify the disruptant activity of pheromone, particularly useful at high population densities, and prevent male attraction from nearby fields (Witzgall *et al.*, 1999).

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Control of the Codling Moth, *Cydia pomonella* (L.) (Lepidoptera Tortricidae), by disorientation

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Abstract: A new pheromone-based control technique, the so-called disorientation method, has been recently proposed for Codling Moth (CM), *Cydia pomonella* (L.), control. Dispensers used for disorientation (Ecodian CP) were characterized in relation to their release rate and life span. Pheromone release rates from new and field aged dispensers were evaluated using SPME (directly). EAG recordings showed that males respond to field aged dispensers even though responses decreased with dispenser age. Wind tunnel observations demonstrated the activity on CM behaviour of aged Ecodian CP. Traps lured with aged dispensers are able to attract males throughout the season, and their efficacy is higher during the last period of the season when their release rate is comparable to that of the standard lure. Field trails carried out in 2002 and 2003 in replicated blocks confirmed the efficiency of the Ecodian CP dispensers for CM control. The efficacy of disorientation was not significantly reduced when double purpose dispensers for the simultaneous control of *C. pomonella* and *C. molesta* (Ecodian Star) were used.

Keywords: E8,E10-dodecadien-1-ol, Ecodian dispenser, SPME, EAG, wind tunnel, field trial.

Introduction

The Codling Moth (CM), *Cydia pomonella* (L.), is the most important pest in pome fruit orchards in Europe. Its control still relies largely on insecticide applications. Because of their negative side effects on beneficials and the development of resistance towards the most widespread used pesticides, the interest in developing alternative control strategies is increasing. The most widely used technique is Mating Disruption (MD), which consists in the application of a relatively low number of dispensers (500-1,000) loaded with 130-210 mg of pheromone. Mating disruption has been adopted successfully (50% of the Trentino-South Tyrol apple growing area) to control CM in the most infested area. In areas where the pressure of the pest is less intense, less pesticides are applied, and MD applications are economically less convenient. Recently, a new pheromone-based control technique, called disorientation, has been proposed for the control of a number of species of Lepidoptera (Rama *et al.*, 2002; Angeli *et al.*, 2002). According to Sanders (1996), the success of this method depends on three factors: each dispenser should be as potent or more potent than a calling female moth; there must be enough dispensers to consistently decrease the probability of a male finding a female instead of a synthetic source; males must spend sufficient time visiting the dispensers to avoid that they find the calling female moths. As a consequence, the pheromone release rate of the synthetic source must be comparable to or higher than that of the females. This study aimed at characterizing the dispensers applied for disorientation (Ecodian CP and Star, Isagro, Italia) in relation to their release rate and life span, and at verifying the efficacy of integrated strategies in controlling the Codling Moth in field conditions.

Material and methods

Insects

The CM strain used was collected as overwintering larvae in the apple orchards of the Agricultural Institute of S.Michele all'Adige (IASMA) in October 2002. Rearing cages and experiments were housed in climatic chambers (22±2°C, 70±10% R.H., 18L:6D inverted photoperiod).

Dispensers

Two types of hook-shaped dispensers were evaluated in relation their pheromone loading: Ecodian CP loaded with 10 mg E8,E10-12OH, the main pheromone component of *C. pomonella,* and Ecodian Star loaded with 10 mg E8,E10-12OH and 10 mg Z8-12Ac, the main pheromone component of *Cydia molesta* (Busck). In both 2002 and 2003, dispensers were placed in orchards located in Gardolo (200m. a.s.l.; 2 appl./year) and Rumo (800m. a.s.l.; 1 appl./year) (Trento, Italy) in April and May, respectively. Field aged dispensers were collected every other week from the application date on.

Release rate of dispensers

SPME in static air was used for effluvia collections (Rotundo *et al*., 2001; Bordereau *et al*.. 2002). Volatiles were adsorbed from the headspace on a fibre coated with a solid sorbent (pdms; 100 µm - Supelco), first conditioned for 5 minutes in a GC injection port at 220°C. The SPME fibre was directly inserted into the GC for thermal desorption (5 min) and analysis. Chemical analyses were performed on a Hewlett-Packard 5890 gas chromatograph, with a polar Innowax column (30 m x 0.32 mm) programmed from 60°C (hold 3 min) at 8°C/min to 220°C (hold 7 min), interfaced with an electroantennogram apparatus (GC-EAD). Identity confirmation for the compound was obtained by comparing the GC retention time with that of the standard synthetic chemical E8,E10-12:OH (Sigma-Aldrich; purity≥97%), and by verifying the EAG response of a CM virgin male antenna. The amount of pheromone collected in the headspace was determined comparing the GC areas (GC-EAD2000; Syntech, Hilversum, NL) obtained with direct injections of synthetic E8,E10-12:OH solutions; this amount was corrected according to the efficacy of the fibre in recovering the synthetic compound, which has been previously evaluated.

The straight part of the Ecodian hook (2,8 cm), with the cut end sealed in paraffin, was confined in a vial (2 ml) for the collection (n=10). The needle of the SPME was inserted into the vial by piercing the Teflon septum of the vial. Based on recovery data, the extraction lasted 60 minutes, and was preceded by 10 minutes of equilibration. The extract was then injected into the GC. The overall release rate was determined according to the total length of the dispenser. Pheromone release was measured 55, 81 and 111 days after the application in the field (2003; Rumo) of the Ecodian CP dispensers.

Electroantennographic (EAG) responses

The amount of pheromone released by Ecodian CP aged dispensers (2002, 1st application; Trento) was also evaluated using EAG analyses carried out on virgin males. We used a technique similar to that described in a previous study (De Cristofaro *et al*., 2000). The stimuli were provided by inserting the straight part of the Ecodian hook (2,8 cm), with the cut end sealed in paraffin, into a Pasteur pipette. EAG responses were normalized according to the reference stimulus (a piece of filter paper soaked with 10 µl of E8,E10-12OH solution at 1µg/µl in hexane, inserted into a Pasteur pipette) and subjected to the analysis of variance (ANOVA) followed by Duncan test.

Wind tunnel (WT)

Tests were carried out in a glass wind tunnel (250x60x60 cm) at 22±3°C, 45±10% R.H. and at an air speed of 15 cm/s. The laminar air flow was generated and purified (activated charcoal) using an air speed and humidity-conditioning unit (De Cristofaro *et al.*, 2003). The tunnel was illuminated from above by a neon tube with a scattering screen (6 lux). WT sessions started at the scotophase and continued for 4 h. Single, virgin, 2-3-day old males (n=10) were used . Ecodian CP aged dispensers (2002, 1st application; Trento) were vertically hung onto a metal holder in the center of the wind tunnel section. Five behavioural responses were recorded: activation (walking and wing-fanning), taking flight, oriented flight, flight near the odour source (15 cm), landing (landing on the source). Males had 15 min to respond. The behavioral responses obtained with dispensers of different age were compared using 2x2 contingency table-χ^2 tests.

Field trapping

In order to evaluate the activity of the Ecodian dispensers in the field, two different trials were carried out in an apple orchard in Trento (Italy) treated with conventional insecticides. In the first experiment, the attractiveness for CM of new Ecodian dispensers used as lures in delta traps (n=4 Carpotrap; Isagro) was evaluated; Ecodian CP (n=2) and Ecodian Star (n=2) were compared with the standard monitoring lure. In the second experiment, delta traps (n=8) were baited with Ecodian CP; in four traps, the Ecodian CP hooks were renewed at three week intervals, while in the other four traps the lure was not renewed. Four standard lured traps (Carpotrap) were used as reference. In both experiments, traps were checked weekly. The number of males captured was log(x+1) transformed, and compared across treatments by means of one-way ANOVAs, followed by Tukey test for posthoc comparisons of means.

Efficacy evaluation in the field

In 2002 and 2003, in an orchard in Borgo (Trento; Italy) two field trials were conducted in order to evaluate the efficacy of Ecodian CP (2000/ha) and Ecodian Star (2000/ha) dispensers for CM control. Fruit damage, trap shut down, and overwintering population were recorded, and data were compared to those of a organic reference orchard (3 granulosis virus) and of an untreated plot. Ecodian CP and Star were applied twice (the first time at the biofix i.e. 150°D, and the second time at the beginning of the summer generation) in 2002, while in 2003 two applications of Ecodian Star were compared with only one application of Ecodian CP. Each treatment was replicated three times in 6,700 m² randomized plots. In every plot, 2 traps baited with a monitoring rubber septum loaded with E8,E10-12:OH (2.5 mg for disorientation treatments, 1.0 mg for reference treatments), were placed, and the number of captured males was determined weekly. Thousand fruits per plot were checked at the end of each generation, and the damage was addressed as percentage of injured fruits. For the collection of overwintering larvae, a cardboard band was placed around the apple trunk (50/plot).

Results

Release rate of dispensers

At the end of the season (September 6, 2003), 111 days after the single application of the dispensers, the release rate of the Ecodian CP measured with SPME was 258.4±33.8 ng/h/dispenser; this release rate is still around 40 times higher than that obtained for individual calling *C. pomonella* females (Backman *et al.*, 1997).

Table 1. Release rates of E8,E10-12:OH of field aged dispensers (Ecodian CP) used for disorientation in Rumo (800 m. a.s.l.).

Field age of dispensers (days)	Release rate (ng/h/disp.)
55	1602.2±144.6
81	450.8±75.2
111	258.4±33.8

Electroantennographic (EAG) responses

The antennal responses of virgin CM males to aged Ecodian CP decreased gradually over time. Nevertheless, the responses to the Ecodian CP still amounted to 60.5% of the initial response after 60 days of ageing in the field, i.e. when the first flight period of CM ends (end of June); the dispenser was still electrophysiologically active at the beginning of August, after 103 days of field ageing.

Table 2. Attraction of virgin (n=10) *C. pomonella* males to field aged Ecodian CP dispensers.

Field age of dispensers (days)	% activation	% taking flight	% oriented flight	% flight near the source	% landing
0	100	90	30	30	0
11	100	60	30	20	0
26	90	50	20	20	0
39	80	60	50	40	0
60	80	70	60	50	0
75	90	80	40	40	0
88	90	80	40	30	0
103	100	80	20	20	0

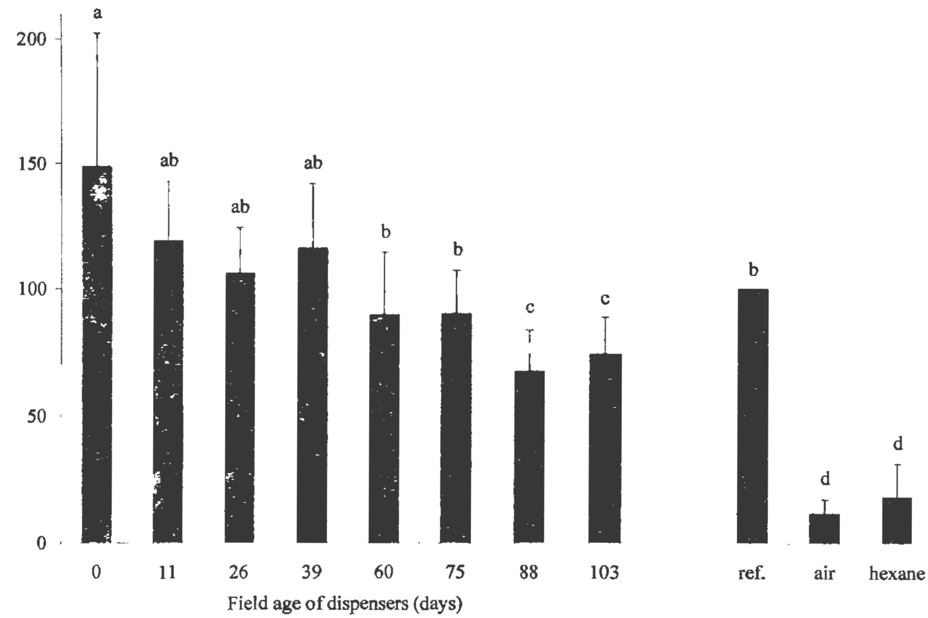

Figure 1. Mean normalized EAG response of virgin (n=10) *C. pomonella* males to field aged Ecodian CP dispensers. Reference stimulus: E8,E10-12:OH (10 µl of solution at 1µg/µl in hexane). Vertical bars represent S.D. Different letters indicate significant differences (Duncan test: P<0.05).

Wind tunnel (WT)

The percentage of oriented flights of virgin CM males was always very high and did not depend on the age of the dispenser; for none of the behavioural responses analized, significant differences among dispensers of different field age emerged (2x2 contingency table-χ^2 test after Yates correction: P>0.05 in all cases).

Field trapping

In the first experiment, Ecodian CP used as lure in monitoring traps was able to attract CM males throughout the season, from early May to early September; Ecodian Star was able to attract CM throughout the season, either, even though at lower numbers than Ecodian CP. Both Ecodian CP and Ecodian Star were significantly less attractive than the standard monitoring lure. In the second experiment, renewed Ecodian CP dispensers did not capture significantly more males than not-renewed dispensers. Nevertheless, the lures remained attractive throughout the entire CM flight period, and their efficacy was higher during the last period of the season, when their release rate was comparable to that of the standard lure. In both experiments, differences among treatments in the number of males captured were significant (ANOVA; P<0,05).

Efficacy evaluation in the field

Field trials carried out in 2002 and 2003 in replicated blocks confirmed the efficacy of the Ecodian dispensers for CM control. The infestation level recorded in the plots treated with disorientation (Ecodian CP and Star) was lower than that observed in the plots treated with the biological insecticide.

Table 3. Total number of CM males captured, percent fruit damage (%), and number of overwintering CM larvae/cardboard in the different treatments (2002).

Treatment	Total no. males captured	% fruit damage	Larvae/cardboard
Ecodian CP (2 appl.)	3	0.82	0.06
Ecodian Star (2 appl.)	2	0.65	0.05
Granupon	38	3.10	0.16
Untreated	147	27.10	0.57

Table 4. Total number of CM males captured, percent fruit damage (%), and number of overwintering CM larvae/cardboard in the different treatments (2003).

Treatment	Total no. males captured	% fruit damage	Larvae/cardboard
Ecodian CP (2 appl.)	6	2.90	0.07
Ecodian Star (2 appl.)	1	0.90	0.03
Granupon	82	7.80	0.70
Untreated	96	28.80	3.10

Discussion

The results of this study demonstrate that the characteristics (life span, release rate, attractiveness) of Ecodian dispensers are compatible with an efficient CM control throughout the season under the climatic conditions of the Trentino Region. The disorientation technique was able to control CM, confirming the results of previous field trials carried out in other areas (Angeli *et al.*, 2003).

Further investigations are needed in order to evaluate the efficacy of this new technique under higher population density of the pest.

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Impact of increased point source densities on communication disruption of tortricid moth pests in Michigan tree fruit

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Abstract: A series of experiments investigating the importance of competitive attraction as the primary mechanism of mating disruption for codling moth (*Cydia pomonella*) and oriental fruit moth (*Grapholita molesta*) were performed in Michigan apple orchards during the 2002-2004 growing seasons. One set of experiments entailed varying the numbers of point sources per area, while maintaining a constant amount of pheromone applied per area. Treatments included an untreated check and at least five point source densities. Levels of trap shutdown within the center and on the edges of plots increased with increasing numbers of point sources. Very high levels of at least 97% inhibition of catches were only recorded at the high point source densities. Substantially better disruption of orientation to traps was achieved in the center compared to the plot edges at all point source densities. Two additional experiments completed in 2004 included the deployment of tethered Oriental fruit moth females in plots treated with wax pheromone drops at four different point source densities for 24 hours on five dates coinciding with peak flight times, and visual observations of male moth visits to pheromone wax drops were conducted on 26 individual nights. The number of females mated decreased as the number of wax pheromone point sources increased; no mating was recorded only at the 2 highest densities where orientation disruption exceeded 99%, 8,200 drops (99.4% orientation disruption) and 27,300 drops (99.2% orientation disruption). Males were observed visiting all pheromone treatments except the 8,200 drop treatment.

Key words: *Cydia pomonella*, codling moth, *Grapholita molesta,* oriental fruit moth, competitive attraction, mating disruption, point sources

Introduction

Pheromone-based mating disruption has been deployed as a pest management tool for the control of agriculturally important pest insects for over two decades, but the main mechanism(s) by which mating disruption works is still not determined (Cardé and Minks, 1995). To optimize the use of this important control tactic, a better understanding of how disruption works under field conditions is essential. Here we describe a series of experiments investigating the importance of competitive attraction (false-plume-following) as the primary mechanism of mating disruption for codling moth (*Cydia pomonella*) and oriental fruit moth (*Grapholita molesta*). The data suggest false-plume-following may be an important mechanism mediating mating disruption.

Materials and Methods

2003 Point Source Trials Experimental Design

The experimental design was a randomized complete block, replicated at four locations. Treatments were randomly assigned to 0.4ha plots within each block. Treatments included an untreated check and at least five point source densities: 0, 10, 40, 200, and 400 points/0.4ha for codling moth, and 0,10, 40, 100, 200 points/0.4ha for Oriental fruit moth. In all treatments there was a total of 200 Isomate® M Rosso polyethylene tubes for Oriental fruit moth (OFM) and 400 Isomate® C+ tubes for codling moth (CM). Isomate® dispensers were attached to 19 gauge galvanized metal hardware cloth with 1.3 cm squares, and the hardware cloth was attached to tree limbs with 6" self-locking nylon ties with 40-lb. tensile strength. Three large plastic delta traps (Suterra™, Bend, OR, U.S.A.) were placed in a transect across each treatment for each moth (CM and OFM) and baited with either 1 mg CM red septa or 0.1 mg OFM red septa lures (Suterra™).

2004 Tethered Female Moth and Male Moth Observation Trials

Mating disruption trials were conducted in 2004 comparing four densities of 100 µl wax drops containing 5% Shin-Etsu Oriental fruit moth pheromone (820/ha, 2700/ha, 8200/ha, and 27300/ha), Isomate® M100 at 300 /ha, and a no pheromone control. Treatments were applied 3 times/year, prior to the start of each moth generation. The experimental design was a randomized complete block with treatments applied to 0.04 ha plots and replicated 5 times. Two large plastic delta traps/0.04ha, with red septa lures replaced once/generation, were used to measure orientation disruption. In addition, tethered females were deployed for 24h on 5 dates coinciding with peak flight times. Females were secured to branches with polyester thread tied to the base of the left wing, and stickum was spread on the branch to deter moth predation. After 24 h, each female's bursa copulatrix was dissected, and the presence or absence of a spermataphore was noted. Finally, visual observations of male moth visits to pheromone wax drops were conducted on 26 individual nights, and results recorded using an electronic voice recorder, and later transcribed. For the observation trials, the control plot had one pheromone drop placed in one tree.

Results and Discussion

2003 Results & Discussion

Levels of trap shutdown within the center and on the edges of plots increased with increasing numbers of point sources (Figs 1 and 2).

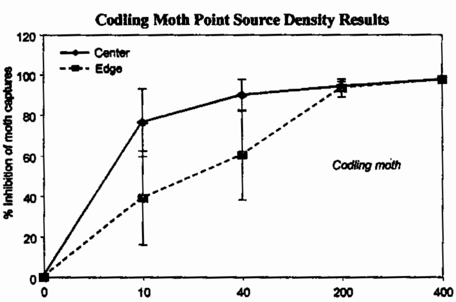

Fig. 1. Effect of point source density on codling moth capture

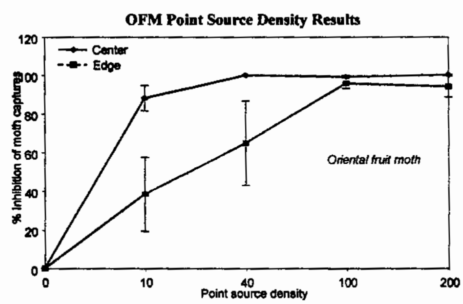

Fig. 2. Effect of point source density on Oriental fruit moth capture

Very high levels of at least 97% inhibition of catches were only recorded at the high point source densities. Substantially better disruption of orientation to traps was achieved in the center compared to the plot edges at all point source densities (Figs 1 and 2), indicating that borders are being left unprotected as point source density is decreased, and also points out the importance of trap placement in monitoring pheromone disrupted blocks. Increasing the amount of pheromone released from a point source, while decreasing the overall number of point sources, did not provide the levels of orientation disruption seen in plots with high densities of point sources releasing lower amounts of pheromone. These findings are consistent with studies on the disruption of Oriental fruit moth conducted with emulsifiable wax dollops in Michigan during the 2002-2004 growing seasons, and other investigations on the importance of high point source density to disruption success.

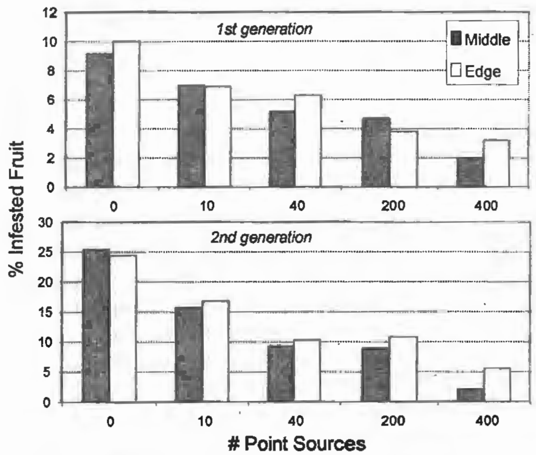

Figure 3. Point Source Density Effects on Fruit Injury

Effect of Dispenser Density on Orientation Disruption

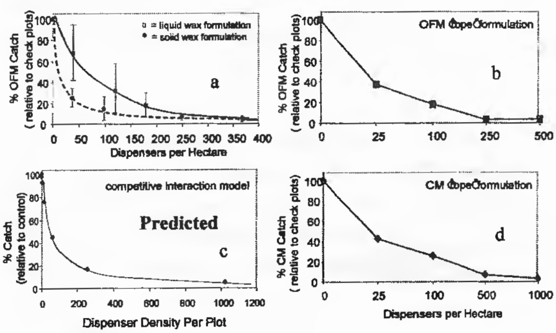

Figure 4. Predicted vs. actual orientation disruption

Orientation Disruption Outcomes Based Solely on Competitive Interactions: Effect of Moth Density

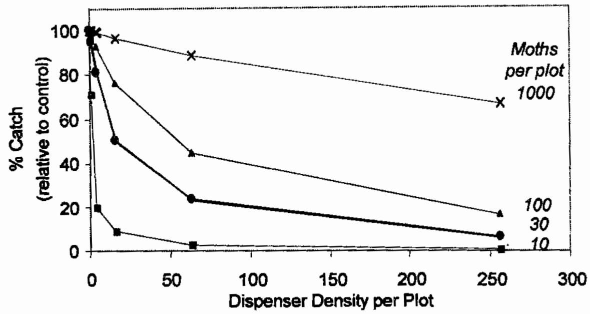

Figure 5. Predicted orientation disruption with fluctuating moth density

Point source density also impacted levels of fruit injury due to feeding by codling moth and Oriental fruit moth larvae. Figure 3 shows decreasing levels of fruit injury at both mid-season and pre-harvest with increasing numbers of point sources distributed throughout the plots.

Based on data showing that orientational disruption was superior when pheromone was released at many rather than few point sources (Fig 4), Dr. James Miller developed a predictive model based on elementary probability theory for competitive attraction (Figs 4c). Plots of data collected in field studies aimed to determine the effect of point source density (Fig. 4a, b, d) on orientation disruption closely match the plot generated by the model (Fig. 4c), providing support for competitive attraction is the mechanism of pheromone disruption.

The possibility that mating disruption might operate primarily by competitive attraction raises the specter that its effect will be dependent upon pest density. Calculations that vary moth densities show that mating disruption outcomes based on competitive attraction would vary
greatly with fluctuations in pest density (Fig. 5). At 10 or 30 moths per plot and 256 pheromone
point sources (females, trap lures, and dispensers), trap-catch shutdown would be 99 and 95 % respectively, while it would be only about 80 and 30 % at 100 and 1000 moths per plot, respectively. Thus, the density of point sources required for nearly complete orientational shut-down is highly dependent on pest population density
under this model.

2004 Tethered Female Moth and Male Moth Observation Trials

The number of females mated decreased as the number of wax pheromone point sources increased; no mating was recorded only at the 2 highest densities where orientation disruption exceeded 99%, 8,200 drops (99.4% orientation disruption) and 27,300 drops (99.2% orientation disruption) (Figs. 6 and 7). Males were observed visiting all pheromone treatments except the 8,200 drop treatment (Fig. 7). Even in plots with levels of orientational disruption reaching 95.7%, 15 males were observed visiting individual wax drops, *ca.* 20 % of total visits (Fig. 7).

The results from these tethered female trials, together with field trials showing increased orientation disruption as the density of point source dispensers increases, provides further evidence for competitive attraction as the principle mechanism by which mating disruption is operating in the field for these species.

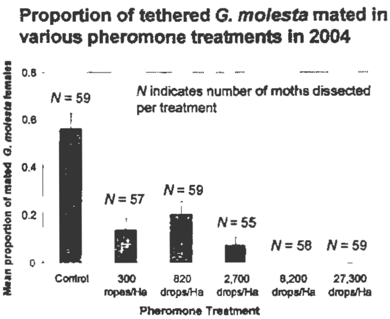

Figure 6. Percent mating by pheromone treatment

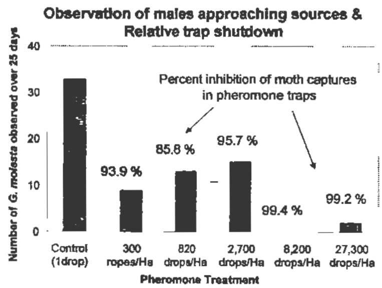

Figure 7. Observation of male visits to pheromone drops in different pheromone treatments.

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Mating disruption for the control of European grapevine moth *Lobesia botrana* (Den. Et Schiff.) in a plastic film greenhouse table grape vineyard

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Abstract: Results of *Lobesia botrana* (Den. & Schiff.) mating disruption trials on table grape under greenhouse condition by means of Isonet L dispensers (Shin-Etsu Chemical Co. Ltd.) are reported.
The trial was located in a table grape vineyard in Central Italy (Latium region) and the dispensers were applied before the first moth trap catch. The grapevine cultivar was Superior seedless trained with Geneva Double Curtain (GDC) system. The recommended application rate on head training system is 750 dispenser/ha while on espalier training system is 500 dispensers/ha.
The aim of the trial was to verify the effectiveness of a 500 dispensers/ha application rate in comparison with the farm standard control strategy.

Key words: *Lobesia botrana*, mating disruption; greenhouse; table grape, Latium Region, Italy

Introduction

European grape berry moth, *Eupoecilia ambiguella* (Hb.), and European grapevine moth, *Lobesia botrana* (Den. & Schiff.), are the major pests in European vineyards. In middle-south Italy, *E. ambiguella* is less common and rarely causes economically relevant damage (Bagnoli, 1990). On the contrary, damage caused by *Lobesia botrana* on table grapes is particularly severe due to the commercial depreciation of the final product.

In the last few years mating disruption technique has been increasingly applied on several different crops, above all in viticulture, throughout the whole Europe, thus allowing to completely or partially replace chemical products, which have represented the main control strategy until present (Varner et al., 2001; Charmillot & Pasquier, 2000).

The recommended application rate for mating disruption against *L. botrana* is of 750 dispensers/ha on head training systems and 500 dispensers/ha on espalier training systems.

The aim of this trial was to verify the effectiveness of a 500 dispensers/ha application rate in comparison with the farm standard control strategy on a table grape curtain training system

Materials and methods

The trial, carried out during 2003, was located in a table grape vineyard in Central Italy (Latium region). The grapevine cultivar was Superior seedless, trained with Geneva Double Curtain (GDC) system under plastic film greenhouse conditions. The acreage of the greenhouse was 3.5 hectares.

Dispensers were applied on March 6th before moth emergence. The moth flight (Fig. 1) was monitored weekly with pheromone traps, both in the control and in the disrupted greenhouse. Chemical control strategies were the same in both greenhouses, as the farmer didn't want to differentiate insect management techniques. Treatments were based on the use of organophosphates against the first and the second generation.

The assessment on the first generation consisted in counting larval nests on bunches and was carried out on May 7th. On June 25th, almost ten days before harvest, second generation damage was assessed.

A total of 250 bunches were observed for each assessment respectively in the centre and on the edges of the disrupted and control greenhouses.

The Attack Degree was determined according to following formula:

AD = a x b

where:

a = % of damaged bunches

b = no. of attacked berries per bunch

After calculating the attack degree, the % of effectiveness compared to the control plot was determined according to following formula:

% of effectiveness = (1 – AD in disrupted greenhouse/AD in control greenhouse) x 100

Results and discussion

In the greenhouse where mating disruption had been applied, a complete shut-down of male catches in pheromone traps was observed (Fig. 1).

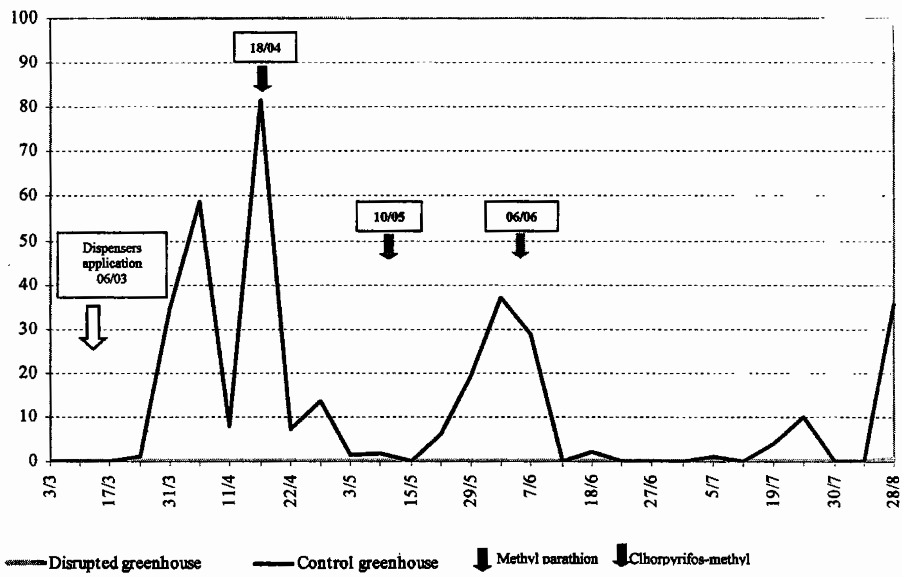

Figure. 1. Male captures of *Lobesia botrana.*

Table 1 summarizes the results of first generation assessment. Damage caused by first-generation larvae had no direct correspondence with second-generation population density.

Table 1. Infestation data of the first generation of *Lobesia botrana*

Date	Position	% bunches with larval nests	
		Edge	Centre
May 07, 2003	Disrupted Greenhouse	9,5	1,0
	Control Greenhouse	18,0	

Table 2 and Figure 2 summarize the results of second-generation assessments at harvest.

Table 2. Infestation data of the second generation of *Lobesia botrana*

Position		Average no. of berries/bunches	% of damaged bunches	Attack Degree	% Effectiveness
Disrupted Greenhouse	edge	1,6	11,1	18,3	78,4
	centre	1,0	1,2	1,2	98,6
Control Greenhouse		2,0	42,3	84,6	-

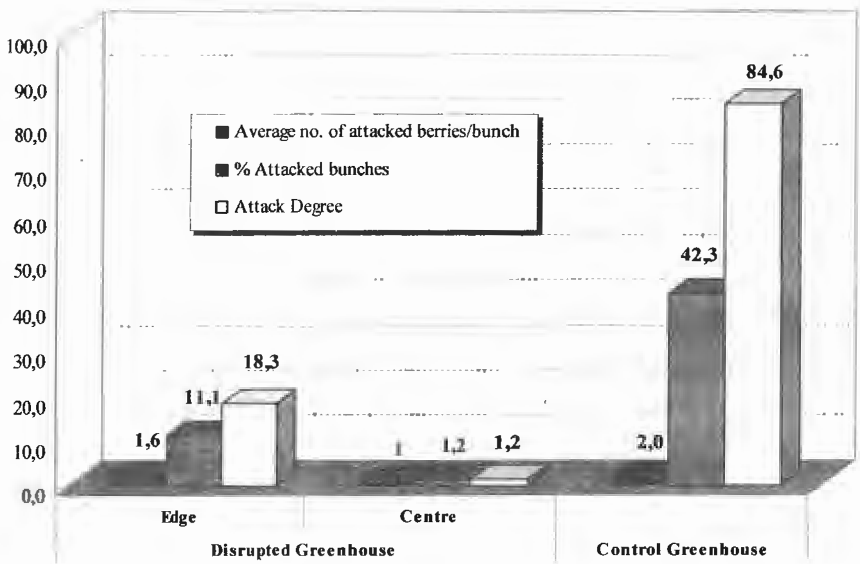

Figure 2. Infestation data of the second generation of *Lobesia botrana*

Data registered at harvest show that damage caused by larvae in the control greenhouse was much higher than in the MD-greenhouse, regardless of precautionary treatments.

Combined strategy effectiveness (mating disruption + chemical treatments) reached respectively 98% and 78% in the centre and on the edges of the MD-greenhouse.

Fig. 3 shows the dispenser release rate of a.i. expressed as % of the initial content (100 %).

Figure 3. Isonet L release rate (Latium, 2003)

As it always happens in fields where mating disruption technique is applied, edges are the most high-risk areas. In this case too, damage caused by both first and second generation was higher in these areas than in the centre of the greenhouse. Results show that a combined application of Isonet L and chemical treatments for the control of European grapevine moth on table grape under greenhouse conditions, at a rate of 500 d/ha, lower than the recommended standard application rate (750 d/ha), produced optimum insect control, far better than the one obtained with standard control strategies alone. These results allow us to presume that at least part of the supplementary insecticide treatments applied could be avoided, thus limiting negative impacts on beneficial insects and reducing chemical residues on the final product. It's important to stress, however, that constant monitoring of moth populations with the help of pheromone traps and constant larval assessments are necessary for an effective application of mating disruption technique. This allows to timely plan supplementary chemical treatments which may be needed during the season.

Acknowledgement

An acknowledgement to Shin-Etsu Chemical Company Ltd. that provided the dispensers for the trials.

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Preliminary investigation on the mating behaviour of the peach twig borer, *Anarsia lineatella.*

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Abstract: The peach twig borer (PTB), *Anarsia lineatella,* is an insect pest of stone-fruit orchards, where larvae cause damage by tunnelling into shoots and fruits. Considered as a secondary pest of peaches, PTB has recently gained in importance due to increasing early infestation in Northern Italy. Sex pheromone-baited traps are used as a tool for monitoring the flight of male moths and the size of populations. Nevertheless, the management of this pest is not without problems. The low performance of some commercial pheromone lures seems due to a lack of basic investigation on the mating and the olfactory behaviour of the PTB. This pilot study provides preliminary results from observation of the mating behaviour of groups of male *A. lineatella* using virgin females and experimental lures as odour sources, in a wind tunnel. Tested lures consisted of two major components of female sex pheromone, *trans*-5-decenol and *trans*-5-decenyl acetate, in two different ratios. Preliminary results of the bioassays are discussed.

Introduction

The peach twig borer (PTB), *A. lineatella* Zeller (Lepidoptera: Gelechiidae), is a pest of peach, almond and other stone fruits (Hathaway et al., 1985). Originating from the Mediterranean region, PTB is reported in many areas of the world, from Europe to North America, from the Middle East to part of Asia (Dickler, 1982). The larvae feed primarily on buds and tender shoots of the host tree after emergence from refuges where they over-winter (Tremblay, 1990). The larvae feed directly on fruit with the later generations.
PTB has recently gained in economic importance as the outbreak of new infestation is reported especially in the areas of North-East Italy (Molinari and Cravedi, 2001). Main problem is the increasing activity of over-wintering larvae and as a consequence of the first generation on green shoots.
As for other lepidopterans, sex pheromone-baited traps are used as a tool for monitoring flights of male moths and size of populations (Millar and Rice, 1992). Nevertheless, the efficacy of the sex pheromone-based technique for the control of PTB appears as suboptimal. Pheromone blend consist of two major constituents, *trans*-5-decenol and *trans*-5-decenyl acetate, as identified by Roelofs et al. (1975). Improper ratio of the two major constituents, as well as the possible presence of minor antagonist constituents (Millar and Rice, 1996), could be responsible of the low efficacy shown by some commercial pheromone lures.
Here we compare the olfactory response of group of virgin male PTB to virgin females PTB and to two experimental pheromone lures, with a different ratio of the two major constituents.

Materials and Methods

Insects. PTB were reared on artificial medium based on wheat and soy (Arthro Feeds©, Bryan, TX), for 20 generations. On emergence, males and females were placed together in a plastic cylinder (1.5 lt.) with access to honey solution. Mated females oviposited on a cotton strip put inside the cylinder. Eggs were collected by a brush and placed individually on a bioassay trays, BIO-BA-128 (C-D International©, Pitman, NJ), containing artificial medium. Etched larvae completed their life cycle on the trays till adults emergence. The rearing was carried out in a climatic chamber at 23°C, L16 : D8, 60-70% r.h. For the bioassay, virgin adult males were never exposed to odour cues and therefore considered as naïve. Individuals were tested 3-5 days after emergence and used only once.

Bioassays

The olfactory response of male PTB was observed in dual choice experiments using a wind tunnel, as described by Hern and Dorn (2004). Horizontally positioned acetate transparencies painted with insect glue were used as traps and placed adjacent to the odour sources. The bioassay used two traps (21 cm wide x 30 cm long) with a gap of 10 cm between them. These traps were placed on the floor of the wind tunnel 5 cm from the upwind end. Charcoal-filtered air was sucked trough the wind tunnel at a rate of 21 cm/s. Experiments were carried out under constant light intensity, room temperature and relative humidity (2100 lux, 24 ± 1 °C, 50 ± 10% r.h.). The moths were released in groups of 10 virgin males at the downwind end, 3 h before the beginning of the scotophase (6:18 D:L), and the trial lasted 17 ± 1 h.

Response to a virgin female

Two cylindrical cages (1.5cm, Ø 3cm) with fine mesh all around the cage wall, were positioned at the upwind end of the 2 traps. One of the 2 cages contained a virgin female, the other was empty. Response of group of males to the virgin female was observed in comparison to a blank. Number of replicates was five.

Dose-Response to synthetic pheromone.

Artificial blends were released from different numbers of disposable capillary pipettes "end to end" (Hirschmann®) of 5 ul, baited and placed on a Petri dish positioned at the upwind end of the trap. Two pheromone lures provided by Shin-Etsu were tested in as many experiments. Lure A had the two main components, *trans*-5-decenol and *trans*-5-decenyl acetate, in a ratio similar to the natural blend. Lure B, had the two main components in a ratio different then the natural blend, with a high concentration of acetate. Each lure, three doses with 5, 15, and 25 ul were tested in comparison to a blank. Number of replicates was five.

Results and Discussion

Previous investigation on the mating behaviour of *Anarsia lineatella* were carried out in field trials for captures of male with pheromone traps. This pilot study investigated the mating behaviour of PTB using a wind tunnel. After a number of preliminary test for a suitable set up, male PTB showed a positive response to virgin females.

Response to a virgin female

Virgin females were attractive to male PTB (Fig. 1). Number of males choosing a virgin female was significant high as compared to blank (9 vs 2, $P < 0.05$, χ^2 Test). The responsiveness was 22%.

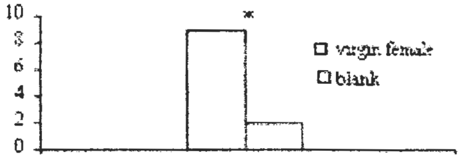

Figure 1. Response of group of 10 male PTB to a virgin female in a wind tunnel. N = 5, * $P < 0.05$ ($\aleph^2$ test).

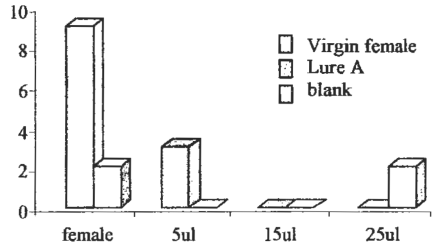

Figure 2. Dose-response of a group of 10 male PTB to lure A as compared to a virgin female in a wind-tunnel. N = 5

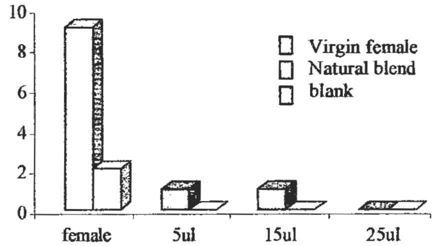

Figure 3. Dose-response of a group of 10 male PTB to lure B as compared to a virgin female in a wind-tunnel. N = 5

The responsiveness of male PTB in this lab experiment was generally low, while in recent field experiments using traps baited with a virgin female PTB, responsiveness was higher (CBC, pers. comm.). As reported for other insect species (e.g. Ruther et al., 2002), plant volatiles may play an important role in the sexual communication of PTB, with some component working as sexual kairomone.

The response of male PTB to both tested lures, A) mimicking the natural blend, and B) with a higher concentration of acetate, was not statistically significant.

Dose-response to lure A

The lure A was slightly attractive to male PTB at low dosage with 5 ul (3 vs 0), not attractive at intermediate dosage with 15 ul, and slightly repulsive at high dose with 25ul (0 vs 2) (Fig. 2). Responsiveness was particularly low (6%, 0, and 4% of total responding males).

Dose-response to lure B

The lure B was slightly attractive to male PTB at low dosage with 5 ul (1 vs 0), and intermediate dosage with 15 ul (1 vs 0), and not attractive at high dose with 25ul (0) (Fig. 3). Responsiveness was very low (2%, 0, and 2% of total responding males)

The slight attractiveness of the lure A might depend on the possible presence of impurities represented by minor components (Millar and Rice, 1992).The low attractiveness of the lure B seems due to high concentration of *trans*-5-decenyl acetate in it, which would work as an antagonist of the pheromone, as previously reported (Millar and Rice, 1996).

Further observations will better investigate the role of host plants on the mating and the searching behaviour of PTB in suitable bioassays, and the pheromone composition via e new re-examination of natural blend.

Acknowledgments

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The use of the aphid sex pheromone and plant volatiles to enhance control of *Dysaphis plantaginea* in apple

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Abstract: The objective of this research is to determine the practicability of exploiting the responses of aphids and their natural enemies to aphid sex pheromone and to plant volatiles in order to enhance biocontrol of the pest. Laboratory and field experiments were used to determine the ratio of the main sex pheromone components of *Dysaphis plantaginea.* The optimal ratio was determined to be approximately 4:1 of cis, trans-nepetalactol: cis, trans-nepetalactone. In a field experiment the pheromone components were successfully used to monitor the autumn migration period of *D. plantaginea*. In field experiments to determine if aphid sex pheromone components or plant volatiles were attractive to beneficial predators and parasitoids, none of the compounds tested were effective attractants for beneficials.

Keywords: *Dysaphis plantaginea*, aphids, predators, parasitoids, sex pheromone, plant volatiles, monitoring, control.

Introduction

The rosy apple aphid, *Dysaphis plantaginea*, is the most serious aphid pest on apple in Europe, causing stunting and malformation of leaves and fruit. The aphid has a host alternating holocyclic life cycle, with apple being its primary host and herbaceous plants of the genus *Plantago* the secondary hosts. Because the aphid is difficult to monitor early in spring, growers often make a routine insecticide application against the pest. If it were possible to monitor the population return to apple in autumn, different methods of control of this pest could be developed, e.g. autumn applications of insecticide could be targeted at the pest and could be timed optimally. Further work would be needed to predict the risk of damage in the spring from known populations returning in the previous autumn (Solomon *et.al.*, 2003). Oviparae, the sexual stage of aphids, produce a sex pheromone which is used by male aphids to locate their mates. The sex pheromone of most aphids investigated comprises two main components: cis, trans-nepetalactol and cis, trans-nepetalactone with specificity depending on different ratios of the two major components plus more specific minor components (Hardie *et al.*, 1999). Host plant volatiles also affect the aphid species that alight on a particular host. It is also possible that predators and parasitoids may be attracted to aphid sex pheromones, or to volatiles produced by the plants as a result of aphid feeding; this effect could be exploited to increase biocontrol of the pest. This paper outlines the results of laboratory and field experiments to optimise the pheromone blend for *D. plantaginea* and considers the possibilities of using sex pheromones and host plant volatiles to enhance control of the pest.

Methods

Dysaphis plantaginea ***sex pheromone: Laboratory experiments***
Dysaphis plantaginea males used in the bioassays were collected from laboratory cultures. Oviparae were collected from the leaves of apple plants maintained in a glasshouse with natural light and heat in autumn, or from field infestations.

Olfactometry
The 'Pettersson-style' olfactometer used was similar to that described by Pettersson (1970) and Vet *et al.* (1983). An aphid was placed in the centre of the arena, using a fine paintbrush. The position of the aphid within the olfactometer was recorded for 16 minutes; time spent in each of the four distinct 'arms' of the olfactometer, as well as time spent in the central area, was recorded. The olfactometer was rotated through 90° every two minutes to prevent directional bias. The olfactometer was prepared so that one arm contained a treatment and the three remaining arms contained controls. Therefore, experiments recording responses to several treatments tested each consecutively. After each bioassay the olfactometer was washed thoroughly in order to remove any contaminants.

Entrainment

Batches of oviparae were placed on a single apple leaf which was enclosed in a glass cylinder with a slot for the petiole. The control was a second container enclosing an uninfested apple leaf. Air was drawn through the vessels (350 cm^3 / min) over two tubes containing Porapak Q 50/80 (50 mg) for 24 hours. These air entrainments were eluted and analysed by GC.

Dysaphis plantaginea ***sex pheromone: Field experiments***
Water traps consisted of 14.5 cm diameter transparent plastic petri dishes. A 2 cm length of pvc water pipe (2.5 cm outside diameter) was glued perpendicularly in the centre on the inside of the petri dish; two small holes were drilled into the end of the pipe to allow the attachment of the pheromone lures. A 10 cm length of pipe was glued in the centre of the base of the dish to allow attachment to supporting canes.

The traps were set out on canes at a height of 60 cm above ground and filled with water containing a trace of detergent (Lipsol). The experiment was replicated in five blocks. Treatments are shown in Figure 1. Traps were positioned close to adjacent apple trees (approx. 1 m) and each trap was a minimum of 6 m from another trap. The traps were emptied daily and the treatments re-randomised.

Effect of plant volatiles on potential biocontrol agents in the field
Water traps were set out as described above and there were three replicate blocks. The traps were baited with plant volatiles in combination with the sex pheromone components:

1. Nepetalactol + Nepetalactone (4:1)
2. Nepetalactone
3. Ocimene + treatment 1
4. Ocimene + treatment 2
5. Methyl salicylate + treatment 1
6. Methyl salicylate + treatment 2
7. Control

The traps were emptied daily and the treatments re-randomised.

Results

Laboratory experiments

The response of *D. plantaginea* males to different ratios of sex pheromone components is shown in Table 1. The optimal ratio of components from this experiment was 5: 1 nepetalactol:nepetalactone.

The analysis of entrainments of leaves infested with oviparae gave a ratio of approximately 4:1 nepetalactol:nepetalactone.

Table 1: Response of *D. plantaginea* males to ratios of sex pheromone components in an olfactometer

Treatment ratio (lactol:lactone)	Mean time (s) Treatment	Mean control	Significance
0	168	177	0.910
1:1	240	177	0.381
5:1	333	150	0.035
10:1	231	206	0.778

Field experiments

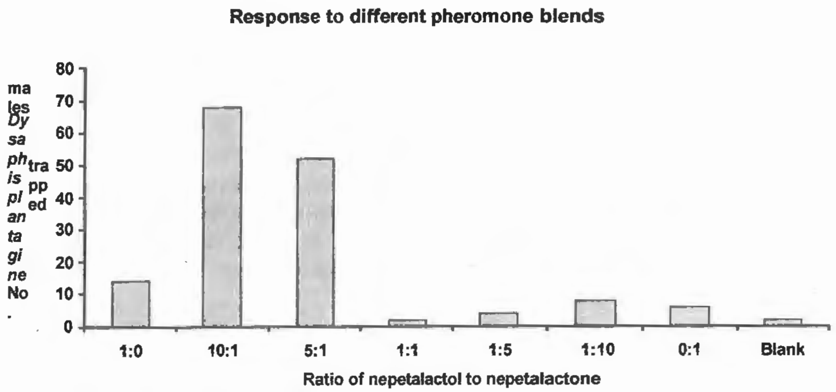

Figure 1. Mean number of *D. plantaginea* caught in water traps baited with different combinations of sex pheromone components.

Mean numbers of male *D. planaginea* caught in water traps baited with different ratios of the sex pheromone components are shown in Figure 1. From these results the optimal ratio appears to be in the region of 10:1 to 5:1 nepetalactol:nepetalactone.

These water traps were able to monitor the autumn migration of *D. plantaginea* males. In this trapping year (2003) there was a particularly high returning population of aphids (Figure 2).

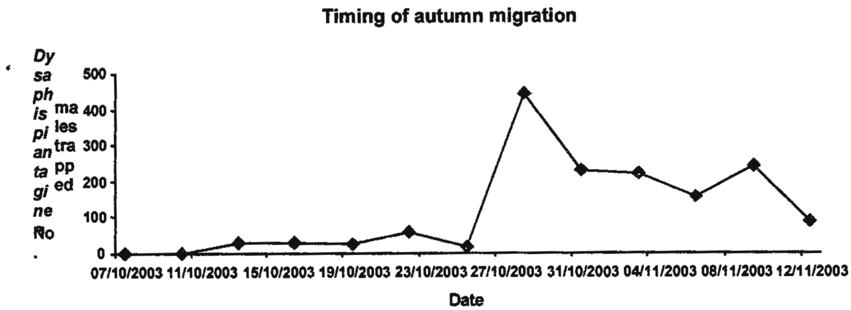

Figure 2. Total numbers of *D. plantaginea* caught in all traps.

Effect of plant volatiles on potential biocontrol agents in the field

Very low numbers of beneficial species were caught in the baited traps. Total numbers of parasitoids caught over a six week period in June/July ranged from 24-41 in the different treatments but there were no significant differences between treatments. The greatest total number of anthocorids (15) was caught in the 4:1 sex pheromone blend.

Discussion

These experiments have enabled us to optimise the blend of nepetalactol and nepetalactone to attract male *D. plantaginea* returning to apple in autumn. From the field trapping we were able to monitor the flight period of the aphids. Experiments using insecticides at this time have proved successful at reducing aphid damage the following spring (J V Cross unpublished results). However, several other aphid species were also attracted to these traps. Result of experiments in which plant volatiles are combined with the sex pheromone to further increase the specificity of the blend for *D. plantaginae* will be reported elsewhere.

The low catches of beneficial species in the different volatile-baited traps during the summer suggest that it may not be possible to use any of the tested volatiles to attract beneficials into orchards to increase biocontrol. Different volatiles may be more effective and will be tested in future experiments.

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Impact of the kairomone ethyl (2E, 4Z)-2,4-decadienoate (DA 2313) on the oviposition behaviour of *Cydia pomonella* on pear

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Abstract: ethyl (2E, 4Z)-2,4-decadeonoate) (DA 2313) is a pear derived compound able to attract both sexes of *C. pomonella* L., and thus very interesting for monitoring practices. The effects of DA 2313 on the spatial distribution of the eggs laid by *C. pomonella* was evaluated. The trials were carried out in 2002, 2003, and 2004 in several orchards, on different crops (pear and apple), varieties (early and late) and during different phenological phases (spring and summer). The experiments were carried out in semi-field conditions: *C. pomonella* couples (one per cage) were released inside cages each including one single branch bearing one fruit. The assess for egg distribution along the branch, the number of eggs laid on branch sections of 20 cm in length (starting from the fruit) was counted on DA-treated and untreated branches.

On the DA-treated branches, significantly more eggs were laid at greater distances from the fruit than on the untreated branches This indicates that DA 2313 could modify the distribution of the eggs laid by *C. pomonella* (*host location disruption*), which could directly affect larval survival and enhance the activity of some natural larvicides.

Key words: *Cydia pomonella*, kairomone, pear ester, oviposition, host location

Introduction

The economic importance of the codling moth, *Cydia pomonella* L. (Lepidoptera Tortricidae), on pome fruits is growing continuously. Because of the poor performances of some insecticides and the appearance of resistance, there is increasing interest in the development of "alternative", non-chemical control strategies, particularly in the use of semiochemicals. Among semiochemicals, sexual pheromones are well-known, whereas studies on volatile substances produced by plants (plant volatiles = kairomones) are limited. As suggested by laboratory studies of Hughes et al. (2002) on α-farnesene, field applications of kairomones could modify the oviposition behaviour of *C. pomonella* or affect neonate larvae.
It has recently been shown that DA2313: ethyl (2E, 4Z)-2,4-decadienoate = Et-E,Z-DD, a pear ester, attracts both *C. pomonella* males and females (Light et al., 2001). In Italy, a thorough monitoring study with this substance has been conducted (Ioriatti et al., 2003). The direct effects of DA2313 were investigated on two orchard crops (apple and pear), on one early and one late cultivar (apple: 'Gala' and 'Golden Delicious'; pear: 'William' and 'Abate Fétel'), during two phenological stages (fruitlet and ripe fruit). Some results of this study, in particular the effects of DA2313 on the oviposition behaviour *C. pomonella* on pear, are reported.

Materials and Methods

In 2002, 2003, and 2004, semi-field trials were conducted on pear trees (cv. 'Abate Fètel') in the fruitlet stage. On different trees, eight branches were selected. All branches were oriented vertically (length: 110 cm in 2002, 90 cm in 2003, 50 cm in 2004), and bore one fruit. Four

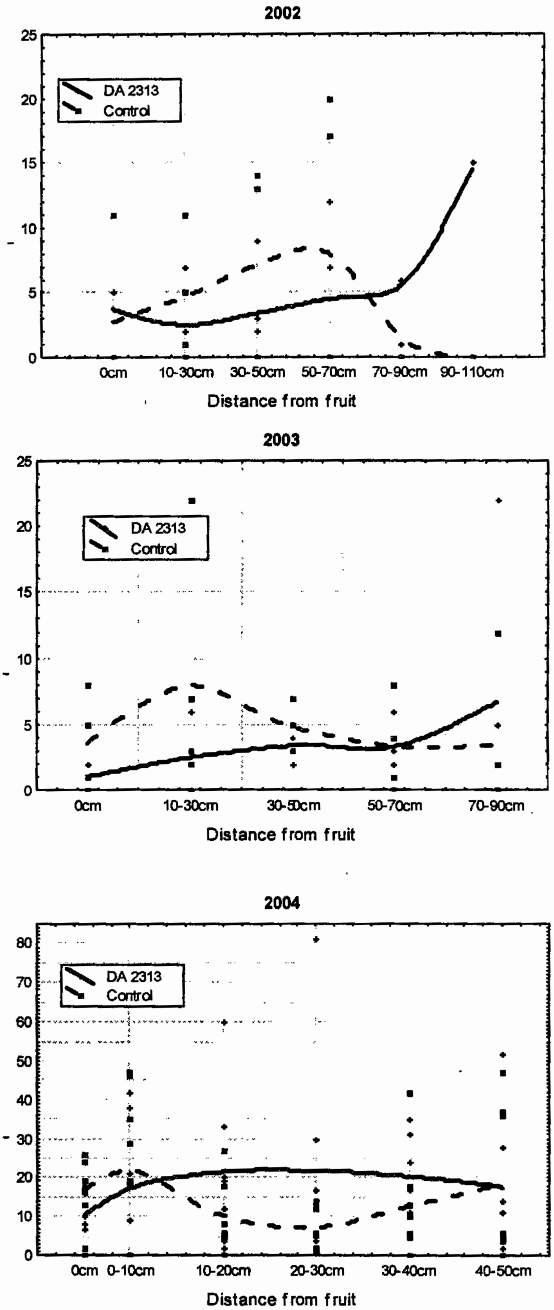

Fig. 1 - Distribution of the eggs on DA 2313-treated and untreated branches in 2002 (top), 2003 (centre), and 2004 (bottom) (Kolmogorv-Smirnov Test: P<0.001 in all cases).

branches were treated with DA 2313 provided by Trecé Inc., Salinas, CA (USA). The other four branches were used as untreated control. Both treated and untreated branches were enclosed in 1.20 x 0.5 m white nylon net bags (mesh size: 1 mm^2). Mated *C. pomonella* couples (5 in 2002 and 2004; 2 in 2003) were released in each bag. After four days, bagged branches were brought to the laboratory. Each branch was then cut into sections (20 cm long in 2002 and 2003; 10 cm in 2004), the section bearing the fruit included. Each section, leafs and ramifications included, was examined under a binocular. The number of eggs on each section was counted, and the distance of the eggs from the fruit was measured. The distribution of the eggs on treated and untreated branches was compared using the Kolmogorov-Smirnov test, which considers both the number of eggs laid and their distance from the fruit (Statistica® 6.0).

Results

In all three years, the distribution of the eggs on DA 2313 - treated branches differed significantly from that on untreated branches (Kolmogorov-Smirnov Test: $P<0.001$ in 2002, 2003 and 2004; Fig. 1), and the mean distance of the eggs from the fruit was bigger in treated than in untreated branches (Table 1).

Table 1 - Mean distance (m±s.e.) of eggs from fruit (cm) in DA 2313-treated and untreated branches.

Year	DA 2313	Control
2002	35.5 ± 3.1	20.2 ± 1.7
2003	54.3 ± 3.2	35.5 ± 3.2
2004	22.5 ± 0.6	19.5 ± 0.7

Discussion and Conclusion

DA 2313 seems to affect the oviposition behaviour of *C. pomonella* females: the spatial distribution of the eggs laid by *C. pomonella* females on DA 2313 - treated branches significantly differed from that on untreated branches. The kairomone probably impairs the ability of the mated females to locate the regular oviposition sites (i.e. fruits and their immediate surroundings), thus disrupting the host location behaviour of the females.

Host location disruption has recently been observed also in *C. pomonella* larvae (Pasqualini, *pers. com.*). In conclusion, if the eggs are more distant from the fruit, neonate larvae may have more difficulties in locating their host, which may be a precondition for improving *C. pomonella* control techniques.

Aknowledgments

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Control of the Grapevine Moth, *Lobesia botrana* (Den. *et* Schiff.) (Lepidoptera Tortricidae), by disorientation

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Abstract: Disorientation, a new pheromone based control technique, has been recently developed for the control of the Grapevine Moth (GM), *Lobesia botrana* (Den. *et* Schiff.) (Lepidoptera Tortricidae), in isolated and small vineyards. In this study we characterized the Ecodian® disorientation dispensers according to their release rate and life span. The pheromone release rates of new and field aged dispensers were evaluated using SPME. In addition, EAG recordings showed that males respond to field aged dispensers. The activity of aged dispensers on GM behaviour has been observed in a wind tunnel. The mode of action of disorientation was investigated in the field using traps lured with pheromone and females. The efficacy of disorientation was confirmed in the field.

Key words: E7,Z9-dodecadienylacetate, Ecodian dispenser, SPME, EAG, wind tunnel, field trial.

Introduction

The control of the Grapevine Moth (GM), *Lobesia botrana* (Den. *et* Schiff.), is a serious challenge for European vine growers. Insecticides represents nowadays the most widespread control method, but the future scenario is not completely clear. Some of the most toxic insecticides are being withdrawn from the market, and the need for alternative techniques is increasing. Among the latter, the most frequently used technique is pheromone mating disruption. However, its cost is not comparable to that of chemical control, and the efficacy of the method is not always satisfactory when the treated area is limited in size. Recently, Disorientation, a new pheromone technique, has been developed to overcome the limits of mating disruption. In this case, the mechanism acting on the mating behaviour is the competition between natural and synthetic sources of pheromone, sometimes referred to as "false-trail following technique". In order to achieve a satisfactory control level, the release rate of the synthetic dispensers has to be comparable or above that of a calling female. The aim of this work was to characterize the Ecodian® (Isagro, Italy) disorientation dispensers according to their release rates and life spans, in order to verify their efficacy in controlling the Grapevine Moth (GM) in the Trentino Province (Northern Italy). We also investigated the mode of action of disorientation using traps lured with pheromone and females. The efficacy of this strategy for GM control was evaluated in the field, and compared to that of mating disruption and conventional insecticides.

Material and methods

Insects

Insects were collected from the vineyards of the IASMA and reared on a semi-artificial diet. Rearing cages were housed and experiments were conducted in climatic chambers (22±2°C, 70±10% RH, 17L:1T:6D inverted photoperiod).

Dispensers

The hook-shaped Ecodian® dispensers were evaluated in relation to their pheromone loading: 10 mg of E7,Z9-dodecadienylacetate, main GM pheromone component. The dispensers were applied in vineyards located in Faedo (400 m a.s.l. – Trento, Italy), and applications were carried out twice during the season, on April 15 and June 25, respectively, both in 2002 and 2003. Field aged dispensers were collected every other week from the application date on.

Release rate of dispensers

SPME in static air was used for effluvia collections (Rotundo *et al.*, 2001; Bordereau *et al.*, 2002). Volatiles were adsorbed from the headspace on a fibre coated with a solid sorbent (pdms; 100 µm – Supelco, Bellefonte, PA), first conditioned for 5 minutes in a GC injection port at 220°C. The SPME fibre was directly inserted into the GC for thermal desorption (5 min) and analysis. Chemical analyses were performed on a Hewlett-Packard 5890 gas-chromatograph (GC), with a polar Innowax column (30 m by 0.32 mm) programmed from 60°C (hold 3 min) at 8°C/min to 220°C (hold 7 min), interfaced with an electroantennogram apparatus (GC-EAD). Identity confirmation of the compound was obtained by comparing its GC retention time with that of the standard synthetic chemical E7,Z9-12:Ac (purity 97%; Sigma-Aldrich, Milan, Italy), and by verifying the EAG response of a GM virgin male antenna. The amount of pheromone collected in the headspace was determined comparing the GC areas obtained with direct injections of synthetic E7,Z9-12:Ac solutions; this amount was corrected according to the efficacy of the fibre in recovering the synthetic compound, which has been previously evaluated.

The straight part of the Ecodian® hook (2,8 cm), with the cut end sealed in paraffin, was confined in a vial (2 ml) capped with a Teflon® septum (n=10). The needle of the SPME was inserted into the vial by piercing the Teflon septum. Based on recovery data, the extraction lasted 60 minutes, and was preceded by 10 minutes of equilibration. The extract was then injected into the GC. Quantification of the overall emission was calculated according to the total length of the dispenser. Pheromone release was measured after 0, 18, 53, 74, 97, 136 days for dispensers of the first application and after 8, 31, 70 days for those of the second application.

Electroantennographic (EAG) responses

The amount of pheromone released by aged Ecodian® dispensers (2002, 1st application) was evaluated using EAG analyses carried out on virgin males. We used a technique similar to that described in a previous study (De Cristofaro *et al.*, 2000). The stimuli were provided by inserting the straight part of the Ecodian® hook (2.8 cm), with the cut end sealed in paraffin, in a Pasteur pipette. EAG responses were normalized according to the reference stimulus (a piece of filter paper soaked with 25 µl of a Z3-hexen-1-ol solution at 100µg/µl in mineral oil, and inserted into a Pasteur pipette) and subjected to the analysis of variance (ANOVA) followed by Duncan's test.

Wind tunnel (WT)

Tests were carried out in a glass wind tunnel (250x60x60 cm) at 22±3°C, 45±10% R.H., and at an air speed of 15 cm/s. The laminar air flow was generated and purified (activated charcoal) using an air speed and humidity-conditioning unit (De Cristofaro *et al.*, 2003). The tunnel was illuminated from above by a neon tube with a scattering screen (6 lux). WT sessions started 1 h after the onset of the scotophase, and continued for 2 h. We used single, virgin, 2-3-day old males (n=10). Aged dispensers (2002, 1st application) were vertically hung on a metal holder at the upwind end of the tunnel. Five behavioural responses were recorded: activation (walking and wing-fanning), taking flight, oriented flight, flight near the odour source (15 cm), landing (landing on the source). Males had 15 min to respond. The behavioral responses obtained with dispensers of different age were compared using 2x2 contingency table-χ^2 tests.

Field trapping

Disorientation, mating disruption, and a conventional control were compared in a two year field experiment (Telve, 2003; Faedo, 2004 – Trento, Italy). Each treatment was applied to plots of 20,000m^2 nearby in vineyard of 6 hectares. The vineyards were grown in the Pergola trailing system (trial 2003), and Guyot system (trial 2004). Ecodian® disorientation dispensers were placed in the vineyards with a rate of 2,000 per hectare, and applied twice (first application prior to the beginning of the 1° flight period of GM; second application at the beginning of the 2° flight period). Mating disruption dispensers (Isonet® L) were applied at a rate of 500 per hectare in only one application. One area treated with conventional insecticides (2 treatments with Organophosphate) acted as control.

In order to collect large and homogeneous trapping data in an area with a low GM population density, GM males marked with fluorescent powder were regularly released. At the beginning of the 2nd GM flight period, 30 fluorescent males were released (replaced every 2 days; total 420) by placing them into an artificial nest hanging on a rope within one row in the centre of each plot. Pagoda traps were installed at the corners of two concentric squares (one small and one large square) in the surrounding of the nest. Four traps were baited with 5 virgin females (replaced every 2 days), and other four traps were baited with 1 mg of the main sex pheromone component, E7,Z9-12:Ac. The traps in the small square were 28.3 m apart and at 20 m from the nest. The traps in the large square were 70.7 m apart, and at 50 m from the nest. The number of marked males in the traps was checked weekly.

Efficacy evaluation in the field

The same experimental design, previously described for the trapping experiment, was used in the field trial. Thousand grape clusters per plot were checked at the end of each GM generation, and damage was expressed as percentage of injured clusters.

Results

Release rate of dispensers

The release rate of the Ecodian® dispensers decreased over time. However, at the end of the season (September 02, 2003), after 136 days in the field for dispensers of first application and after 70 days for dispensers of second application, the release rates were still respectively 45 and 930 times higher than that calculated for individual calling *L. botrana* females (Anfora *et al.*, 2004).

Table 1. Release rates of E7,Z9-12:Ac of field aged dispensers (Ecodian®) used for disorientation.

1st application		2nd application	
Field age of dispensers (days)	Release rate (ng/h/disp)	Field age of dispensers (days)	Release rate (ng/h/disp)
0	2010.4±88.2		
18	1405.2±141.7		
53	293.9±27.9		
74	99.4±12.2	8	1728.7±177.7
97	62.1±15.3	31	866.3±79.1
136	13.8±1.3	70	280.6±17.6

Electroantennographic (EAG) responses

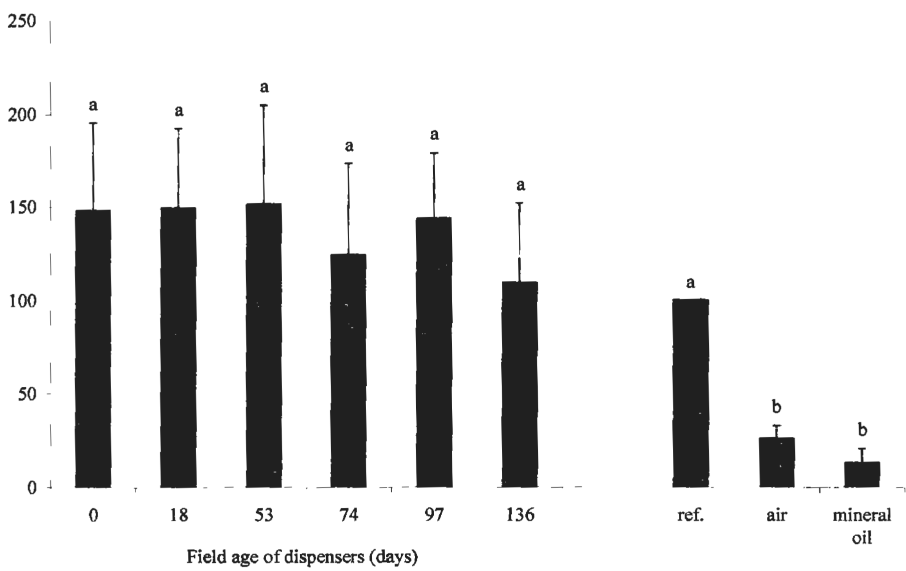

Figure 1. Mean normalized EAG responses of virgin (n=10) GM males to field aged Ecodian® dispensers. Reference stimulus: Z3-hexen-1-ol (25 µl of solution at 100µg/µl in mineral oil). Vertical bars represent S.D. Different letters indicate significant differences (Duncan's test: P<0.05).

All tested Ecodian® aged dispensers highly stimulated virgin male antennae. The EAG responses to new dispensers and to those aged for 136 days in the field were not significantly different (P>0.05).

Wind tunnel (WT)
The percentage of oriented flights and landing on the pheromone source was comparable to that observed in WT with optimized release of synthetic E7,Z9-12:Ac (El-Sayed *et al.*, 1999). For none of the behavioural responses analyzed, significant differences among dispensers of different field age emerged (2x2 contingency table-χ^2 test after Yates correction: P>0.05 in all cases).

Table 2. Attraction of virgin (n=10) GM males to field aged Ecodian® dispensers.

Field age of dispensers (days)	% activation	% taking flight	% oriented flight	% flight near the source	% landing
0	100	90	80	60	20
18	100	100	50	40	0
53	90	90	50	50	20
74	100	90	50	40	20
97	100	90	60	50	10
136	100	90	70	60	10

Field trapping
While in the control plots 24.8% of the released marked males were recaptured, in the mating disruption plots and in disorientation plots only 0.24% and 0.71%, respectively, were recaptured. Traps baited with virgin females were attractive only in the control plots. Traps baited with the synthetic pheromone were most effective in the control plots; only a small number of males entered the pheromone traps in the vineyards treated with pheromone.

Table 3. Marked GM males, recaptured in the field trapping trial.

Treatment	Trap (males/trap)	
	Virgin females	Synthetic pheromone
Control	4.6±3.6	21.8±12.0
Mating disruption	0	0.3±0.5
Disorientation	0	0.8±1.0

Efficacy evaluation in the field
The GM infestation level recorded in the pheromone treated vineyards was lower than that in the field treated with conventional insecticides. The lowest percentage of clusters injured by GM larvae was observed in the plots with disorientation.

Table 4. Percentage of clusters injured by *L. botrana* larvae at the end of the 1st and 2nd generation in a two-year field experiment.

Treatment	1st generation		2nd generation	
	2003	2004	2003	2004
Control	6	-	5	2.4
Mating disruption	2	0	0	1.2
Disorientation	0.5	0	0	0

Conclusion

Our results demonstrate that in the Trentino Region the life span of the Ecodian® dispensers for disorientation can cover the entire season, and that at the end of the season their release rate can be considered still competitive with calling *L. botrana* females.

The field trapping experiments showed that in the vineyards where the disorientation treatment was applied, the Ecodian® dispensers competed with natural and synthetic sources of pheromone: the released GM males were not able to reach virgin females.

In our experimental conditions, the control of the grapevine moth by disorientation was satisfactory also when compared with mating disruption and conventional insecticides.

Further investigations are needed in order to test this new technique with high population densities of the target pest.

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Field evaluation of the sex pheromone of the Lackey moth *Malacosoma neustrium* (L.) in two Italian regions

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Abstract: The attractant power and selectivity of delta traps baited with different dispensers (rubber septum, polyethylene vial, multilayer cellulose dispenser) loaded with two doses (250 and 1000 µg) of (E,Z)-5,7-dodecadienal (E5,Z7-12:Ald) alone or in combination with (E,Z)-5,7-dodecadienol (E5,Z7-12:OH) (9:1, 3:1 and 1:1, respectively), the two sex pheromone components of the Lackey moth Italian strain, were investigated in field tests. Trials were carried out in two Italian regions, Calabria and Sardinia Island, where the population density of Lackey moth was estimated to be low and high, respectively.
At the tested doses, the 9:1 and 3:1 blends of E5,Z7-12:Ald and E5,Z7-12:OH were more attractive than the other lures. Polyethylene vials proved to be the most effective dispensers. They allowed for capturing male moths in both regions while rubber septa and multilayer cellulose dispensers worked only in Sardinia but were less efficient than polyethylene vials. The attractant power of different lures did not change according to the dose. At the present time, several samplings per week of delta traps baited with polyethylene vials loaded with 250 mg of a E5,Z7-12:Ald and E5,Z7-12:OH blend from 9:1 to 3:1 allow for monitoring the short flight period of Lackey moth.

Key words: *Malacosoma neustrium*, Lepidoptera, Lasiocampidae, sex pheromone, (E,Z)-5,7-dodecadienal, (E,Z)-5,7-dodecadienol, dispensers, field tests.

Introduction

The Lackey moth, *Malacosoma neustrium* (L.) (Lepidoptera Lasiocampidae), is a common pest of many forest and cultivated broadleave trees in Europe and Asia. The insect is univoltine and the adult presence is limited to a few weeks between June and July. In Italy, recent outbreaks in Sardinia Island and Calabria have caused heavy defoliations of cork oak (*Quercus suber* L.) and holm oak (*Quercus ilex* L.) forests (Luciano and Roversi, 2001).

Pheromone methods could be used to monitor the Lackey moth population in order to undertake timely and localized control measures. The sex pheromone of the Lackey moth Italian strain was recently characterized as a mixture of (E,Z)-5,7-dodecadienal (E5,Z7-12:Ald), the main component, and (E,Z)-5,7-dodecadienol (E5,Z7-12:OH), the minor one (Rotundo *et al.*, 2004).

In order to evaluate the effect of (1) composition of the pheromone blend, (2) type of dispenser and (3) bait dose on Delta trap catches field tests were carried out in two Italian regions with different population densities of Lackey moth.

Materials and methods

Pheromone dispensers

Polyethylene vials (0.35 ml), green rubber septa and multilayer cellulose dispensers loaded with 250 and 1000 µg of synthetic E5,Z7-12:Ald alone or in combination (9:1, 3:1, 1:1) with E5,Z7-12:OH (Novapher, Milan, Italy) were tested.

Field tests

Field trials were carried out during 2003 at Savelli (950 m above sl, Calabria) from June 13 to 30 and at Bottidda (300 m above sl, Sardinia) from June 6 to 27. The Lackey moth population density was estimated to be low at Savelli (no evident defoliation) and high at Bottidda. In this latter, the mean number of *M. neustrium* larvae per 40 twigs ranged from 121 to 484 during 2002-2004 (Luciano *et al.*, 2004).

Insects were trapped in green delta traps (13x11x20 cm) containing a white adhesive base (12.7x19.5 cm) (Novapher, Milan, Italy). Traps were suspended from trees 2-3 m above ground level 50 m apart. In each area, treatments were replicated three times. Three unbaited traps were used as a control. Trap bases were replaced and male catches recorded at four day intervals in Calabria and at 1 week intervals in Sardinia.

Data analysis

Data were expressed as total male catches per trap. Data were transformed to $\sqrt{x+0.5}$ and subjected to analysis of variance (ANOVA) followed by a Duncan test (P=0.05) or a Student's *t*-test.

Results

In Calabria, a total of 167 males were caught from June 13 to 21. In this area, only polyethylene vials allowed to capture male *M. neustrium* moths. Traps baited with rubber septum, multilayer cellulose dispensers and unbaited traps (control) did not catch any male.

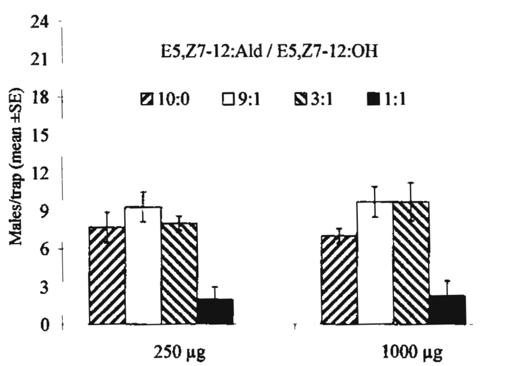

Fig. 1. Mean number (±SE) of *M. neustrium* males caught per trap in Calabria (June 13 – 21, 2003) by polyethylene dispensers loaded with 250 and 1000 µg of different blends of sex pheromone components.

Male catches by E5,Z7-12:Ald alone and in 3:1 and 9:1 ratios with E5,Z7-12:OH were similar among them and significantly (P=0.05; Duncan test) greater than that of the 1:1 blend (Fig. 1). For each lure, the attractant powers of 250 and 1000 μg doses were not significantly different (P=0.05; *t*-test).

In Sardinia, all baited traps caught male *M. neustrium* moths from June 6 to 18. The total number of males captured by polyethylene vials, rubber septa and multilayer cellulose dispensers was 150, 113 and 120, respectively. In unbaited traps male moths were not found.

At the tested loading, polyethylene vials caught, on the average, more males than either rubber septa or multilayer cellulose dispensers; however these differences were not always statistically significant (Tab. 1).

The mean number of male catches by different pheromone blends did not significantly (P=0.5; Duncan test) vary when deposited on multilayer cellulose dispensers or polyethylene vials even though, for the latter, the 9:1 blend was more attractive than the other ones. On rubber septa dispensers, at the 1000 μg dose, the 3:1 and 9:1 blends of E5,Z7-12:Ald and E5,Z7-12:OH were significantly (P=0.05; Duncan test) more attractive than the other ones; these differences were not observed at the 250 mg dose.

Increasing the dose of different lures from 250 mg to 1000 μg male catches were not significantly (P=0.05; *t*-test) affected.

In both areas, non-target insects were not caught during field trials.

Tab. 1. Mean number (±SE) of *M. neustrium* males caught per trap in Sardinia (June 6 – 18, 2003) by multilayer cellulose, polyethylene vials and rubber septa dispensers loaded with 250 mg and 1000 μg of different blends of sex pheromone components.

Attractants (μg)		Dispenser		
E5,Z7-12:Ald	E5,Z7-12:OH	Multilayer cellulose catches (mean±SE)	Polyethylene vial catches (mean±SE)	Rubber septum Catches (mean±SE)
1000	-	3.7 ± 0.9 ab	8.3 ± 2.6 b	1.3 ± 0.9 a
900	100	3.3 ± 2.8 a	15.0 ± 4.6 b	9.0 ± 1.5 ab
750	250	7.8 ± 3.2 a	13.3 ± 2.6 a	8.3 ± 1.0 a
500	500	5.3 ± 2.3 ab	7.0 ± 1.5 b	1.3 ± 0.3 a
250	-	3.0 ± 0.6 a	6.7 ± 1.2 b	3.0 ± 1.0 a
225	25	4.7 ± 2.3 a	14.7 ± 3.8 a	6.7 ± 2.7 a
188	62	3.7 ± 1.5 a	8.0 ± 1.7 a	4.3 ± 0.9 a
125	125	4.3 ± 1.7 a	7.3 ± 2.8 a	4.0 ± 1.2 a
Unbaited trap		0	0	0

Means within each line followed by the same letter are not significantly different by Duncan test (P=0.05).

Conclusions

The 9:1 and 3:1 blends of E5,Z7-12:Ald and E5,Z7-12:OH were better than other lures. At the tested doses (250 to 1000 μg), the higher attractant power of these two blends was more evident when they were deposited on polyethylene vials and rubber septa dispensers.

Polyethylene vials proved to be the most effective dispensers. They allowed for capturing male moths in both regions while rubber septa dispensers worked only in Sardinia and were slightly less efficient than polyethylene vials.

It is generally assumed that polyethylene vials have higher intrinsic release rates when compared to rubber septa (Cork *et al.*, 2001). This characteristic may explain the high efficiency of polyethylene vials at Savelli where, due to the high altitude (950 m above sl), night-time temperatures drop drastically. The greater effectiveness of polyethylene vials in respect to the rubber septa could be due to a better release of sex pheromone components. The release of (Z)-7-dodecenal (Z7-12:Ald) and (Z)-7-dodecenol (Z7-12:OH) from rubber septa is initially rapid and then drop markedly within a few days; in contrast, initial releases from polyethylene vials increase to reach a fairly constant level after three to four days (Beevor *et al.*, 1999).

In Sardinia Island, the high population density, in addition to environmental factors, leads to capture male moths by rubber septa and probably to the saturation by moths of delta traps baited with polyethylene vials. These effects made less evident the differences on the dispenser efficiency in this area.

The total number of *M. neustrium* males captured by delta traps baited with polyethylene vials in both areas was similar. This further suggests the saturation of delta trap by moths in Sardinia. As a consequence, for this trap more samplings per week are needed. Water traps represent a possible alternative because they are unlikely saturated by moths but they are more expensive and difficult to use in the forest environment.

At the present time, several samplings of delta traps baited with polyethylene vials loaded with 250 mg of a E5,Z7-12:Ald and E5,Z7-12:OH blend from 9:1 to 3:1 allow for monitoring the short flight period of Lackey moth.

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Electrophysiological responses of two different species of apple gall midges (Diptera: Cecidomyiidae) to host plant volatiles

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Abstract: The Apple Leafcurling Midge, *Dasineura mali* (Kieffer) (Diptera, Cecidomyiidae), damages apple trees, because it makes leaf galls. In 2003 and 2004, 80% of the midges that emerged from apple leaf galls collected in Trento Province (Italy) belonged to a new species of the genus *Macrolabis* (Diptera Cecidomyiidae). Only the remaining 20% was *D. mali*. The identification of this new species, and studies on its biology are in progress. We recorded the EAG responses of *D. mali* and *Macrolabis* sp. adults to volatile compounds previously identified in the headspace of immature apple leaves; some volatile compounds were able to stimulate mated female antennae of both species. Preliminary olfactory responses of both species to the EAG-active compounds were recorded.

Key words: semiochemicals, *Dasineura mali*, *Macrolabis*, EAG, behaviour.

Introduction

The Apple Leaf Curling Midge (ALCM), *Dasineura mali* (Kieffer) (Diptera Cecidomyiidae), damages apple trees in Europe, Great Britain, North America, and New Zealand. The larval feeding causes the leaf to curl up (galls) and to drop prematurely. ALCM control requires pesticide applications, especially in nurseries and young plantations. In 2003 and 2004, also midges of the genus *Macrolabis* (Diptera Cecidomyiidae) emerged from apple leaf galls collected in Trento Province (Italy). The presence of a *Macrolabis* species was recorded in apple orchards from South Tyrol to SW Germany (Carl, 1980); the identification of this species and studies on its biology are therefore necessary. Laboratory trials aiming at clarifying the biological role of the *Macrolabis* species were carried out. Most Cecidomyiids are highly specialized, monophagous insects, and host plant selection and oviposition site location are supposed to be mediated by specific odour blends (Galanihe and Harris, 1997). Volatile compounds released by young apple leaves and buds, were collected and identified, and the data were compared with those reported in literature. The olfactory sensitivity of *D. mali* and *Macrolabis* sp. mated females to the identified volatile compounds was studied using electroantennography (EAG). Some EAG-active compounds were used in an olfactory bioassay in order to preliminarily investigate possible behavioural effects on mated females.

Material and methods

Insect rearing

Apple galls (cv. Golden, Stark, Fuji) infested with midge larvae were collected from orchards in Trento Province in 2003 and 2004. Galls were placed in cubic plastic cages (length 45 cm) with a 3-cm layer of horticultural soil. Rearing cages and experiments were housed in climatic chambers (26±2°C, 60±5% RH, 16L:8D photoperiod).

Studies on biology

Test 1: infested apple galls were collected and transferred individually (50 replicates) into cylindrical pots (height 10.5cm; diameter 8 cm) with a 3-cm layer of horticultural soil; emerging adults were collected daily, and specimens were identified.

Test 2: potted apple plants with young leaves and buds (cv. Golden) were isolated individually in cylindrical plastic cages (height 26 cm; diameter 12 cm). Six newly emerged pairs of adults were placed in each cage (N=10/species). Plants were checked after 2 weeks.

Headspace collection and chemical analysis

The top of a little apple plant (cv. Golden) with immature leaves was enclosed into a 1,000-ml plastic bag; charcoal-filtered air was pulled through the bag at 150 ml/min and over a Porapak Q cartridge containing 50 mg of active adsorbent (Sigma-Aldrich). Collections (n=5) lasted 24 h. Volatiles were desorbed by eluting the cartridge with 500 μl redistilled hexane. Collected volatiles were identified by using a Hewlett-Packard 5970B mass spectrometer (MS), interfaced with a Hewlett-Packard 5890 GC and equipped with a HP-INNOWax column, with the following oven temperature program: 5 min at 50°C, 10°C/min to 60°C, 1 min at 60°C, 2.5°C/min to 200°C, 30 min at 200°C. To identify the compounds collected, they were compared with standard synthetic compounds (Sigma-Aldrich purity>98%).

Electroantennography (EAG)

Twenty-nine of the identified volatile compounds and/or of those that had been previously reported (Bengtsson *et al*, 2001) were selected (Table 1). EAG analyses were carried out on mated females of both species using technique similar to that described in a previous study (De Cristofaro *et al.*, 2000). The responses (mV) of 10 antennae of each species were recorded. Stimuli were provided by soaking a piece of filter paper (2 cm^2) in 25 μl of compound solution (1/10 v/v in mineral oil), and inserting it into a Pasteur pipette. EAG responses were normalized according to the reference stimulus (Z3-hexen-1-ol) and subjected to the analysis of variance (ANOVA) followed by Duncan test.

Olfactory bioassay

The olfactory bioassay was carried out in a plastic Petri-dish (diameter 15 cm) placed in a white, uniformly illuminated box (2,000 lux). The bottom of the dish was covered with filter paper. Two pieces of filter paper (2 cm^2) were placed on two opposite sides: one was soaked with 10 μl of a compound solution (1/10 v/v in mineral oil), whereas the other one was soaked with 10 μl of mineral oil, thus acting as control. Mated females of both species (n=10 per species) were then inserted individually through a little hole in the lid of the Petri dish and observed for 10 min. The time spent in the sector (half dish) with the compound was recorded. For each species, four compounds were tested, and the time spent in the compound sector was compared with that spent in the control sector (*t*-test).

Results

Studies on biology

Test 1: adults of both species emerged from 76% of the galls, whereas only *D. mali* adults emerged from the remaining 24%. In the galls with mixed populations, 72% were *Macrolabis* sp. adults.

Test 2. 80% of the plants caged with *D. mali* adults showed midge galls after 2 weeks. In the plants caged with *Macrolabis* sp. adults, no galls were observed.

Chemical analysis

Table 1. Volatile compounds identified in this study and/or previously reported by Bengtsson *et al.* (2001) for apple leave headspace collections.

Compound	IASMA	Bengtsson *et al.*, 2001	Compound	IASMA	Bengtsson *et al.*, 2001
acids			*esters*		
acetic acid	*	*	Z3-hexenyl-acetate	*	*
hydrocarbons			geranyl-acetate	*	
pentadecane	*	*	*aromatic compounds*		
hexadecane	*		ethyl-benzoate	*	
heptadecane		*	benzyl alcohol		*
heneicosane		*	methyl salicylate		*
alcohols			benzyl-acetate		*
hexanol	*		benzaldehyde		*
Z3-hexen-1-ol	*	*	*monoterpenes*		
2-ethyl-hexanol	*		β-pinene	*	
octanol	*		(S)-(-)-limonene	*	
1-octen-3-ol	*		Δ3-carene	*	
aldehydes			β-linalool	*	*
E2-hexenal	*		β-ocimene	*	*
octanal	*	*	α-terpineol	*	
nonanal	*	*	*sesquiterpenes*		
ketones			β-caryophyllene	*	*
6-methyl-5-hepten-2-one	*		(E,E)- α-farnesene	*	*

Electroantennography (EAG)

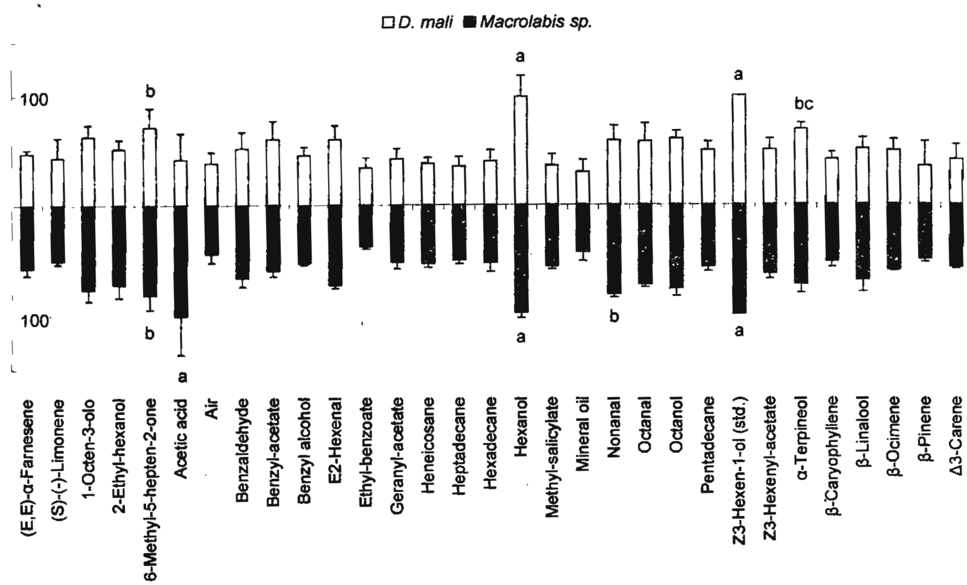

Figure 1. Mean normalized EAG response of mated *D. mali* and *Macrolabis* sp. females (n=10) to volatile compounds (25 μl of solution at 1:10 v:v in mineral oil) released by young apple leaves. Reference stimulus: Z3-hexen-1-ol. Vertical bars represent S.D.; Letters indicate the two higher response groups (Duncan test; P<0.05).

Olfactory bioassay

Table 2. Olfactory responses of mated *D. mali* and *Macrolabis* sp. females to EAG-active host plant volatiles – percentage of time (%) spent in the sector with the compound. (*=significant differences between the two sectors; *t*-test: P=0.01)

D. mali	
Hexanol	41.7±22.5
Z3-hexen-1-ol	13.7±8.3*
α-Terpineol	43.2±15.0
6-methyl-5-epten-2-one	55.9±14.7

Macrolabis sp	
Acetic acid	69.6±11.2*
Z3-hexen-1-ol	46.0±17.6
Nonanal	43.8±6.3
6-methyl-5-epten-2-one	55.5±21.6

Discussion

The study on the biology of the *Macrolabis* species showed that the species is unable to make galls; larvae of *D. mali* and *Macrolabis* sp. live in the same gall. The new species does not seem to be a real phytophagous, but it seems to dwell as an inquiline in *D. mali* galls, as other species of the genus (Fedotova, 1998), and during the season its population becomes larger than that of the host. The new species is being described. Both mated *D. mali* and *Macrolabis* sp. females showed olfactory sensitivity to volatile compounds identified from apple leaves with considerable differences between the two species: *Macrolabis* antennae were mainly stimulated by hexanol, Z3-hexen-1-ol, and acetic acid, while *D. mali* antennae were stimulated by hexanol and Z3-hexen-1-ol. In preliminary olfactory bioassays, using the four most EAG-active compounds, acetic acid was attractive to *Macrolabis* females, and Z3-hexen-1-ol was repellent to *D. mali* females. Further researches are in progress in order to screen the olfactory sensitivity of the apple gall midges to the host plant volatiles and to investigate the role of the active compounds in interspecific communication.

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Effect of Madex® (granulovirus) on codling moth egg laying and larval damages on two apple varieties – Relationships with plant surface metabolites.

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Abstract: Differences in Madex® (granulovirus) treatment efficiencies against codling moth, *Cydia pomonella* (*C. pomonella*) larvae were observed in San Michele orchard according to apple tree varieties. Our hypothesis is this can be due to egg laying preferences and/or to differences in neonate larval behaviour and damages. Water-soluble metabolites: sugars and sugar-alcohols have been already identified from surfaces of apple tree organs. Among them fructose, sorbitol and *myo*-inositol stimulate particularly *C. pomonella* egg laying and examination of the substrate by the neonate larvae. Our aim here is to compare the effect of Madex® on apple damages on two varieties (Golden Delicious and Red Chief) by looking at: i) egg laying, ii) fruit damages and iii) primary metabolites on leaf surface already known as influencing egg laying and neonate larvae behaviour. Treatment with Madex® decreased codling moth fruit damages on the two varieties: this is due to both activities on the egg laying and on the larvae. The decrease in the number of eggs, after treatment with Madex®, was similar on the two varieties, however on the larval damages, the effect of Madex® was higher on Golden Delicious than on Red Chief. The differences in the composition of sugar-alcohols on the surface of apple leaves may explain the decrease in the number of eggs by Madex® and also the differences in larval damages between the two varieties.

Key words: *Cydia pomonella*, Madex®, egg laying, larval damages, primary metabolites.

Introduction

The codling moth, *C. pomonella* is the main pest of apple worldwide. Management of the insect benefits from a complex of natural enemies but still depends on selective insecticides or microbial sprays such as *Bacillus thurigiensis* (B. t.) and granulovirus.

Differences in treatment efficiencies against *C. pomonella* larvae with Madex® was observed in 2002, in San Michele orchard, on two apple tree varieties: Golden Delicious and Red Chief. In our hypothesis, this can be due to differences in egg laying and/or to differences in neonate larval behaviour and damages.

Water soluble metabolites: sugars and sugar-alcohols have been already identified from surfaces of apple tree organs. Among them fructose, sorbitol and *myo*-inositol stimulate particularly *C. pomonella* egg laying and examination of the substrate by the neonate larvae. The purpose of this study is to compare the effect of Madex® on apple damages on two varieties (Golden Delicious and Red Chief) by looking at: i) egg laying, ii) fruit damages and iii) primary metabolites on leaf surface already known as influencing egg laying (Lombarkia & Derridj, 2002) and neonate larvae behaviour.

Material and methods

Egg laying and larval damages sampling

Two varieties: Golden Delicious and Red Chief in San Michele orchard were treated with Madex® nine times throughout the season (2003). Dosage used was: 7 mL/1 hl of Madex® and 30 mL/1 hl of UV-protect: vaporgard. Comparisons between treated and non-treated trees were carried on by numbering eggs at the maximum of first (19^{th} to 28^{th} May) and second (15^{th} to 25^{th} July) egg laying periods. Trees were five years old and are between two and three meters high. Eighteen trees per variety, per treatment and per moth flight were sampled. Nine branches per tree, with or without fruits, of 10 to 20 cm long, taken randomly on the tree, were sampled and observed on each tree for each treatment and for each flight. At the same time the number of damaged fruits by larvae was recorded.

Collect of leaf surface metabolites and chemical analyses in GC-FID

Metabolites were collected on both varieties non-treated and treated with Madex® during the egg laying periods at twilight. Collecting process consists in spraying ultra-pure water on leaves and fruit (Fiala *et al.*, 1990). Four replicates per variety consisting of one cluster per tree were sampled. A cluster composed of the same number of leaves (five biggest leaves) and fruit (one) on one side of each tree in one tree zone (median). Each tree corresponding to a tree sampled for egg laying (same rank). Leaf and fruit surfaces were sprayed at 10 cm distance with a flow of nitrogen gas, and pressure of 17 L/min. Leaves were laid with a slope of 60° and the foliar surfaces were sprayed with approximately 10 mL of ultra-pure water per 100 cm^2. Fruit surfaces were sprayed with 10 mL of ultra-pure water per 300 cm^2. Collection of internal leaf and fruit fluid was avoided by sealing the wounded part in liquid paraffin (liquid at 44-46°C). Collection was followed by filtration of the samples through a 0,25 µm filter to remove impurities and epiphytic microorganisms. Chemical analyses of metabolites were carried out on derivated samples by gas chromatography coupled to the flame ionization detector (FID) Delsi Nermag D N 200 apparatus.

Statistical analyses

Egg laying and fruits damaged by larvae data as well as quantities (ng/cm^2) of metabolites collected on leaf and fruit surfaces were compared by Student t-test. The level of significance taken was equal to 0,05.

Results and discussion

Comparison of the number of eggs between the two varieties:

Treatment with Madex® decreased the number of eggs on the two varieties. The ratio: non-treated/treated was almost the same for the two varieties: 2,28 for Golden Delicious and 2,57 for Red Chief. The first graph showed also a difference in the egg laying preference between the two varieties, which persists after treatment with Madex®.

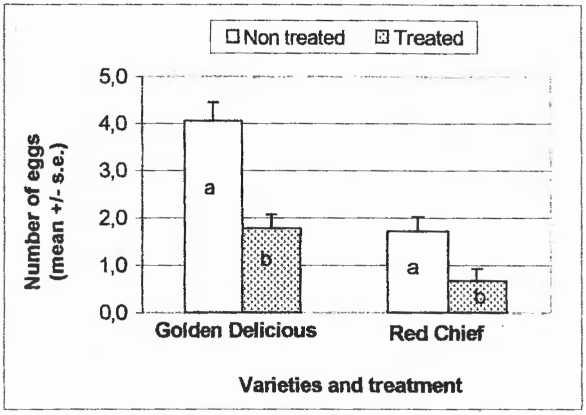

Figure 1: Number of eggs (per tree) on non-treated and treated Golden Delicious and Red Chief.

Comparison in the larval damages between the two varieties:

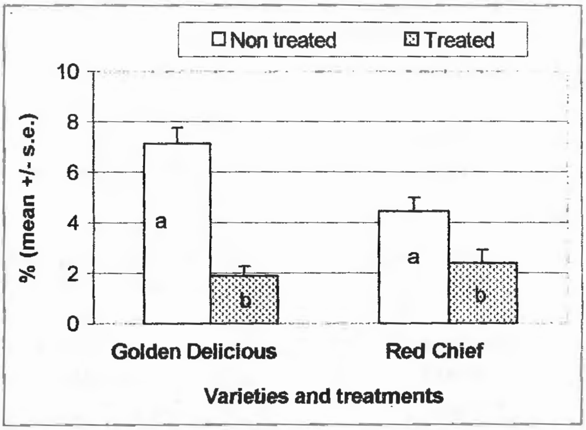

Figure 2: Percentage of damaged fruits on non-treated and treated Golden Delicious and Red Chief.

Treatment with Madex® decreased the percentage of damaged fruits by larvae of both varieties (figure 2). The ratio non-treated/treated was higher on Golden Delicious than on Red Chief with respectively: 3,79 and 1,87.

There is a difference in the larval damages between the two varieties. But this differential effect between the two varieties disappears after treatment with Madex®. These data confirm the differences in Madex® efficiencies, between the two varieties, already observed in 2002 in San Michele orchards.

Comparison in the leaf surface sugar-alcohols between the two varieties:

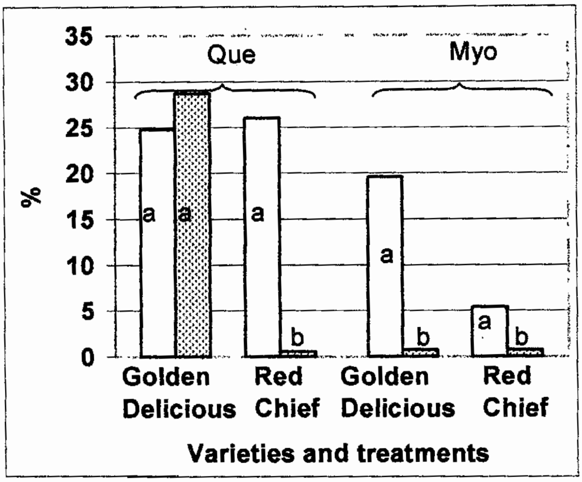

Figure 3: Proportions of sugar-alcohols on the lower leaf side of □ non-treated and ▨ treated Golden Delicious and Red Chief. Que: Quebrachitol, *Myo*: *Myo*-inositol.

But could we explain, by plant surface metabolites, the differences observed in the egg laying and larval damages between the two varieties and the effect due to treatment with Madex® on these two factors?

The differences in the composition of sugar-alcohols on the surface of apple leaves may explain the decrease in the number of eggs by Madex® and also the differences in larval damages between the two varieties. Indeed, treatment with Madex® decreased the number of eggs on both varieties. This is correlated to the decrease in the proportions of *myo*-inositol on the two varieties: 19,52% on non-treated trees against 0,71% on treated one for Golden Delicious (figure 3). This observation is also valid for Red Chief: 5,37% on non-treated against 0,74% on treated one. Previous studies showed that subtraction of *myo*-inositol from a blend of five metabolites: glucose, fructose, sucrose, sorbitol and quebrachitol decreased by half the number of eggs laid on artificial substrate (Lomabrkia & Derridj, 2002). Considering this effect of the virus on the insect egg laying, it may be useful to take advantage of the reduction of the egg laying by Madex® in order to increase the effect on larvae. The aim is to act on the two main steps of the insect life cycle before penetration into the fruit: eggs and neonate larvae.

The knowledge of the activity of metabolites in the egg laying preference and larval damages of *C. pomonella* could explain variations of the efficacy of treatments with Madex®.

The efficacy of the granulovirus can be improved by the addition of primary metabolites. Charmillot *et al.* (1998) showed that the granulovirus had a good initial efficacy on *C. pomonella* larvae: average efficacy of 46,7%, but a poor persistence due to quick inactivation by UV light. In general, addition of products such as sugars increased the persistence of the virus. Indeed, the two adjuvants: skimmed milk powder and Nufilm-17 did not improve Madex® efficacy, but when sugars were added to the two adjuvants, the efficacy increases very clearly, reaching 61,8% with skimmed milk powder and 89,3% with Nufilm-17.

This study showed also that apple tree susceptibility and Madex® effect on *C. pomonella* egg laying and larval damages could be considered as integrated protection method of apple trees. Studies of the relationships with primary metabolites could open new directions of research to improve apple tree egg laying resistance and granulovirus efficacy.

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Effects of the (E; Z)-2,4-ethyl decadienoate (DA2313) and synthetic pheromone blends to monitor *Cydia pomonella* adults*

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Abstract: Trials to assess possible synergic activity of (E; Z)-2,4-ethyl decadienoate (DA2313) and the synthetic pheromone (E,E)-8,10- dodecadienol to monitor both sexes of *Cydia pomonella* adults were carried out. Field trials were carried out in four Italian and one French areas. Traps baited with DA or synthetic pheromone or with different blends of the two compounds were placed in the fields and trap catches were recorded weekly. Similar results were obtained from all the examined areas: synthetic pheromone caught more males than DA, while regarding females DA was more attractive. Sinergy between the two substances is absent with the doses tested: the blends with 2 different doses have lower performances than using the pheromone for males and DA for females separately.

Key words: *Cydia pomonella,* plant volatiles, monitoring

Introduction

Codling moth, *Cydia pomonella* (L.) is the most important pest in apple, pear and walnut orchards. Damaged fruits show penetration holes and galleries going into pulp to the pips that are devoured by larvae. Besides, fruits can fall in advance to the ground and be declassed because of aesthetic damages. Traditional control tactics is based on repeated insecticide applications (up to six/ year) with high environmental impact and increase of insecticide induced resistance.

The identification of Codling Moth sexual pheromone (E,E)-8,10-dodecadienol (E8E10-12:OH) by Roelofs et al., (1971) led to the development of more environmentally safe control tactics. Pheromone was mainly applied succesfully in baiting traps to monitor flight of adult males. On the contrary adult females behavoiur is not usefully modified by the pheromone, so the finding of new attractants can improve the defence strategies; among these, the identification of (E,Z)-2,4-ethyl-decadionate (DA2313), a pear esther able to attract both males and females, (Light et al., 2001) started experimentations to define the potential applications of this substance on insect monitoring.

Preliminary work was carried out in Italy to evaluate the DA efficacy since 2000, within a research project coordinated by Trécé, using different E8E10-12:OH and kairomone doses

and blends, on apple and pear orchards of different northern Italy production areas. Ioriatti et al. (2003) confirmed that DA2313 can be an interesting attractant in monitoring *C. pomonella.* Trials to verify a potential synergism between DA2313 and E8E10-12:OH were performed too. The E8E10-12:OH/DA2313 (1.0:0.1 mg) blend showed higher performance, attracting more insects than 1.0 mg of the pheromone only. Besides, from these trials it was seen that high doses of DA2313 usually reduced pheromone efficacy in monitoring. So it was supposed that an increase of the attractant potential can be obtained using low dose blends, both for males and total catches.
Within the same research project (Bioinnova) we wanted to evaluate the effects of low doses of these substances, separately or blended, for monitoring male and female adults of Codling Moth.

Material and methods

The trials were carried out, during the whole flight period of 2003, in 5 different geographic areas, 4 in Italy (Gardolo in Trento, Cuneo, Massa, Campobasso) and 1 in France (Chem) (table 1). The trials were performed on apple fruits, the only exception was represented by a pear orchard (Massa).

Table 1: Data concerning the experimental orchards

Site	**Crop**	**Varieties**	**Control strategy**
Trento	apple	Golden Delicious	chemical
Cuneo	apple	Golden Delicious	chemical
Chem (Montpellier)	apple	Different varieties	chemical
Campobasso	apple	Different varieties	organic
Massa	pear	Abate Fétel	chemical

To verify the combination effects of low doses of E8E10-12:OH and DA2313, we compared the catches obtained with DA2313 (0,1 mg), E8E10-12:OH (0,1 and 1 mg) and with the two possible blends among them.
5 thesis were considered in each orchard:
- 0.1 mg DA2313,
- 1 mg E8E10-12:OH,
- 0.1 mg E8E10-12:OH,
- 0.1 mg E8E10-12:OH + 0,1 mg DA2313,
- 1 mg E8E10-12:OH + 0,1 mg DA2313.

Pherocon II B (Trécé) traps were adopted. The traps were 20 meters apart from each other and were installed at the top of the trees out of the vegetation (80 cm). Baits were replaced every 2 weeks; the traps were checked weekly: traps with insect catches were replaced with new ones and observed in the laboratory to verify the number of *C. pomonella* males and females. A randomized block scheme with 4 replications/thesis was adopted.

Data of each site were analized by ANOVA and the means were separated by Tukey test.

Results and discussion

The results obtained were the same for all the considered areas: global catches (males + females) with synthetic pheromone (E8E10-12:OH) were higher than those obtained with the plant volatile attractant DA2313. On the other hand the DA2313 resulted more effective for females monitoring (tab. 3): the number of females caught was generally negligible, with the only exception of DA2313 alone; in this case, on a total of 357 insects (tab. 2) females were 158, the global percentage being 44,26%. With the blend E8E10-12:OH + DA2313 more females were caught than with E8E10-12:OH alone, but less than with DA2313 alone (tab 3). With the blend 0,1 mg DA2313 + 1 mg E8E10-12:OH 61 females were caught, while 71 with 0,1 mg DA2313 + 0,1 mg E8E10-12:OH.

Regarding adult male catches (fig. 1), 1 mg of pheromone alone showed the best attractant power. The addition of DA2313 (0,1 mg) caused a reduction of male catches. The number of males caught with 0.1 mg of pheromone and with the blend 0,1 mg DA2313 + 0,1 mg E8E10-12:OH did not show meaningful variations.

Finally, no synergistic effect of DA2313 on E8E10-12:OH was noticed, both in terms of total catches and with regard to each sex.

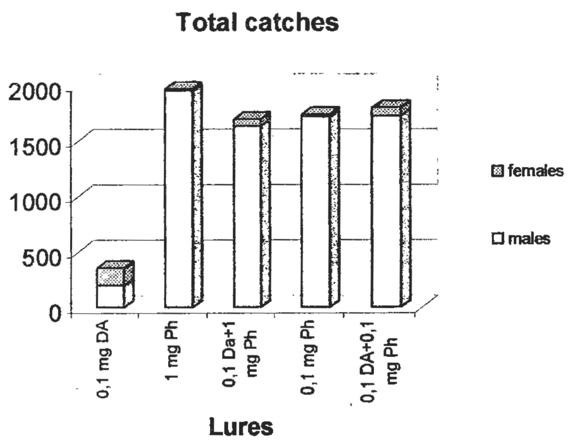

Figure 1. Total catches/ lure in the season

Table 2. Total catches (males + females) in the season for each site with different lures.

Site	0,1 mg DA	1 mg Ph	0,1 Da+1 mg Ph	0,1 mg Ph	0,1 DA+0,1 mg Ph
Trento	52	381	320	257	236
Cuneo	36	350	209	221	256
Chem (Montpellier)	153	742	921	885	906
Campobasso	111	339	184	205	268
Massa	5	161	64	171	137
total	357	1973	1698	1739	1803

Table 3. Female catches in the season for each site with different lures. The percentage refers to the number of females on the total catches (table 2).

Site	0,1 mg DA	(%)	1 mg Ph	(%)	0,1 Da+ 1 mg Ph	(%)	0,1 mg Ph	(%)	0,1 DA+ 0,1 mg Ph	(%)
Trento	24	46,2%	0	0,0%	4	1,3%	0	0,0%	10	4,2%
Cuneo	11	30,6%	5	1,4%	10	4,8%	2	0,9%	8	3,1%
Chem (Montpellier)	71	46,4%	1	0,1%	30	3,3%	9	1,0%	28	3,1%
Campobasso	47	42,3%	7	2,1%	14	7,6%	4	2,0%	22	8,2%
Massa	5	100,0%	0	0,0%	3	4,7%	2	1,2%	3	2,2%
total	158		13		61		17		71	

As example the graphic with male and female catches recorded in Campobasso is reported (fig. 2). Different letters indicate statistically significant differences.

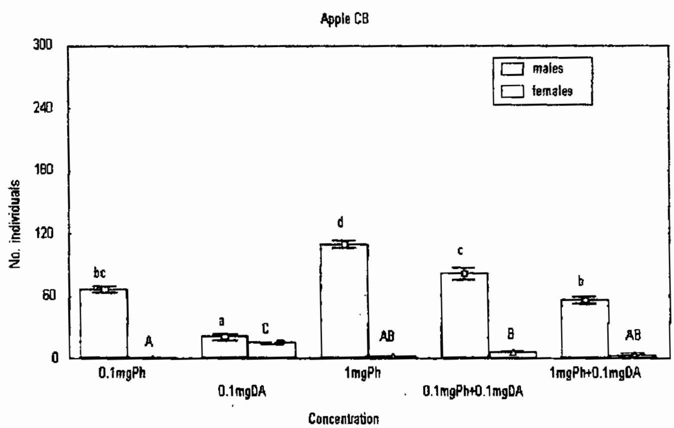

Figure 2. Mean of catches (4 replicates) for each thesis in Campobasso.

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Electrophysiological and olfactory responses of *Lobesia botrana* (Den. et Schiff.) (Lepidoptera Tortricidae) to odours of host plant*

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Abstract: Electrophysiological responses (EAG) to 50 volatile compounds (38 identified in *Vitis vinifera* L. and 12 used as comparison) were recorded on both virgin and mated sexes of the European Grapevine Moth, *Lobesia botrana*. In addition, olfactory responses (double choice test) from males and females to grape ethanolic extracts, were recorded in a self-made glass Y olfactometer. EAG patterns of males and females to the volatile compounds were different. Virgin and mated females showed higher responses to aldehydes and alcohols, while virgin males to aldehydes and acids; the sensitivity to acids was lower in mated males. Comparing the absolute (mV) mean EAG responses virgin and mated females showed significantly lower responses than males to several tested compounds. Significant differences were also observed among virgin and mated males and females; virgin insects showed higher responses than mated ones to almost every compound. Compounds evoking highest responses were used to determine EAG dose-response curves. All tested substances evoked dose-dependent responses in both sexes. Olfactory responses showed that females of *L. botrana* are oriented to the odour source by volatile compounds released by host plant.

Keywords: Grapevine moth, *Vitis vinifera*, semiochemicals, EAG, olfactometer.

Introduction

European Grapevine Moth (EGM), *Lobesia botrana* (Den. *et* Schiff.) (Lepidoptera Tortricidae), is one of the most serious pest of the European vineyards. Alternative control strategies (i.e. mating disruption, *Bacillus thuringiensis* Berliner, viruses) could be enhanced using attractant or repellent semiochemicals. Volatile compounds from grape have been widely studied, especially with respect to aromas from flowers or ripe fruit important for wine and other use, but not enough is known about their effect on the EGM host-finding process and the selection of the oviposition site. In recent studies the attraction of EGM females in wind tunnel to the blend emitted from the host plant was showed (Tasin *et al.*, 2005).

In the present study we investigated the electrophysiological (EAG) responses of *L. botrana* to a broad range of generic plant volatiles identified in different plant organs and developmental stages of *V. vinifera*. In addition, an olfactory bioassay in a self-made glass Y olfactometer (double choice test) was set up with the aim to investigate the effects on the EGM behaviour of the single EAG-active compounds. Preliminary responses from males and females to ethanolic plant extracts were recorded.

Materials and methods

Insects

Insects were reared on a semi-artificial diet at 22±2°C, 70±5% R.H. and L17:D7 inverted photoperiod. Insects were originally collected from the vineyards of Agricultural Institute of San Michele all'Adige (Trento; Northern Italy). Three-five day-old males and females were isolated in Petri dishes to avoid exposure to testing stimuli and then used for EAG recordings and olfactory tests. Matings were allowed in polystirene containers (200 ml, Ø 60x80 mm) covered with a gauze.

Stimuli

The EAG responses to 50 volatile compounds were recorded. Thirty-eight compounds were identified in the headspace of *Vitis vinifera* L. (Schreier *et al.*, 1976; Welch *et al.*, 1982; Buchbauer *et al.*, 1994; Rosillo *et al.*, 1999); 12 addictional compounds were used as comparison. Compounds (Sigma-Aldrich, purity >97 %) were diluted in mineral oil (1:10, v/V). The mineral oil solutions of the compounds evoking highest EAG responses were diluted in log steps (from 100 μg/μl to 0.01 μg/μl).

Electroantennography (EAG)

EAG responses were recorded as described in a previous paper (De Cristofaro *et al.*, 2000). Each stimulus was prepared by absorbing 25 μl of a solution on a piece of filter paper (1,5 cm^2) inserted into a Pasteur pipette. Virgin and mated male and female (n=5) responses (mV) were compared by *t*-test (P=0.05; P=0.01) and normalized according to the reference stimulus (Z3-hexen-1-ol). The mean EAG normalized data, within each group, were submitted to analysis of variance (one-way ANOVA) followed by Duncan test (P=0.05). Compounds evoking highest responses were used to determine EAG dose-response curves (n=5); doses of different chemicals were applied in ascending order (from 0.01 μg/μl to 100 μg/μl). As comparison, the same analysis was carried out using E7,Z9-dodecadienyl acetate, the main EGM sex pheromone component (Sigma-Aldrich, purity>97 %).

Field Extraction

Four field extractions were made, at dusk, in a vineyard (*cv.* Sangiovese, Tendone rearing system) located in Larino (Campobasso, Southern Italy) in four different phenological stages during the season 2004: pre-flowering extended clusters (27.V), end-flowering clusters (22.VI), growing berries (21.VII), ripening clusters (08.IX). As a solvent, 200 ml of ethanol (Fluka Chemie, 99.8%) were used. An uncut cluster was immersed for 30' into a Pyrex® graduated beaker containing the solvent. The extraction was repeated until the solution reached 100 ml. Rough extracts were stored at –25 °C.

Olfactory Test

Olfactory responses were recorded in a self-made glass Y olfactometer (stem and arms: 150 cm long, section: 40x40 cm). Purified air was supplied by a proper self-projected air flow conditioning unit equipped with paper filters, activated charcoal filters, laminar air flow generator and membrane humidifiers, able to regulate wind speed (0.2±0.02 m/s), temperature (23±2°C) and relative humidity (60±2%) (De Cristofaro *et al.*, 2003). Light intensity was 10±3 lux. In each arm, a white Delta trap (Pherocon® IIB Trap, Trécé Incorporated) was suspended in the center of the arm section. One trap was baited with 1 ml of ethanolic extract, in a pierced polyethylene 2 ml vial (5 holes, Ø 1mm), while the other trap was baited with a blank control (ethanol). Insects of the same sex were placed in a cylindrical cage (200 ml, Ø

60x80 mm), sealed with gauze on both sides, and suspended at the center of the end stem section. A preliminary screening on the 4 ethanolic extracts was recorded, testing 10 insects of the same sex per each extract. Insects were released from their cage after the introduction of the stimuli upwind in the arms. On the basis of preliminary observations, sessions started at the beginning of scotophase and continued for 4 hours. The number of insects caught in the traps and being in the arms at the end of the experiment was recorded. Data were analyzed as percentage of released moth collected in the arms.

Results

Electroantennography (EAG)

Comparing the absolute (mV) mean EAG responses, virgin and mated females showed significantly lower responses than males to several tested compounds; fourteen substances showed differences between the virgin sexes at the lower significant level (P=0.05), eight compounds at the higher level (P=0.01); in the mated adults, differences were recorded at the lower significant level for sixteen compounds and at the higher level for three of them.

Significant differences were also observed among virgin and mated sexes; virgin insects showed higher responses than mated ones to almost every compound. However the number of EAG response groups (Duncan test, P<0.05) decreased from virgin (24) to mated males (19) while slightly increased from virgin (21) to mated females (22).

Virgin and mated females showed higher responses to aldehydes and alcohols, while virgin males to aldehydes and acids; the sensitivity to acids was lower in mated males. The most EAG-active compounds were hexanal, E2,E4-decadienal, hexanoic acid, heptanal, 1-octen-3-ol for virgin and mated females and hexanal, E2,E4-decadienal, hexanoic acid, E2,E4-nonadienal, E2-nonenal for virgin and mated males. These compounds were used to determine EAG dose-response curves (from 0.01 μg/μl to 100 μg/μl). All tested substances evoked dose-dependent responses in both sexes.

Olfactory Test

As a general tendency, olfactory responses showed that both EGM males and females had a similar percentage of activation in the bioassays (about 50%). EGM males didn't show a preferential choice between ethanolic extracts and control, while EGM females oriented significantly to the ethanolic extracts (75%) (Fd=1, χ^2 test, P< 0.05).

Conclusions

EAG responses demonstrate the olfactory sensitivity of EGM both sexes to a wide range of volatile compounds released from *V. Vinifera*. However, the EAG patterns of males and females to the tested compounds were different. Lepidoptera male antennae well-equipped for perceiving plant compounds were already reported (i.e. Den Otter et al., 1996; De Cristofaro *et al*., 2000; De Cristofaro *et al*., 2003; Avilla *et al*., 2003).

On the basis of the number of EAG response groups, the olfactory selectivity in mated females did not decrease and it might be related to the need of the oviposition site location (De Cristofaro *et al*., 2000; De Cristofaro *et al*., 2004).

All the compounds, screened for their EAG-activity, evoked EAG dose-dependent responses in both sexes. This relationship, similar to that obtained with the pheromone on male antennae, will be considered in the behavioural bioassays.

The preliminary bioassay in Y olfactometer showed that, on the whole, the blend released by host plant may be attractant to the EGM female moths, as observed in wind tunnel experiments (Tasin *et al.*, 2005). Further observations and more replicates are needed to evaluate moths' responses to rough ethanolic extracts and to concentrated one. Bioassays to record olfactory responses to water extracts from grape are still in progress. This olfactory bioassay will allow the evaluation of the influence of the single EAG-active compounds emitted by host plant on the EGM behaviour, starting from the threshold doses recorded by EAG, for their possible use in the EGM control.

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Table 1. Mean EAG response of *L. botrana* (n=5) virgin females (VF), virgin males (VM), mated females (MF) and mated males (MM) to host plant volatiles (25 μl of a solution 1:10 v:V in mineral oil). Significant differences between groups: * P=0.05; *** P=0.01 (*t* test).

Compounds	VF	VM	MF	MM	VF/VM	MF/MM	VF/MF	VM/MM
2-pentanone	-0.75±0.23	-1.39±0.34	-0.69±0.15	-0.59±0.42	*			***
2-hexanone	-1.32±0.39	-1.47±0.44	-0.92±0.19	-0.96±0.26			*	
2-heptanone	-0.74±0.26	-1.59±0.22	-1.10±0.21	-1.49±0.43	***			
2-octanone	-1.23±0.40	-1.80±0.55	-1.39±0.18	-1.31±0.70				*
2-nonanone	-1.56±0.43	-1.37±0.58	-1.19±0.46	-1.39±0.27				
hexanal	-2.05±0.48	-3.30±0.95	-1.64±0.20	-2.44±0.98	*			
heptanal	-1.99±0.32	-2.24±0.80	-1.73±0.43	-2.28±0.44				
octanal	-0.75±0.18	-1.18±0.23	-1.21±0.37	-1.43±0.41	***	*	***	
decanal	-1.05±0.38	-1.97±0.56	-1.14±0.26	-1.97±0.60	*	*		
E2-hexenal	-1.70±0.22	-2.52±0.69	-1.59±0.20	-2.27±0.76	***	*		
E2-octenal	-1.20±0.26	-1.69±0.48	-1.25±0.32	-1.63±0.25	*	*		
E2-nonenal	-1.81±0.36	-1.64±0.51	-1.40±0.57	-2.49±1.06				
2,4-hexadienal	-1.02±0.34	-1.20±0.45	-1.20±0.24	-1.52±0.29		*		
E2,E4-heptadienal	-0.66±0.18	-0.89±0.27	-0.88±0.20	-0.98±0.27				
E2,E4-nonadienal	-1.18±0.32	-3.03±1.07	-1.51±0.31	-2.72±1.02	***	*		
E2,E4-decadienal	-2.06±0.58	-1.72±0.44	-1.57±0.61	-2.52±0.89		*		
benzaldehyde	-0.38±0.16	-0.53±0.10	-0.49±0.09	-0.54±0.23				
furfural	-0.43±0.18	-0.93±0.39	-0.44±0.10	-0.54±0.23				*
1-butanol	-0.44±0.23	-0.93±0.24	-0.32±0.07	-0.88±0.47	*	*		
1-pentanol	-1.45±0.32	-1.50±0.56	-0.83±0.15	-1.03±0.23		*	*	
1-hexanol	-1.64±0.29	-1.88±0.55	-1.64±0.41	-2.10±0.31		***		
2-hexanol	-0.78±0.27	-1.26±0.26	-0.82±0.17	-1.30±0.30	***	*		
3-hexanol	-0.99±0.28	-1.49±0.69	-0.91±0.10	-1.17±0.59				
2-methyl-1-butanol	-0.73±0.26	-1.09±0.45	-0.63±0.22	-1.04±0.45				
3-methyl-1-butanol	-1.23±0.44	-1.49±0.52	-0.71±0.15	-1.20±0.24		***	*	
1-octen-3-ol	-2.40±0.50	-2.06±0.49	-1.74±0.54	-1.91±0.39			*	
Z2-penten-1-ol	-0.76±0.17	-1.17±0.56	-0.27±0.17	-0.54±0.25		*	***	
E2-hexen-1-ol	-0.70±0.23	-0.96±0.33	-0.77±0.17	-1.37±0.33		*		***
Z3-hexen-1-ol	-1.23±0.42	-1.86±0.46	-1.14±0.43	-2.00±0.43	*	***		
linalool	-0.99±0.32	-1.37±0.61	-0.89±0.28	-0.90±0.29				
geraniol	-0.61±0.28	-1.18±0.49	-0.53±0.15	-0.62±0.36				
eugenol	-0.43±0.15	-0.80±0.27	-0.33±0.11	-0.50±0.22	*			
β-citronellol	-0.44±0.07	-0.89±0.36	-0.28±0.14	-0.50±0.22	*		*	
naphtalene	-0.54±0.17	-1.00±0.29	-0.54±0.13	-0.73±0.24	*			
(+)-3-carene	-0.55±0.21	-0.93±0.31	-0.63±0.09	-0.66±0.29				*
(+)-E-caryophyllene	-0.97±0.38	-0.89±0.40	-0.74±0.38	-0.79±0.22				
myrcene	-1.78±0.44	-1.32±0.41	-1.25±0.48	-1.15±0.29	***		*	
E-β-farnesene	-0.74±0.25	-1.90±0.67	-0.52±0.15	-0.70±0.30	*			*
R-(+)-limonene	-0.85±0.37	-1.01±0.39	-0.66±0.17	-0.75±0.29				
(+)-α-pinene	-0.57±0.29	-0.68±0.29	-0.37±0.15	-0.42±0.15				
1,2,3-trimethylbenzene	-0.36±0.16	-0.52±0.20	-0.32±0.15	-0.42±0.16				
propionic acid	-0.40±0.26	-1.00±0.37	-0.38±0.08	-0.73±0.29	*	*		
N-butyric acid	-0.74±0.34	-2.04±0.64	-0.76±0.36	-1.45±0.66	*	*		
pentanoic acid	-1.22±0.37	-3.08±0.82	-0.80±0.16	-1.64±0.70	***	*		*
hexanoic acid	-1.21±0.46	-2.61±0.65	-0.69±0.11	-1.23±0.54	*	*		*
heptanoic acid	-0.81±0.23	-1.90±0.80	-0.57±0.13	-0.68±0.37	*			*
octanoic acid	-0.53±0.17	-1.22±0.28	-0.84±0.34	-1.11±0.74	***		*	
benzylacetate	-0.81±0.18	-1.84±0.76	-0.78±0.28	-1.03±0.39	*			
butylacetate	-0.90±0.20	-1.43±0.65	-0.69±0.37	-0.96±0.68				
pyridine	-0.80±0.28	-1.55±0.54	-0.54±0.28	-0.78±0.27	*	*		*

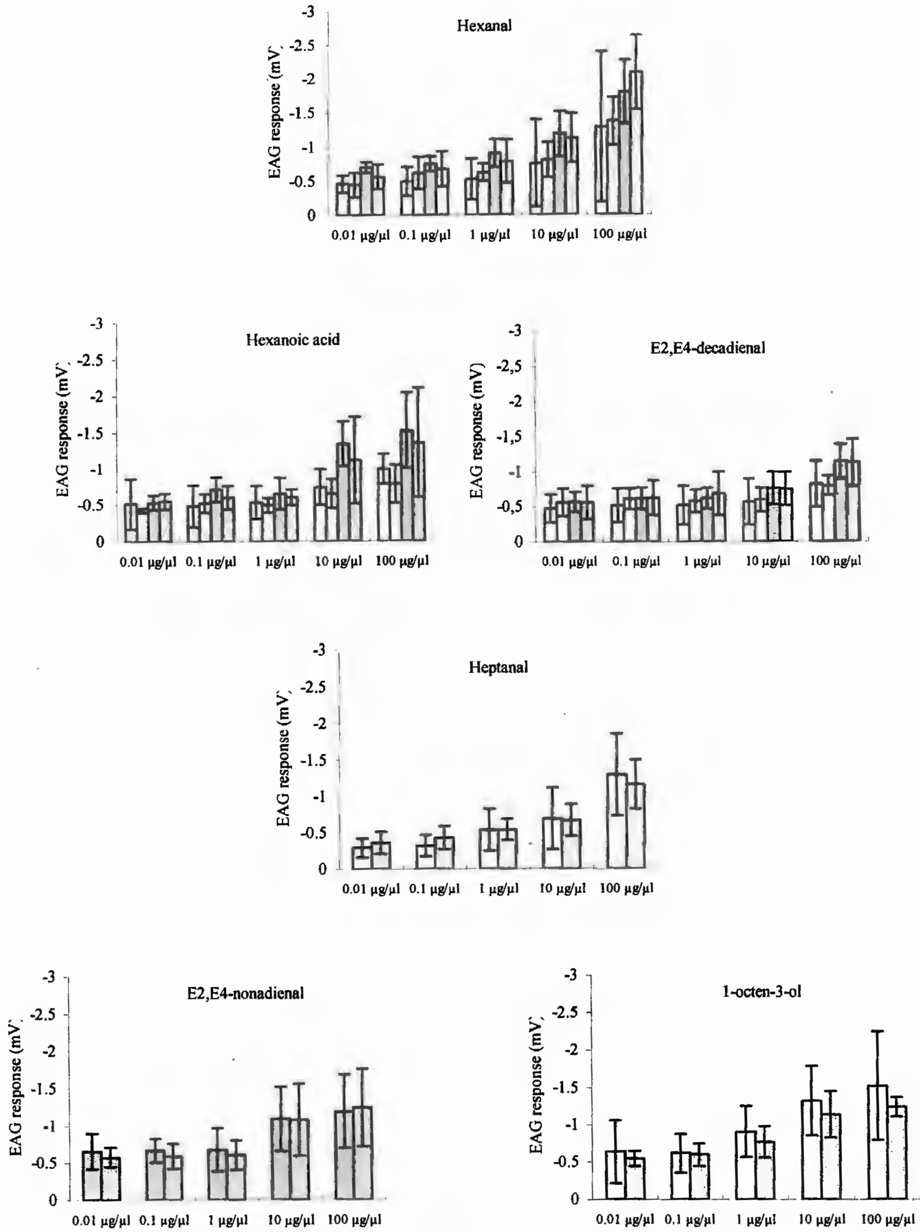

Fig. 1. Mean EAG (mV±SD) dose-response curves of *L. botrana* (n=5) virgin and mated females and males to host plant volatiles and to E7,Z9-dodecadienyl acetate (5 doses, from 0.01 µg/µl to 100 µg/µl in mineral oil). (White bars: virgin females; spotted white bars: mated females; grey bars: virgin males; spotted grey bars: mated males).

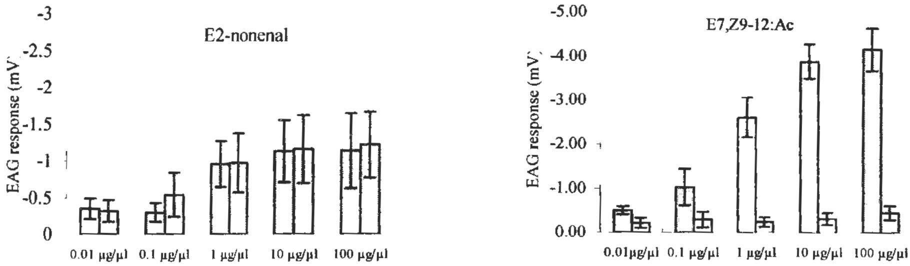

Fig. 1.(continued) Mean EAG (mV±SD) dose-response curves of *L. botrana* (n=5) virgin and mated females and males to host plant volatiles and to E7,Z9-dodecadienyl acetate (5 doses, from 0.01 µg/µl to 100 µg/µl in mineral oil). (White bars: virgin females; spotted white bars: mated females; grey bars: virgin males; spotted grey bars: mated males).

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Allelochemical effect of the fruit trees, *Ziziphus spina christi*, on the searching behaviour of the parasitoid *Bracon hebetor* Say. (Hym., Braconidae)

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Abstract: The genus Ziziphus (fam., Rhamnaceae) includes a wide variety of evergreen trees and shrubs in the tropical and subtropical regions of the world. The Ziziphus plant is one of the most important species because of its edible fruit and medicinal uses. Moreover, the present study shows that the plant extracts, especially the flower-extracts, modify the searching behaviour of the ectoparasitoid Bracon hebetor. The braconid parasitoid responded to hexane-; ethanol-; and acetone-extracts of different parts of the Ziziphus spina christi trees (leaves, fruits and flowers). In a T-multiple choice chamber, it is clear that the flower-extracts contain most modifying-behaviour agents in compare to the other extracts and induce the following: (1) increase significantly the number of visiting of the parasitoids during the time of experiment. (2) increase significantly the number of arrested parasitoids at the end of experiment. (3) The acetone-extract contains most active allelochemicals of the flowers in compare to the other solvents and increases significantly the parasitization rate of the female parasitoids.

Key words: plant allelochemicals, *Ziziphus spina christi*, searching behaviour, ectoparasitoid, *Bracon hebetor*.

Introduction

The *Ziziphus* plants (fam. Rhamnaceae), mostly trees and shrubs, are cultivated in subtropical and tropical regions, especially in Egypt. The *Ziziphus* plants have several physiological and morphological characteristics that contribute to their ability to adapt to arid environments. They can be used for a variety of purposes in arid regions such as food, fuel, fodder, fertilizer, building materials and medical herbs (Boulos, 1983; Arndt *et al.*, 2001).

Chemicals that induce allelopathic influences between plants and other organisms are called allelochemicals. Many plants produce allelochemical compounds that directly or indirectly impact their biological environment and have ecological function (Farmer, 2001). The composition of the allelochemical signals often differs with different plants (Steinberg *et al.*, 1993). They can be pollinators, attractants, or serve as chemical defenses against microorganisms, insect pests and even other plants (Balandrin *et al.*, 1985). Natural enemies (predators and parasitoids) learn to respond to the different blends of allelochemicals that mediate the location of their hosts and prey (Tumlinson *et al.*, 1993; Shonouda & Nasr, 1998). These allelochemicals will be emanated from its host or from host plants. The parasitoid *Bracon hebetor* is one of the most important larval ectoparasitoid of many lepidopterous pests that attaching stored products such as flour, meals, cereals, seeds, dried fruits and bees wax (Farghaly & Ragab, 1985). So, the effect of plant allelochemicals on the searching behaviour of the braconid ectoparasitoid was studied in the laboratory.

Material and methods

Rearing of the parasitoid Bracon hebetor

The parasitoid *B. hebetor* was reared on the flour moth *Ephestia kuehniella* larvae under laboratory conditions at 25 ± 2 ^{0}C and 65 ± 5 % RH (Shonouda & Nasr, 1998). For the biological experiments, the newly emerged parasitoid adults were kept in couples and fed only on honey solution for 24 h.

Biological experiments

The plant extracts were prepared by dissolving each part of the tree as follow: 5 g of leaf powder; 3 g of flower powder in addition to 5 ml fruit juice in 25 ml of different solvent (hexane, ethanol and acetone). The plant extracts were left for 24 h., afterthat, they were filtrated by filter paper. The filtrated plant extracts were kept in the refrigerator for the biological experiments.

The first experiment was done by dropping separately 25 µl of hexane extract of each part of the *Ziziphus* plant on three glass slides and was left for 10 min. to permit complete evaporation of the solvent. Afterthat, each glass slide was put in one chamber of the T-multiple choice chamber. On the other side of chambers, 10 parasitoids were introduced and left for 20 min. During the time of experiment, the number of visiting exhibited by each parasitoid to each chamber was recorded. The second experiment was conducted in the same manner except the number of arrested parasitoids in each chamber was recorded after 1 h. Three replicates were performed for both experiments and for each solvent.

The third experiment was conducted by preparing 2 covered Petri dishes. 25 µl of hexane-flower extract were dropped in the first dish, while 25 µl of the solvent hexane were dropped in the second dish as control. After complete evaporation of the solvent, 2 host larvae were gently put in each Petri dish. One parasitoid female was introduced into each covered dish and was left for 2 h. Ten replicates were performed. At the end of experiment, the parasitization rate, i.e., the number of emerged parasitoid larvae, was recorded. The same experiment was repeated in the same manner but with the flower extracts dissolved in other solvents. Statistical analysis was done by using Student's t-test and analysis of variance (ANOVA) to test the significance of difference between the different values.

Results and discussion

In a T-multiple choice chamber, the parasitoid *Bracon hebetor* was exposed to different hexane-plant extracts. They were visited the hexane-flower extract ($x^\backslash = 8.0$) more than either hexane-leaf extract ($x^\backslash = 3.33$) or hexane-fruit extract ($x^\backslash = 2.0$). The mean number of visiting of parasitoids to the chamber contains hexane-flower extract was significantly higher ($F = 5.46$; $p < 0.05$) in compare to other plant extracts. Also, the parasitoids exposed to ethanol- or acetone-plant extracts were visited the chambers contain flower extracts ($x^\backslash = 11, 11.67$) more than either leaf-extracts or fruit extracts ($x^\backslash = 2.33, 3.33$ and $x^\backslash = 5.0, 3.0$), respectively. The mean number of visiting of parasitoids to ethanol- or acetone flower extract were significantly higher ($F = 12.66, 13.47$; $p < 0.01$) in compare to either leaf-extracts or fruit extracts. It is clear that acetone–flower extract was the most active plant extract that stimulates the parasitoids to increase their number of visiting, (Fig. 1A).

The results also showed that the parasitoids *B. hebetor* were arrested in different chambers contain plant extracts dissolved in different solvents. The parasitoids were arrested in the chambers contain ethanol- or acetone flower extracts (% = 55, 60) more than either leave extracts (% = 15, 25) or fruit extracts (% = 10), respectively. The percentage of arrested parasitoids in chambers contain either ethanol- or acetone-flower extracts were significantly higher ($F = 12.45, 10.83$; $p < 0.01$) in compare to either leaf extracts or fruit extracts. While, there was no significant difference ($F = 3.78$; $p > 0.05$) between the different plant extracts dissolved in hexane, (Fig. 1B). It is clear that the ethanol- and acetone-flower extracts contain the most active ingredients of flowers and the acetone-flower extract was arrested the highest percentage of parasitoids (60 %) in compare to ethanol-flower extract (55 %).

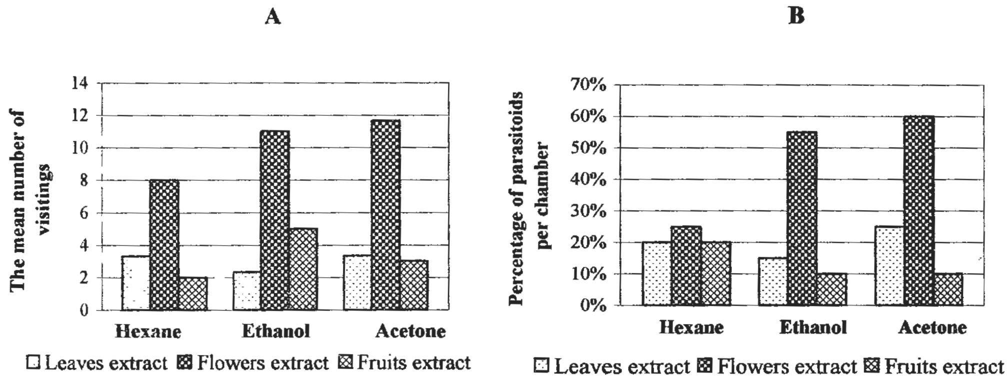

Figure 1. Shows the mean number of visiting (A) and the percentage of arrested parasitoids (B) exhibited by parasitoids in each chamber contains plant extract with different solvents.

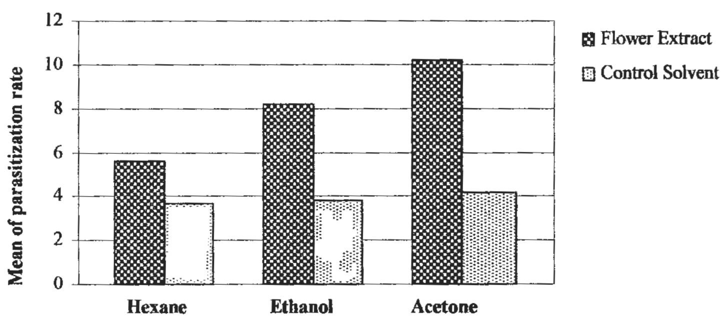

Figure 2. Mean parasitization rate of the parasitoid females under the effect of flower extracts in addition to different solvents as control.

Concerning the third behavioural response, the mean parasitization rate of female parasitoids exposed to hexane-flower extract ($x^{\backslash} = 5.6$) was higher than control ($x^{\backslash} = 3.7$). The difference between means was significantly high ($t = 2.8$; $p < 0.05$). Also, the mean parasitization rate of female parasitoids exposed to ethanol- or acetone-flower extracts ($x^{\backslash} = 8.2, 10.3$) were higher than control ($x^{\backslash} = 3.8, 4.2$). The results showed that there was significant difference between the mean parasitization rate of parasitoids under the effect of ethanol- and acetone-flower extracts ($t = 7.1, 8.5$; $p < 0.001$) in compare to control, (Fig. 2).

Moreover, the aceton-flower extract was induced the parasitoids female to put the highest parasitizatione rate ($x^\backslash = 10.3$).

It could be concluded that plant allelochemicals, especially extracted from flowers, not only increase the rate of parasitism but also arrested large number of parasitoids and increase the èxistence of parasitoids in the treated area by increasing the number of visiting exhibited by parasitoids. The present study confirmed that the flowers of *Ziziphus spina christi* contain allelochemicals that attract natural enemies (Shonouda, 2003). In addition, predators and parasitoids use plant allelochemicals to modify their searching behaviour (Okroy *et al.*, 2001; Hunter, 2002). These plant allelochemicals could be used in integrated pest management by releasing in conjunction with beneficial insects to control different pests. However, further studies are needed to determine and identify the active ingredients that are present in flowers of *Ziziphus* plants.

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ABSTRACTS

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IFP – the state of the art

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A questionnaire survey was sent to Integrated Fruit Production (IFP) representatives in spring 2004 to investigate the extent and quality of IFP in different fruit growing regions of Europe and some other countries (New Zealand, South Africa, South America) and how closely guidelines and practices comply with IOBC guidelines. The aim of this survey was the identification, description and analysis of the IFP programmes/initiatives. Data on IFP and conventional production (area/crops/no. of farm involved) in each IFP region were requested from the representatives. A comparison/evaluation was made of important technical aspects in order to analyse the quality of the different programs. The technical items analysed were inspired by the Checklist used by the IOBC Commission "IP Endorsement & guidelines" for the evaluation of the regional/national guidelines. The intention was to permit comparison of the regional/national guidelines with the IOBC standards. The results of the survey will be reported, the state of the art of IFP in Europe overviewed, and progress since previous survey reviewed.

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Sustainable Winegrowing New Zealand: Technical developments and achievements

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New Zealand's winegrape industry has almost tripled since 1995, with over 16,000 hectares now planted. An integrated fruit production programme for grapes commenced in 1995 as a three-year pilot programme. The programme was reviewed and rebranded Sustainable Winegrowing New Zealand (SWNZ) in 2002. The SWNZ programme has been adopted on over 70% of the planted area, with a national membership of over 350 vineyards. The programme has recently been extended to include certification of wineries, allowing sustainable management of the entire wine production process from vineyard to bottle.
Implementation of the SWNZ programme is monitored using a property self-audit scorecard and the collection and analysis of production input and outcome data. The winery side of the programme also features a self-audit scorecard, with particular emphasis on management of waste streams and energy inputs. Both programmes are subject to independent audit. Membership of both programmes is entirely voluntary and is not supported by any government subsidies.
This paper describes how SWNZ vineyard data is analysed and used to benchmark progress towards the implementation of sustainable practice and also to identify areas where the programme could be further developed. This process is an integral component of all sustainable fruit production programmes in New Zealand. Agrochemical use pattern analysis and use risk assessment must be a component of any IFP system. The agrochemical risk reduction tools developed and implemented for the New Zealand wine industry are described.

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Genetic analysis of plum curculio, a fruit pest of export concern

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Plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), is an endemic pest of pome and stone fruit crops east of the Rocky Mountains in North America. Two morphologically identical strains have been reported: a univoltine northern strain and a multivoltine southern strain. The line dividing these populations runs near the fruit-growing region of western Virginia. Because detection of multivoltine larvae in fruit at harvest can result in the establishment of trade barriers to fruit export from affected regions, definitive identification of the two strains is essential to limit the economic impact of this pest. First generation occurs early in the season; if oviposition occurs while fruit are very small, larvae are usually crushed by the expanding apple fruit. If fruit are sufficiently expanded at the time of oviposition (as may happen late in a first generation or during a second generation) then larvae may successfully complete development in apple.

Randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was used to examine plum curculio collected from several states within the species' range. Of 59 bands that were scored, 21 (35.6%) were informative for distinguishing between univoltine and multivoltine individuals. These genetic analyses of univoltine and multivoltine strains of plum curculio will not only provide the foundation for development of convenient and rapid diagnostic strain assays, but will also provide insights into the reproductive phenology of this weevil at a molecular level. In addition to enhancing our understanding of plum curculio basic biology, a better understanding of reproductive behavior will likely allow for better management of this economically important fruit pest, and address potential or existing trade barriers.

During the course of this study, PCR analysis revealed that plum curculio is infected with *Wolbachia*, an endosymbiotic bacterium. Furthermore, the two strains of plum curculio are infected with *Wolbachia* of differing supergroups; this may account for the degree of reproductive isolation reported in the literature for these geographic strains.

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The ecological infrastructures of the farm to maintain and increase functional biodiversity

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Ecological infrastructures are key elements of the farm to maintain functional biodiversity, but there is a lack of practical information for growers and field technicians. The present state of the art is presented here. The types of ecological infrastructures and their functions are reviewed, with a special emphasis on the IOBC/WPRS list of ecological options. Examples for fruit orchards and vineyards are given. All the information has been compiled in an IOBC publication.

Peach Extrafloral Nectaries Impact Natural Enemies and Biological Control of the Oriental Fruit Moth

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The effects of extrafloral nectaries (EFN) on biological control of the oriental fruit moth [*Grapholita molesta* (Busck)] in peach [*Prunus persica* (L.) Batsch] were investigated in replicated orchard plots during a two-year study. Naturally occurring natural enemy and oriental fruit moth populations were monitored on peach trees with and without EFN over the entire growing season during 2002 and 2003. Rates of oriental fruit moth parasitism were compared for orchards with and without EFN. Data reveal an association between trees providing EFN resources and natural enemy densities, as well as enhanced biological control of the oriental fruit moth, indicating that EFN resources may be crucial for conservation biological control in peach.

Keywords: peach, oriental fruit moth, extrafloral nectaries, conservation biological control

Kaolin particle film as a pesticide in organic fruit production in a cool climate

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Organic fruit production is small in Norway, much due to the many insect pests that cause harm to fruits. In an average organic apple orchard about 25 % of the apples are damaged by various lepidopterious larvae. To increase organic fruit production, there is a need for effective management tools against various insect pests. Kaolin particle film (Surround®) was tested as an insecticide against over wintering larvae of Tortricidae and early hatching larvae of Noctuidae in both apples and plums over two years. One and two applications of kaolin, the first just before green tip and the second just before balloon, were compared in both years. To evaluate the effect of kaolin the damage on leaves as well as the number of larvae was registrated. The effect on apple rust mite (*Aculus schlechtendali*), plum rust mite (*A. fockeui*), European red mite (*Panonychus ulmi*) and various predaceous mites was also evaluated. The results showed that kaolin had an effect on lepidopterous larvae even in a cool climate. Kaolin had an effect on both Tortricids and Noctuids. There was no difference between one and two applications when the precipitation was low, however there was a difference when the precipitation was higher. Kaolin also had an effect on apple rust mite, a reduction of nearly 80 % was found on apple rust mite. No negative effects on predaceous mites were found in these experiments. Thus, kaolin might be an alternative management tool for organic fruit production against early lepidopterous larvae and apple rust mite in a cool climate like Norway.

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Earwigs in stone fruit orchards

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Earwigs feed on a wide range of organisms: fungi, lichens, prey, fruit. They can therefore play a role as a predator, for example on aphids and psylla eggs. But in stone fruit orchards, they can cause severe damage to the fruit as they near maturity. The damage to fruit is caused by feeding, thus allowing disease infection through the open wound leading to rots (caused by Monilia) during storage.

Egg laying takes place twice during the cold months, once in autumn and once at the end of winter. Eggs are laid in a hole at different depths in the earth. The young nymphs stay there under the protection of the female. The four instar stages take between 40 and 50 days. The earwigs move to the trees during the tree's growth period. They come out at night and during the day hide in groups in sheltered spots.

Control measures can be carried out:
Mechanically, with sticky bands on trunks. This efficient method is limited when there are other means of access to the canopy other than the trunks : training posts and wires, tall weeds or branches that touch the ground. In addition the sticky band can become ineffective if it is covered with dust or other debris thrown up during mowing.
Chemically: young nymphs are most sensitive to chemical control. The insect comes out at night which means that treatments applied during the day are not very effective. Effective chemical control is also hindered by the fact that the least sensitive instars are present on the tree: either aged larva or even only adults nearing fruit maturity.

In view of these difficulties, it seemed useful to monitor insect populations in the canopy.
Monitoring was based on the "grouping" phenomenon of earwigs during the day: Refuges were fixed to the trunks (pieces of bamboo measuring 20 cm). These trefuges were checked every fortnight over a year.
The results show that in 2003 the insects were present during the whole growth period, from mid April to the end of November. The insects rapidly spread to the trees in spring, populations remaining stable from mid May, 3 or 4 weeks after moving to the canopy. The insects disappeared totally from the trees in winter (reproduction period), except for a few males.

Taking these biological elements into consideration should improve the efficacy of control measures in the orchard:
Sticky bands should be placed on the trunks before the insects move to the canopy, before mid April (in 2003).
When using chemical control, it would be interesting to check if modifying the application strategy would improve efficacy, knowing that treatments are more effective when applied to the first instar. There is a higher proportion of first instar when trees are first infested. Trials need to be carried out on early application, targeting insects as soon as they spread to the trees for the first 3 to 4 weeks (from mid April to mid May in 2003).

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The impact of Integrated Fruit Production on pest and disease management in New Zealand's apple industry

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Integrated Fruit Production, (IFP), was readily adopted by New Zealand's apple growers between 1996 and 2001, when it became the minimum export standard. The implementation of IFP pest management resulted in a 50% reduction in insecticide use and a 95% reduction in organophosphate (OP) insecticide use. This was achieved thorough the adoption of pest monitoring systems, selective pest management and greater use of biological control. IFP pest management strategies brought significant changes to the status of key pests including leafrollers and mealybugs that had previously accounted for approximately 80% of total insecticide use. Both pests had acquired variable levels of resistance to OP insecticides and by the mid 1990's were the primary reasons for downgrading of export consignments. Insecticide tolerance was also a factor in the elevated pest status of Froggat's apple leafhopper and apple leafcurling midge.

With the development of IFP, an insecticide resistance management strategy based on four classes of selective insecticides was implemented as a component of leafroller control. Selective mealybug control was promoted and this facilitated unexpected biological control. Together with post-harvest disinfestation, the IFP programme substantially reduced the incidence of mealybug infesting fruit. Removal of OP insecticides also increased activity in a number of parasitoid species that contributed to a reduction in the pest status of both leafhoppers and leafcurling midge. In contrast, the removal of OP insecticides increased the pest status of woolly apple aphid and scale insects and control of these has proved challenging despite considerable biological control activity.

The primary disease management objectives were the implementation of resistance management strategies and fungicide compatibility with integrated mite control. While there has been widespread adoption of the strategy, fungicide use has also declined and the incidence of some diseases (e.g. apple black spot) has increased in many crops. Changes in fungicide product use may be linked to the increase of some diseases (e.g. Elsinoe spot) but growers' reliance on disease monitoring technology, and their perception that reduced fungicide use is a an IFP goal, are key factors in the increased incidence of these diseases under IFP management.

This paper discusses the IFP crop protection outcomes, the benefits and difficulties arising from the widespread adoption of IFP in New Zealand apple production. These outcomes: the impact of pesticide changes, enhanced biological control, growers' interpretation of IFP disease management guidelines and less effective disease control are presented.

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Developments in integrated pome fruit production in Portugal

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Since 1993, after the publication of guidelines for integrated pome fruit production (IFP) in Portugal, it is possible to use this method of production. In the last 10 years there is a substantial increased in the area of (IFP) in Portugal. Concerning the development of IFP we discuss the importance of changes in the production system, and results of research and development activities.

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Information Technology and the dissemination of fruit pest management information in Virginia, U.S.A.

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Fruit pest management information has been available in a web based format in Virginia since 1997, maintained in the Department of Entomology at Virginia Tech in Blacksburg (http://www.ento.vt.edu/Fruitfiles/VAFS.html). Information is presented on the biology, identification, monitoring and control of fruit pests of apple, pear, stone fruits, grape and small fruits (strawberry, caneberries and blueberries). Biological information is also presented for the major arthropod predators in eastern U.S. fruit systems. Information on establishing mating disruption programs in orchards (codling moth, oriental fruit moth, peachtree borer and lesser peachtree borer) and vineyards (grape berry moth) is presented in appropriate crop pages. In addition to IPM pages, the "What's Hot" section for each crop delivers recent phenological events, regulatory changes, meeting dates and sites and other timely information. Weather pages contain maximum and minimum temperatures, biofix dates and accumulated degree days for key pests in several fruit regions within the state. Annual visits to the Virginia Fruit Home Page in 2003 totaled 15,168. However, total visits to all component html files within the site are much higher: 732,121. The site continues to be used by both commercial and home fruit producers, reflected by use statistics for pages based on Virginia Tech pest management recommendations.

In order to extend the use of electronically distributed information into orchards and vineyards, the web site has been streamlined for access using personal digital assistants (PDAs). These small handheld computers not only contain much of the biological and control information of the full web site, but allow growers to enter pest trapping data while in the field which is archived on the grower or extension agent's desktop upon synchronization, and is also automatically uploaded to an IPM specialist's computer. This two way communication facilitates interpretation of trapping data for the individual grower, and allows a broader view of trapping trends in the state. Using PDA's synchronized to our site, growers and agents will have in their pockets current pest and predator biological information, sampling information and thresholds, updated chemical control recommendations and related restricted entry and preharvest interval data, and a way to effortlessly archive and share trapping data with specialists.

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Predicting the risk of brown rot in peaches after harvest

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Postharvest diseases in peaches are essentially due to *Monilia* (*Monilia laxa, fructicola, fructigena*). Contamination of the fruits takes place mainly in the orchard. The contamination level of the plot strongly affects the efficiency of the control measures, which are exclusively prophylactic. However, rots do not necessarily manifest themselves in the orchard, and the absence of symptoms is not always a sign that the fruits are healthy: damage may appear after harvest.

It is important for commercial operators to know the risk of post-harvest rot. This allows them to direct the batches towards short-term or long-term post-harvest circuits. To this purpose, we propose a predictive test that may allow an earlier knowledge of the risk of the fruits developing Brown rot – whether sooner or later. The aim is to obtain information as soon as harvest starts.

Principle of the predictive test

It is considered that fruit contamination takes place in the orchard by *Monilia* conidia being deposited on the fruits throughout their life on the tree. The extent of the risk of post-harvest rot depends on:

the quantity of spores (conidia) present on the fruit at the time of harvest

the surface of the ports of entry (wounds, microcracks…)

the wetting conditions of the fruit.

The test we are proposing consists of taking a sample of fruits a week before the first picking and placing it in conditions that are favourable for germination of the conidia (point 3-) in order to get a quick idea of the rot potential (related to points 1- and 2-).

Method

Taking a sample of 30 fruits (2 alveolate trays of 15 each) per homogeneous plot.

Soaking the fruits in water or spraying them abundantly on the tray.

Packing the trays airtight in order to maintain a confined moist atmosphere.

Bringing them to room temperature (22°-26°C) with natural or artificial light 12h/24h

Counting the rotten fruits every 2-3 days, systematically removing fruits infected with *Rhizopus* which might rapidly contaminate the entire tray.

Counting the rotten fruits makes it possible to draw up a curve for rot development.

Results and perspectives

In this experiment, we compared predictive samples with batches from the successive pickings (24 plots in 2000, 11 in 2001, 28 in 2002). The results show that, in most cases (18 out of 24 in 2000, 7 out of 11 in 2001, 19 out of 28 in 2002), rot development in the samples is consistent with that in the batches harvested subsequently. Interpretation of the result must take weather conditions during harvest into account.

This simple method remains to be validated by use in on-farm routines. It seems able to give a reliable indication of the storage potential of peach batches originating from identified plots. We have noticed that the plot is a key factor: sometimes enormous storage differences are observed for the same variety.

Susceptibility citrus leaf miner, *Phyllocnistis citrella* in Punjab, Pakistan, to different insecticides

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Trials were carried out to determine the efficacy of nine different insecticides viz. Match, Fastac, Pirate, Cascade, Aflix, Hostathion, Methyl-Parathion, Azodrin and Systoate against the citrus leaf miner *Phyllocnistis citrella* (Stnt.), a pest in citrus orchards in Punjab, Pakistan. Insecticides were applied at the dose rates of 100 ml, 50 ml, 70 ml, 100 ml, 200 ml, 150 ml, 150 ml, 100 ml and 100 ml per 100 liters of water, respectively. The percentage reduction in leaf infestation caused by citrus leaf miner and the percentage larval mortality were assessed. All the insecticides significantly controlled citrus leaf miner and also proved to be effective in increasing yield.

Preliminary study of the control of the leafhopper *Jacobiasca lybica* using a system with cover crop and mating disruption for *Lobesia botrana*.

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The green leafhopper *Jacobiasca lybica* (de Bergevin) (Hemiptera: Cicadellidae) has been an increasing pest in vineyards in southern Portugal. It might be possible to control this pest through its natural enemies, such as the egg parasitoid *Anagrus atomus* (L.) and the predators *Chrysoperla carnea* (Stephens) and different species of spider. A cover crop system can improve the development of these beneficials, especially when *Lobesia botrana* (Denis & Schiffermüller) is controlled by mating disruption, which doesn't affect the populations of beneficials. The purpose of this preliminary study, in 2004, is to evaluate how the populations of *J. lybica* and its natural enemies evolve in response to two different treatments for the control of *L. botrana*: mating disruption and chemical control, both with a cover crop.

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Effect of groundcover management on apple orchard arthropods

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Effects of different groundcover management (bare-ground, periodically mowed natural grasses, flowering herbs in the alleys) and different insecticide regimes (IPM versus broad spectrum insecticides) on the on the pests and some natural enemies were investigated in an apple orchard in Hungary.
The previously conventionally treated orchard was divided into four parts each of 1 ha:
- Conventionally treated plot, treated with broad spectrum insecticides and with mowed grass in the alleys (CON+Grass)
- IPM plots, treated with selective insecticides
- and with bare ground in the alleys (IPM+Bareground)
- with mowed grass vegetation in the alleys (IPM+Grass)
- with flowering herbs in the alleys (IPM+Flower)

The flowering herbs (with the coverage in the brackets) in the alleys of the IPM+Flower plots were: *Sinapis alba, Fagopyrum esculentum, Lupinus sp.* and *Trifolium pratense* in 2002 and *Sinapis alba, Fagopyrum esculentum, Trifolium repens* and *Phacelia tanacetifolia* in 2003. This plot was overgrown with weeds (*Amaranthus retroflexus, Chenopodium album, Echinochloa crus-galli*) in the second part of the summer in 2003 so had to be mowed to a height of 15cm.

The CON+Grass plot was characterised by higher *Adoxophies reiculana* and *Leucoptera malifoliella* damage compared to the IPM plots in 2003. The number of spiders decreased.

When comparing the plots with different groundcover management practices, we found lower *Adoxophyes reticulana* infestation in the IPM+bareground plot. The IPM+Flower plot had higher woolly aphid infestation, higher *Leucoptera malifoliella* parasitation and a more abundant spider population compared to other two IPM plots.

In case of phytophagous and predatory (*Amblyseius andersoni, Typhlodromus pyri*) spider mites, aphids (*Aphis pomi*) and codling moth we found no clear differences.

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Use of Spray Adjuvants to enhance the performance of BOTRY-Zen® in grapes

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BOTRY-Zen® is a biological control agent based upon the fungal antagonist, *Ulocladium oudemansii* and has been developed for the early season control of *Botrytis cinerea* in grapes. It is applied as a spore suspension through conventional vineyard spray equipment and achieves suppression of *B. cinerea* through competitive colonisation of senescent and necrotic floral debris and aborted fruitlets, thereby reducing *B. cinerea* inoculum potential in grapevine bunches. Good coverage of the target tissues is essential in order to achieve *B. cinerea* suppression, yet grapevine bunches over flowering are surprisingly difficult spray application targets and this could compromise *B. cinerea* control.

A study was undertaken to determine the effects of different adjuvants on the deposition and spray coverage potential of BOTRY-Zen® conidia. These properties were assessed by measuring droplet spreading on immature grape berries and spray coverage on leaves for different adjuvant mixtures. Stability of spores within each mixture was also assessed. Selected adjuvants were further tested to ensure they had no adverse effects on germ tube vigour, tissue colonisation and *B. cinerea* suppression capabilities.

Organosilicone and traditional non-ionic surfactants significantly improved droplet spreading on berries and increased spray coverage on leaves compared to the nil adjuvant treatment. However, these adjuvants had no effect on spore suspension stability. In contrast, a Xanthun gum based adjuvant had no affect on droplet spreading and coverage but greatly improved spore suspension stability.

In a field trial in 2002, combining BOTRY-Zen® with various adjuvant/application volume combinations showed that *Botrytis* disease control improved when an organosilicone spreader was used.

Results from a field study to monitor effects of Spinosad on the natural community in an apple orchard

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Spinosad (NAF-85) (SC, 480g/l) is an insecticide derived naturally from a bacterium through fermentation. It is active against a variety of insect pests, especially caterpillars, and is an interesting candidate to be used in orchards to control e.g. leafrollers and codling moth. Spinosad is known to be rather selective to many predators. According to our own investigations and considering different exposure routes, spinosad, is harmless for *Chrysoperla carnea* and *Coccinella septempunctata.* There exists, however, data that it can harm parasitoids.

The objective of our field study, carried out in 2000 and 2001, was to monitor potential effects of spinosad on the natural community of arthropods on apple trees, when used as insecticide to control the leafroller *Adoxophyes orana.* According to the recommendations of the supplier (Dow AgroSciences) in 2000, the application against the first generation of *A. orana* was split and within one week at the end of June two treatments, each with 100 ml spinosad/ha, took place. In 2001, two treatments per *A. orana* generation were made with 200 ml/ha in June/July and August. As a reference, the product ME 605 Spritzpulver (WP, a.i. 405 g methylparathion/kg) was used (500 ml/ha). The control plot was sprayed with water. Plot size per treatment was 0.2 ha. The apple fauna was assessed with beating samples: five samples per treatment and 25 branches per sample at each sampling date. Beating samples were taken one day before and one day after each treatment, as well as two weeks and four weeks after the last treatment.

Arthropods collected with the beating samples covered a wide range of taxa. However, only few groups were present in constant higher abundances. These were especially Araneae, mites (Anystidae, Oribatei), Dermaptera (*Forficula. auricularia*), Formicidae (*Lasius niger*) and Cicadellidae. In comparison to the water treated control, the abundance of arthropods of most taxa was not or only slightly reduced in the spinosad treatment. In contrast, the toxic compound resulted in clearly reduced numbers of some arthropod groups.

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Effects of neem oil and NeemAzal® on the life-cycle of the vine moth, *Lobesia botrana* (Lepidoptera Tortricidae)

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The vine moth, *Lobesia botrana* (Lepidoptera Tortricidae) is one of the most important pests of grape in Southern and Central Europe, North Africa and several areas of Asia. Damage can be important: the caterpillars attack ripe fruits and various moulds, in particular *Botrytis*, can develop very rapidly on the wounds; the attacked fruits turn brown at the place of attack and rot. The presence of larvae and rotten fruits lowers the quality of the crop; moulds render wine making difficult.

The purpose of this work was to evaluate the effects of neem oil and of an Azadirachtin based compound (NeemAzal®) on the life-cycle of *L. botrana*. In particular, we evaluated the effects of proposed compounds on the larval development and longevity, and on adult fertility and fecundity.

Larvae were obtained from untreated eggs and fed on grapes treated with neem oil or with a 50.000 ppm NeemAzal® solution in concentrations ranging from 0,25 to 3%. Couples of eggs were put in small plastic containers with 10 grapes and allowed to develop in a climatic chamber (24°C 8/16 D/L cycle) for 25 days (the average development period for *L. botrana* larvae). Containers were inspected every 24h during the first two days, to monitor egg hatching and first larval penetration into grapes; containers were then inspected every 10 days and treated grapes were renewed concurrently.

Both compounds had a large impact on larval development of the moth: only larvae fed with untreated grapes (control) were able to pupate; all larvae fed on treated substrates showed a marked size reduction, could not complete their development and either died prematurely or remained in the larval state for a long time showing senescence features.

Furthermore, life duration of larvae fed on treated substrates was proportional to the neem oil / NeemAzal® concentration.

Pairs (male and female) of adults obtained from untreated larvae, were put in small plastic containers and allowed to feed on small tampons soaked with a water + sucrose solution to which were added neem oil or a 50 000 ppm NeemAzal® solution in concentrations ranging from 0,25 to 3%. Preliminary tests showed no differences in the oviposition rate between fed and unfed females, and the absence of repellence related to both compounds tested.

Feeding activity as well as the number of eggs laid were recorded daily; fecundity was evaluated indirectly counting unhatched eggs.

Results showed differences between neem oil and NeemAzal® effects: neem oil evidenced effects on fecundity only at higher concentrations and scarce effects on fertility; on the other hand, NeemAzal® elicited a more marked effect on fecundity proportional to concentration, while the effects on fertility were significative only at the higher concentrations.

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Prospects for developing eco-friendly biopesticides from natural plant species in Nigeria

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The antimicrobial potentials of natural plant products were investigated. Aqueous and ethanol extracts from plant materials inhibited spore germination and radial growth of some pathogenic organisms in culture and checked disease incidence and severity in food crops including tomato, potato, yam, cassava, rice, and cowpea. The efficacy or potency of the plant products was affected by the type of plant material or the part of the plant from which it was extracted, extraction solvent and time of application. The reduction in disease incidence and severity was greater when crops were treated with the extracts before spray-inoculating them with pathogens. This result is consistent with the extracts being effective as protectants, inhibiting infection rather than than action as eradicants once the dissease is established. Tested plants were *Ocimum gratissimum, O. sanctum, Azadirachta indica, Xylopia aethiopica, Carica papaya, Cymbopogon citratus, Piper nigrum, Afremomum meleguata* and *Agaratum conyzoidis.*

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Production and marketing of organic hazelnuts: the case of "Tonda Gentile Romana"

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Organic farming can no longer be considered a niche of the agrifood sector. Organic production has now gained widespread attention and acceptance. In the last ten years the number of organic farms and the area of organically cultivated land have dramatically increased and the market share of organic products has shown a similar trend. Hazelnuts have fully experienced this situation and interest in organic hazelnuts is rapidly increasing among producers, processing firms and consumers.

In Italy the cultivation of organic hazelnut has gained a quite relevant dimension, exceeding 3.600 hectares of orchards and reaching a share of more than 5%. More specifically, in the Monti Cimini district, one of the main Italian hazelnut production areas where "Tonda Gentile Romana" is almost exclusively grown, the organic cultivation is increasing continuously thanks to the important role played by local organic hazelnuts processors and traders.

An analysis of the organic hazelnut sector was carried out with particular reference to this district. In this area, which accounts for about 4-5% of the world hazelnut production, the share of organically cultivated land is close to 5%. The analysis focused on different topics: technical and economic aspects at farm level, industry and market situation and prices evolution.

The survey, conducted through data elaboration and interviews, highlights that organic hazelnut production, being similar to conventional hazelnut production, is characterized by peculiar and specific management, processing and marketing.

Particular attention has to be paid to the cultivation technique that requires well-timed interventions with specific fertilizers and different pruning techniques. Different processing methods are needed to satisfy the demand of organic hazelnuts, not only for tracking requirements, but also for the specific products typologies demanded by importers of Northern Europe.

The distribution network of organic hazelnuts differs from conventional ones: usually, all stocks are sold at the beginning of the campaign through pre-harvest contracts stipulated with regular buyers. This is one of the reason why the price of organic hazelnut appears quite stable and does not follow directly the international trade fluctuations.

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Current status of IFP programs for stone and pome fruits in Oregon, USA

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Efforts have been under way since 1994 to develop grower-supported IFP programs for stone and pome fruits in Oregon. Initially the focus was on developing the infrastructure for these programs including IFP guidelines, certification protocols, and Internet-based information systems. Real-time weather information is now available through an extensive network of automated weather stations. Weather data and predictive models are instantly accessible via the Internet and provide growers with valuable tools to practice information-based pest and disease management. A major aim has been to reduce reliance on organophosphate (OP) and other broad-spectrum pesticides for control of major orchard pests and replace them with selective control tactics. The adoption of more selective control programs has been aided by recent regulatory restrictions of OPs, environmental concerns about OP use and their impact on non-target organisms, especially fish, and the development of OP resistance in key pests such as codling moth. Demonstration orchards and educational projects serve as vehicles to promote the adoption of IFP programs.

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Phenological model of pear psylla *Cacopsylla pyri*

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A phenological model of 1st and 2nd generation pear psylla was developed. The model included a time distributed delay and was based on own observations and literature data of termination of reproductive diapause, oviposition period length and developmental duration of juvenile stages depending on temperature. After calibrating microclimate, the model simulated observations of several years from different orchards of Switzerland and Italy well. The model or simulated output tables can be used to improve timing of monitoring and control measures.

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Toxicity of five fungicides to predatory mites (Acari: Phytoseiidae) in an apple orchard in northern Portugual.

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Five commercial formulations of fungicides used against apple scab, kresoxim-methyl (Stroby ®WG), mancozeb (Mancozan®), dodine (Dodim®), thiofanat-methyl (Tocsin®) and pirimetanyl + fluquinconazole (Vision®), where tested in the field for their toxicity and side-effects on predatory mites (Acari: Phytoseiidae) at three application timings. The experiment was carried out during the summer of 2003. The experimental design was fully randomised, included five replicates for each fungicide and the control plots were treated with water. The fungicides were applied three times at 12 days intervals. Assessments of the phytoseiids were performed two days before the first application, four days after each application and seven days after the last application. The results of toxicity of fungicides to the phytoseiids were calculated with the Henderson-Tilton formula, and classified into four categories corresponding to the Standard for Field Methods of the IOBC Working Group "Pesticides and Beneficial Organisms".
The classical contact scab fungicide mancozeb was confirmed to have a poor selectivity for phytoseiid mites after one, two or three applications, and were harmful, while the remaining kresoxim-methyl, dodine, thiofanat-methyl and pirimetanyl+fluquinconazole, showed a good selectivity and were harmless.

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The production of "green" and "yellow" lists in Integrated Production Guidelines

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The "green" and "yellow" lists of agrochemicals are the most common tools to advise the growers on the control measures to be applied in the Integrated Production programs. However, the practice has led in many cases to the thought that the chemicals included in the green lists can be applied without any limitation. We propose to change the way these lists are produced and that every guideline must contain:
(a) A restrictive list of key pests, key diseases and key weeds that are economically important and require regular control measures in the region concerned.
(b) A list of the most important known antagonist(s) of key pests with supportive importance in each crop. The protection and augmentation of at least 2 antagonists must be mentioned as desirable objective in advanced sustainable production systems.
(c) A list of preventive and highly selective direct control measures to be used in the IP program ("green list").
(d) A list of pesticides to be used with restrictions ("yellow list").

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Adult *Cydia pomonella* L. responses to apple volatiles: sex, mating status and dosage effect

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The codling moth, *Cydia pomonella* L., is a key pest in pome fruit orchards worldwide. Its low tolerance threshold and the endophytic habits of the larvae have traditionally led to a control strategy based on numerous pesticide treatments. The emergence of resistant populations and growing environmental concerns make necessary the use of alternative control strategies. Mating disruption can be an appropriate alternative strategy under some conditions, but it has some drawbacks. The most important of them is the lack of effectiveness of pheromone traps for population monitoring purposes. To solve this problem, attractants other than the pheromone are needed, and host-plant volatiles could be an appropriate source of them.

Several studies have been carried out on host plant-codling moth relationships mediated by chemical cues. Although responses of adults and larvae of *C. pomonella* to host odours have been proved, it is still unknown which are the key compounds responsible for adult attraction to host plants. Actually, the only real success in this area has been achieved with the pear ester, ethyl (E,Z)-2,4-decadienoate, which attracts both males and females in the field, mainly in walnut orchards.

In the present study the electrophysiological (EAG) and behavioural (olfactometer) responses of 2 to 3-day-old adult males and mated and virgin females to a series of apple tree volatiles were evaluated. The volatiles tested had previously been identified by GC-MSD from dynamic headspace collections of apple tree branches in the field. In the tests the stimuli were presented at 3 different dosages, related 100-fold to each other.

The effects of dosage, sex and mating status on the adult *C. pomonella* response to apple tree volatiles are discussed.

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Laboratory and field tests to optimize the attraction of apple fruit moth (*Argyresthia conjugella* Zeller) to volatiles emitted from rowan (*Sorbus aucuparia* L.).

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Apple fruit moth, *Argyresthia conjugella*, can in years with low fruiting in its main host rowan, *Sorbus aucuparia*, cause considerable damage to apple crops. As an alternative control method, volatiles from rowan can be used as attractants in apple crops. An attractive two-component blend of 2-phenyl ethanol and anethol is established. In addition, a field screening of other compounds identified from rowan, revealed several new candidates. In 2004 we will test different combinations of compounds to optimize the attractivity and specificity. Results from GC-EAD, wind tunnel tests and field trials will be presented.

Occurrence of olfactory cells responding to pheromone components and plant volatile compounds in different species of Lepidoptera and Diptera: possible implications for semiochemical applications*

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Sampling by single cell recording (surface contact technique) the antennal olfactory cell responses of different species of Lepidoptera and Diptera, together with cell types stimulated by pheromone (sex, aggregation) components or plant/food volatile compounds, the presence of highly generalist cells sensitive to substances of both categories was found. Insect species in which the occurrence of this cell type was verified as well as chemicals of both categories eliciting electrophysiological responses at low doses are reported in the following table:

Insect species	Pheromone components	Plant or food volatile compounds
Cydia fagiglandana (Zeller)	*E*8*E*10-12:Ac	Chestnut compounds
Cydia splendana (Hübner)	*E*8*E*10-12:Ac	Chestnut compounds
Cydia pomonella (L.)	*E*8*E*10-12:OH	Ethyl (*E*,*Z*)-2,4-decadienoate
Lobesia botrana (Den. et Schiff.)	*E*7*Z*9-12:Ac	Grapevine compounds
Phthorimaea operculella (Zeller)	*E*4*Z*7*Z*10-13:Ac	Potato compounds
Ephestia kuehniella (Zeller)	*Z*9*E*12-14:Ac	Cereal compounds
Bactrocera oleae (Gmelin)	1,7-dioxaspiroundecane	R-(+)-limonene, (-)-β-pinene
Ceratitis capitata (Weidmann)	Ethyl-*E*3-octenoate	R-(+)-limonene, (-)-β-pinene
Musca domestica L.	*Z*9-tricosene	Food compounds
Drosophila melanogaster L.	*Z*11-18:Ac	Ethyl acetate, acetic acid
Drosophila simulans (Sturtevant)	*Z*11-18:Ac	Ethyl acetate, acetic acid

The presence of cells sensitive to the main component of the sex pheromone (*E*,*E*)-8,10-dodecadien-1-ol (*E*8*E*10-12:OH, codlemone) and to the kairomone ethyl (*E*,*Z*)-2,4-decadienoate (Et-2*E*,4*Z*-DD, pear ester) of the Codling moth (*C. pomonella*) (De Cristofaro *et al.*, 2002) is particularly relevant. This finding could explain the pheromonal potency (Light *et al.*, 2001) of Et-2*E*,4*Z*-DD and the recurrent reduction of the codlemone efficiency when mixed to this compound. As a consequence, it is necessary to consider these possible peripheral interactions in odour perception setting up blends of pheromone components and plant volatiles for monitoring or controlling insect pest populations.

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Tactics to use mating disruption of codling moth in pear orchards of Oeste region in Portugal

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Recently, a dissemination project began in 2002 (PO AGRO DE&D n° 30) in order to promote mating-disruption as an area wide management tactic for codling moth control in Oeste region of Portugal. With the use of mating disruption for codling moth in IPM programs, it's possible to reduce the number of insecticides sprayed per year. For several years the results of mating disruption are positive in the control of codling moth, a key pest in pear orchards.
The evaluation of local conditions is essential for the success of mating disruption. In order to define the number of dispensers/ha it is necessary to analysed several factors, namely area of the orchard, pest population in the last year, surroundings and wind action.

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Sex pheromone trapping of *Cossus cossus* (L.) and *Zeuzera pyrina* (L.) (Lepidoptera, Cossidae)

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In Italy, European goat moth (EGM) *Cossus cossus* (L.) and Leopard moth (LM) *Zeuzera pyrina* (L.) (Lepidoptera, Cossidae) can infest orchards as well as other trees. Experiments were conducted, during three years in a plantation of walnut trees (*Juglans regia* L.) for wood production, to evaluate capture efficiency in relation to: 1) different types of traps (funnel with or without different kinds of baffles); 2) position of traps in the tree canopy; 3) effect of multi-baited traps i.e.: EGM sex pheromone dispenser added to LM dispenser in one trap was compared to single-baited sex pheromone traps (standard trap). The number of captured individuals was studied using log-linear analysis and for both species the three-factor interactions were showed. Traps located over the tree canopy were the most efficient for EGM and LM male captures. There was a difference in male captures of both species related to type of trap. The number of males of the two Cossidae species trapped in standard traps was different with respect to multi-baited traps. The standard traps without baffles showed the highest capture rate of *C. cossus* males while the multi-baited with mobile traps with thin baffles showed the highest capture rate of LM males.

Attractive plant volatiles to control the apple fruit moth (*Argyresthia conjugella* Zeller)

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Apple fruit moth, *Argyresthia conjugella* Zeller (Lepidoptera: Argyresthiidae), is the most important insect pest of apples in Fennoscandia. In years when its primary host, rowan (*Sorbus aucuparia* L.), has little or no berries female *A. conjugella* invade apple orchards to lay their eggs. In some years the entire apple crop can be destroyed. Volatiles from apples and rowan have been collected and identified. In GC-EAD tests females responded to several compounds found in both rowan and apple. Some of these compounds were used in field trapping tests during 2002, and a mixture of two compounds trapped significantly more females and males compared to control traps. However, in field test from 2003 the two-compound blend trapped insects too late in the season to prevent egg laying in apples, indicating that the blend was not optimal. Field trapping tests from 2004 with mixtures of the two compounds with several other plant volatiles from rowan, will be discussed in relation to timing of attraction to control *A. conjugella* females.

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The sex pheromone of the apple leaf midge

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The apple leaf midge (*Dasineura mali* Kieffer) is an important pest of apples, especially in the nursery and in young orchards during establishment. The larvae cause the edges of the leaves to curl in characteristic galls, and growth is stunted. Severe attacks in mature orchards can greatly reduce photosynthetic area, adversely affecting fruit size and cropping the following year. The midge, which is believed to have developed resistance to chlorpyrifos and other insecticides, is currently very difficult to control.

Female apple leaf midges produce a sex pheromone that is highly attractive to the male midges. The pheromone was identified and shown to be a novel compound representing a new group of pheromone structures. Volatiles were collected from virgin female midges and analysed by linked GC-EAG. A single response was observed. Collections were then analysed by GC-MS and the component responsible for the EAG response was characterised, even though it was estimated to be produced at only 1.5 picograms per female per hour. The chemical structure of this component was predicted by interpretation of the mass spectra, microchemical reactions and comparison with synthetic standards. The compound was synthesised as the racemate and shown to have identical chromatographic, spectroscopic and electrophysiological properties to the natural pheromone component. It has a single chiral centre and synthesis of the separate enantiomers is in progress.

The racemic pheromone is exceptionally attractive to male apple leaf midges and is effective for pest monitoring. Sticky delta traps baited with 100 micrograms of the pheromone on rubber septa (releasing < 1 ng/h) in lightly infested orchards caught an average of 4037 males over a 10 day period in May 2004. A good relationship between trap catches and midge populations has been demonstrated. The pheromone has an effective range of over 50 m. Work to exploit the pheromone for control of this pest by mating disruption, mass trapping or lure-and-kill approaches is in progress.

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Synthesis from germacrone of (±)-9-methylgermacrene, an active analogue of (S)-9-methylgermacrene-B the sex pheromone of the sandfly, *Lutzomyia longipalpis* from Lapinha, Brazil.

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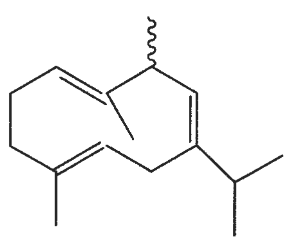
The Phlebotomine sandfly, *Lutzomyia longipalpis* (Lutz & Neiva) (Diptera: Psychodidae) is the vector of the Protozoan parasite *Leishmania chagasi/infantum* (Cunha & Chagas) (Kinetoplastida: Trypanosomatidae) the causative agent of visceral leishmaniasis (VL), a debilitating and often fatal disease of the urban and rural poor in South and Central America. *L. longipalpis* is a complex of at least 4 species. Males of each member of the complex produce a unique sex pheromone from glandular areas underlying the cuticle of tergite 3 or tergites 3 and 4 to attract conspecific females.

Although (S)-9-methylgermacrene-B, the sex pheromone of the most geographically widespread member of the *Lutzomyia longipalpis* species complex has been synthesised by a complex multistep synthesis from methyl (*S*)-3-hydroxy-2-methylpropanoate, it is unstable and readily undergoes acid catalyzed cyclization and a Cope rearrangement to the corresponding elemene structure on heating. The complex synthesis and instability of the compound make it unlikely to be a viable synthetic attractant in possible monitoring or control programmes.

We have synthesised a more stable analogue, (±)-9-methylgermacrene in 4 synthetic steps from germacrone a naturally occurring plant secondary metabolite and have shown in the laboratory that it is as attractive as the sandfly produced and synthetic (S)-9-methylgermacrene-B.



(±)-9-methylgermacrene

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Quantitative method for pheromone delivery in studies of sensory adaptation of moth antennae

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Adaptation of the peripheral nervous system has been proposed as one of several mechanisms of sex pheromone-mediated mating disruption in pest moths. The continual exposure of male moths to synthetic pheromone dispensed from controlled release devices into cropping environments may reduce the sensitivity of the male's antennae to natural pheromone.
A reduction in antennal sensitivity may affect the ability of males to locate sexually receptive females. The responsiveness of moth antennae after exposure to synthetic pheromone has been studied using an electroantennogram. In these studies, male moths have been held in airtight enclosures containing a source of pheromone, or in enclosures containing a source of pheromone with an air exchange system for maintaining a constant concentration of aerial pheromone. A serious limitation of the first method is that pheromone concentration cannot be regulated, and although the second method does permit some degree of regulation of pheromone concentration, accurate estimation of concentration around the antenna is very difficult. The exposure of antennae to precise amounts and ratios of synthetic pheromone compounds can be accomplished using a piezoelectric "pheromone sprayer". This system has been used to deliver pheromone in flight tunnels and field trapping experiments. An aerosol of synthetic pheromone in ethanol is generated at the tip of the sprayer that is inserted into the air delivery tube of the electroantennogram. The method has been successfully used to measure the effect several doses and blends of synthetic pheromone compounds on the responsiveness of the antennae of the obliquebanded leafroller, *Choristoneura rosaceana* (Lepidoptera: Tortricidae), which is not readily controlled using pheromone, and the oriental fruit moth, *Cydia molesta* (Lepidoptera: Tortricidae), which is readily controlled using pheromone.

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Olfactory sensitivity of *Bactrocera oleae* (Gmelin) (Diptera Tephritidae) adults to host-plant semiochemicals and kairomone identification by electrophysiological (EAG, SCR) and field studies*

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Chemical control of the Olive fruit fly [*Bactrocera oleae* (Gmelin) (Diptera Tephritidae)] is particularly difficult. During the last years, studies to set up alternative control methods (i.e. use of semiochemicals) were undertaken. Several researches were carried out on the use of the sex pheromone while only few investigations were focused on the understanding of the biological role of volatile compounds emitted by the host plant and their possible use in modifying Olive fruit fly behaviour. In this paper, the EAG technique was used to determine the olfactory sensitivity of virgin and mated males and females of different ages (1-3; 10-15; 27-32; 57-62; 87-92 days old) to 28 synthetic substances identified in olive leaves and fruits. EAG responses were subjected to ANOVA and cluster analysis. Responses of the different insect categories were compared using *t*-tests (P=0.01; P=0.05). Both sexes, independently of age and physiological state, were able to perceive a wide variety of odours emitted by the olive plant. Considering the mean EAG response to all compounds, the olfactory sensitivity decreases with age advancement in virgin males and females while it is quite constant in mated ones. Virgin insects showed a higher number of EAG response groups than mated ones, tending to decrease with age. In contrast to mated females, a clear reduction of the EAG response groups was observed in mated males. The persistent olfactory sensitivity and selectivity of mated females might be related to the necessity of oviposition site location.
Single cell recordings (surface contact technique) enabled location of cells sensitive to several compounds, particularly terpenes [(+)-α-pinene; (-)-β-pinene; E,E-α-farnesene; (+)-α-copaene; R-(+)-limonene, L-(-)-limonene] on the antennal funiculus. Some cells were sensitive both to the main component of the sex pheromone and terpenes [R-(+)-limonene, (-)-α-pinene]. Some electrophysiologically-active terpenes [(+)-α-pinene, (-)-β-pinene, R-(+)-limonene, L-(-)-limonene)] were able to attract both sexes of *B. oleae* in preliminary wind-tunnel trapping experiments. In an open field study, using sticky tablet traps baited with rubber septa dispensers containing different doses (0.1, 1.0, 10 mg diluted in mineral oil) of a synthetic compound, R-limonene showed a higher catch potency, trapping Olive fruit fly males and females in a 1:1 sex ratio. White traps were less attractant than yellow ones to the Olive fruit fly and, profitably, to parasitoids.
Research focused on practical use of the identified kairomone, alone or mixed with other compounds, for *B. oleae* monitoring and control (mass trapping, lure and kill) techniques, are still in progress.
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Integrating mating disruption and new insecticides in Washington apple orchards, 2001-2003.

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Fifteen apple sites were established throughout Washington in 2001. Each site consisted of an orchard of 8 to 16 hectares that had an identical pest control history. Each orchard was treated with pheromones for control of codling moth (CM), *Cydia pomonella* L. but half of each orchard used conventional insecticides to control pests (COVN program) while the other half used only non-organphosphate insecticides (NO-OP program). Monitoring of selected pests and their natural enemies was conducted using standardized methods. Pest injury to foliage and fruit was documented at selected times throughout the season and just before harvest each year. The insecticides used in each half of the orchards were recorded. A simple economic analysis of the data was performed for the three years of the project.
Both NO-OP and CONV treatment programs were able to maintain injury from CM at acceptable levels in all orchards, even in the face of increasing CM pressure throughout most of the growing areas of Washington. Foliage and fruit injury from leafrollers was lower in the first year in the NO-OP treatment programs but was not different between programs in years 2 adn 3. A noctuid pest, *Lacanoiba subjuncta* (Grote and Robinson), which had become a serious pest in the late 1990s was not a serious problem in either treatment program. The average number of insecticides (including pheromones) and the total cost of insecticides per area (including pheromones) were not statistically different between the two management programs (e.g. NO-OP - $635/ha vs. CONV - $559/ha in 2001; NO-OP $508/ha vs. CONV- $533/ha in 2002; NO-OP $424/ha vs. CONV- $455/ha in 2003). There were no noticeable increase in biological control of aphids, spider mites or leafhoppers from the NO-OP program relative to the CONV program. In part this is probably becuase small areas were treated and because many natural enemies in Washington are tolerant of OP insectiicdes. It was apparent that after three years of comparing these different programs there was no biological or economic differences between them.
This project demonstrated that apples could be produced using a pheromone-based management approach that did not rely on OP insecticides and at no increase in cost to the grower. If even half of the apple orchards in Washington would adopt this management approach OP use could be reduced at least 50%. Along with federal rules that set long reentry intervals for OP insecticides and new state rules on cholinesterase testing for farm workers applying these products it is likely growers asking for pest management programs that eliminate the need for these products. This project has helped provide a realistic basis for alternative pest management programs that Washington growers can adopt immediately without increasing the risk of crop losses or expenses.

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Prevention of Mating by *Auto confusion*™: - New Pest Management Technology Using Electrostatic Powders

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Progress made from concept to registration of the Exosect® *Auto Confusion*™ method will be reported (see abstracts of IOBC meeting Erice, Italy, 2002). Pheromone is formulated with an inert carrier in the form of electrostatically chargeable powder (Entostat™). The carrier powder has the ability to adhere strongly to the insect cuticle, placing innumerable pheromone-emitting sources on the antennae and other parts of the body. This technology is therefore radically different from conventional mating disruption methods, which involve permeating the air above the crop. The main advantage being the highly reduced amount of pheromone used, therefore making mating disruption a commercially viable option for farmers.

When presenting this system at the IOBC meeting in 2002, the challenge that lay ahead was to turn our hypotheses of the mechanics of the disruption effect (listed below) into scientific fact. Since then, a BBSRC grant award has enabled our research team to investigate these hypotheses and provide scientific data explaining exactly how the system works, revealing an additional disruption effect that we were not immediately aware of, see VI. Our poster will detail all the steps below.

The advantages of the Exosect® *Auto Confusion*™ method are:

The Entostat™ powder is retained in dispenser systems to which the insects are attracted

The amount of pheromone used is equivalent to the quantity used in conventional monitoring traps.

The number of Exosex™ dispensers used is typically 20 – 30 per hectare. Hence the total amount of pheromone applied per hectare is of the order of milligrams.

The Exosex™ powder has a multiple effect:

Pheromone sources on the body cause habituation of receptors.

The differential distribution of particles can cause an imbalance of sensory input from the antennal receptors.

A contaminated male will form a mobile pheromone dispenser, producing "false" pheromone trails.

Powder transfer will occur between contaminated males and non-contaminated ones,

Courtship between females and contaminated males is inhibited because the males are emitting the wrong chemical signals.

The females that have been delayed in mating become less fecund; therefore the viability of their eggs is reduced.

The Exosex system is a viable method for use in the integrated management of many lepidopteran crop pests. The Exosex system for Codling Moth (*Cydia pomonella*), has recently been approved for use in the USA and the UK. We expect to receive a further Codling Moth registration in South Africa, and 4 more registrations in the U.S.A. should follow by October 2004. We are now moving onto using the Entostat powder carrier system for delivering minute quantities of active ingredients as part of a *lure and kill* system which we have named '*auto-dissemination*' for the control of fruit flies and other important pest insects where an attractant is available, trials have commenced this year for which we have received further grant funding from the BBSRC.

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Effect of Plant Volatiles on the Growth of *Pythium aphanidermatum*, *Fusarium oxysporum* and *Botrytis cinerea* Strains Pathogenic on Kiwi and Grapevine

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Volatile compounds from plants can both inhibit and stimulate fungal growth, spore formation and germination (Wilson *et al.* 1987). It is also known that these volatile compounds can have attractive or repulsive effects on insects.

In European vineyards, infection of grapes by *Botrytis cinerea* is associated with grape berry moth damage (*Lobesia botrana*: Lepidoptera, Tortricidae) (Fermaud & Le Menn, 1992). Mondy *et al.* (1998) studied the interaction between *L. botrana* and *B. cinerea* and demonstrated a mutualistic relationship between these two organisms.

In our work, thirteen volatile compounds, each known to have a role in feeding host-finding behaviour of herbivores, were analyzed for their inhibition on the growth of *Pythium aphanidermatum*, *Fusarium oxysporum* and *B. cinerea* strains pathogenic on kiwi and grapevine.

Linalool, methyl salicylate, 2-phenyl ethanol, E-2-hexenal and 1-octen-3-ol completely inhibited both strains of *B. cinerea.* Only E-2-hexenal inhibited *F. oxysporum* and *P. aphanidermatum* was completely inhibited by ten of the thirteen volatile compounds when applied as neat compounds.

When diluted at a concentration of 5 μmol/μL, only E-2-hexenal had an inhibitory effect and only on *P. aphanidermatum*. None of the thirteen volatiles had an effect when diluted at 0.5 and 0.05 μmol/μL.

The pear ester, ethyl (E, Z)-2,4- decadienoate, showed an intermediate effect but differed significantly from the control. E-β-farnesene and farnesol had no effect on the growth of all fungi.

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The Systematic and Efficient Use of Mating Disruption

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Over 20 years have passed since mating disruption (MD) was introduced. MD has played an important role in Integrated Pest Management (IPM) for pest control of various fruits and crops. The area applied with MD has been increasing steadily. In 2002, the covered area increased to 600,000 ha, mainly in Europe and North America including use in forests. There are two important factors to realize the efficacy of IPM in agriculture.

1. The first factor is the role of natural predators and parasites (natural enemies). The efficacy of MD cannot be expected unless MD is used in an IPM program that is also friendly to beneficial insects.
In Japan, one of the major peach production areas has applied IPM using MD for more than seven years. This area has had no trouble up to now and the total number of sprays has been reduced to less than half. The other area where MD was applied, in addition to a wide range of insecticides, has encountered numerous pest problems caused by the decimation of the natural enemy populations.
In India, farmers have sprayed insecticides more than 13 times against Pink bollworm (*Pectinophora gossypiella*) and American bollworm (*Helicoverpa armigera*). After such a number of sprays, they are still suffering from insect pests. In a MD plot with an IPM system, Pink bollworm and American bollworm have been well controlled by MD and soft insecticides.

2. The second factor is the area-wide use of MD. The area-wide use is the most important factor for the success of MD. In an area wide application of MD, a stable efficacy, a reduction in the amount of pheromone materials used and the practical use of natural enemies can be expected

Sampling methods in orchard trials: A comparison between beating and inventory sampling

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Two methods to assess leaf dwelling arthropods in the tree canopy of orchards, which are used in studies to investigate effects of pesticides on non-target arthropods, were compared: beating sampling and inventory sampling. Both methods were applied at the same time in the same apple orchard in Southern Germany. Each inventory sample comprised the catches from one tree, each beating sample the catches from 20 branches. In order to investigate if both methods are able to detect changes in arthropod abundances in a comparable way, samples were taken before and after a treatment with a toxic compound. Additionally, samples were taken in a water treated control area. Besides the arthropods collected, supplementary information concerning time, manpower and necessary plot sizes needed for the different methods was assessed.

Excluding spot wise occurring arthropods the variability between replicates was comparable for both sampling methods, with few exceptions. The mean number of arthropods in the inventory samples was 3 times higher than in the beating samples in the pre-treatment sampling, and 2 times higher in the post-treatment samples.
Reductions in arthropod abundances after treatment with the toxic compound, were revealed with both methods in a similar order of magnitude for Opiliones (> 80 %), Dermaptera (F. auriculuria) (>90 %), and Heteroptera (>76 %). With regard to Araneae, inventory samples revealed higher reductions than the beating samples. Highly moblile arthropods, like Hymenoptera and Diptera, were difficult to assess with the beating sampling method. For these groups inventory sampling achieved higher portions in sample composition as well as more information concerning changes in abundance.

Beating sampling is considered an adequate method for studies, which demand a continuous and/or flexible sampling schedule in trials where arthropods are relatively abundant at the test site. Compared to inventory sampling beating is a low input method concerning time and manpower. Highly mobile arthropods should be assessed with additional sampling techniques. Inventory sampling in contrast is of preference for test sites with lower abundance of arthropods and when a comprehensive assessment of the species diversity is demanded. Whether with inventory sampling reliable information concerning highly mobile/aerial arthropods can be obtained, needs further investigation.

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A possibility to enhance natural enemies in apple orchards? - Reduced pesticide treatments in an apple orchard at East Malling, U.K.

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Effects of "zero pesticide residue" pest management system (when conventional pesticides are used up to petal fall and after harvest and rely on cultural and biological control methods during summer) on natural enemies and other arthropods were investigated in an experimental apple orchard at East Malling Research, Kent, UK in 2001 and 2002.
The experimental orchard is divided into 12 plots, where 3 different pest management systems were being compared as treatments with 4 replicates: (1.) conventionally-treated system (CONV), with a season-long broad spectrum routine pesticide programme, (2.) zero pesticide residue system (ZERO), where plant protection was applied in pre-blossom and post harvest periods only, (3.) untreated (UNTR). The orchard contains plots of disease susceptible and disease resistant apple varieties but every plot contains the variety Discovery on which the sampling by the beating method was done.
The species with higher abundance among spiders (Araneae) were Araniella juveniles (mainly Araniella opisthographa, Araniella cucurbitina), Theridion sp. juveniles (mainly Theridion varians) Philodromus sp. juveniles (Philodromus cespitum) and Tetragnatha sp. juveniles. The most dominant species among the Heteroptera were Anthocoris nemorum, Orius (Heterorius) vicinus, Atractotomus mali and Heterotoma meriopterum. Among the coleopteran predators the most common species were Adalia bipunctata and Coccinella septempunctata. Forficula auricularia also was abundant in the canopy and, because of its numbers and large body size, could be the most important natural enemy.

Generally the abundance of the different predator groups was 1.5-4 times higher in the UNTR plots than in the CONV plots in both years. The ZERO plots had intermediate abundances, in case of some species closer to the UNTR and in others closer to the CONV plots. The effect of spring and after-harvest insecticide treatments and prey densities on different species is also discussed.

As a conclusion, our results show that "zero pesticide residue" treatments not only result in safer apples, but also can enhance predator arthropod assemblages. Additional investigations should be done to improve this effect.

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Effects of an azadirachtin-based compound on the host-parasitoid interactions between the Mediterranean fruit fly, *Ceratitis capitata* Wied. (Diptera Tephritidae) and the braconid wasp *Opius concolor* Szepl. (Hymenoptera Braconidae)

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Azadirachtin is probably the most well known bioactive secondary metabolite produced by the neem tree, *Azadirachta indica* Juss (Meliaceae). This compound shows high toxicity for phytophagous insects, acting as a feeding deterrent and sterilant, interfering with insect growth regulation, gland neurosecretory activity and chitin synthesis.

Although neem-based products are widely used in least-toxic, sustainable integrated pest control practices, the effects of azadirachtin on beneficial insects, such as parasitoids and predators, are still unclear, and subject of controversial opinions in the scientific community.

In the present work, the host-parasitoid system between the Medfly *Ceratitis capitata* and the oligophagous braconid wasp *Opius concolor* (also parasitizing the olive fruit fly, *Bactrocera oleae*), has been studied to investigate the existance of indirect effects of an azadirachtin-based treatment of the host, on the life-cycle of the parasitoid.

As a first step, we evaluated the fertility, fecundity and longevity of a laboratory strain of *O. concolor* parasitizing medfly larvae developed on azadirachtin treated larval medium. Next, behavioural choice tests were carried out in order to test host preference of *O. concolor* ovipositing females, between treated and untreated medfly larvae. Third, we tested the direct effects of azadirachtin on fertility and longevity of *O. concolor* adults fed with treated medium. Finally, hystological observations were performed on the ovaries of treated *O. concolor* females, to evidence any damage to the reproductive system caused by azadirachtin.

Results showed that the braconid wasp was able to complete its life-cycle normally on treated medfly larvae, and to produce fertile offspring, even when medfly larval medium was treated with high levels of azadirachtin. On the other hand, the fecundity and longevity of *O. concolor* adults directly treated with azadirachtin through the feeding medium, showed a significant reduction, highlighting similar effects to those reported for phytophagous insects, such as *C. capitata*. These results were confirmed by hystological observations of the ovaries of *O. concolor* treated females which showed high levels of degeneration due to azadirachtin.

In behavioural tests, *O. concolor* ovipositing females used significantly less time to find and oviposit into treated medfly larvae, showing that the treatment with azadirachtin does not affect the chemicals involved in host individuation.

This work showed that there are no significant indirect effects of azadirachtin treatment in the host-parasitoid system between *C. capitata* and *O. concolor*. Remarkable effects of azadirachtin on the braconid life-cycle were evidenced only upon direct treatment of the insects through the feeding medium.

In this framework, it is possible to state that field applications of azadirachtin-based pest control strategies appear to be safe for beneficial insects and therefore, to sustain the synergistic use of this bioinsecticide with biological control agents.

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Current issues impacting on organic apple production in New Zealand

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New Zealand's organic apple industry expanded rapidly between 1997 and 2001 but has since stabilised at 9% of orchards and 6% of the export apple crop. Initial growth of the organic apple sector was triggered by improved returns for conversion fruit with the expectation of high margins for organic fruit, and the opportunity that both conversion and organic fruit provided to avoid controlled 'single desk' marketing of the export crop. With the export sector deregulated in 2001, and improved prices for conventional fruit production (Integrated Fruit Production), these drivers were lost and no additional orchards have been converted to organic production since 2001. While prices for organic apples in export markets have remained firm over this period, both productivity and profitability of organic apple production in New Zealand has declined.

Reduced productivity, in some varieties by 50%, has been due largely to sensitivity to organic disease management practices and biennial bearing. Growers' dependence on sulphur-based disease management contributes significantly to lower productivity in some varieties that exhibit relatively high sensitivity to sulphur treatments. The impact of sulphur-based treatments on photosynthesis and productivity has been identified and new, less phytotoxic disease management strategies are being implemented to minimise these impacts. Lime sulphur is widely used by growers for management of crop load but this has significant impacts on fruit quality by increasing the natural levels of fruit russet. Mineral nutrition has only recently been identified as a significant issue in some of our older organic orchards and most growers supplement composts with frequent applications of a variety of foliar nutrients. Organic pest management has been effective with crop volumes exported to high value and quarantine sensitive markets.

Current organic fruit production in New Zealand is still largely dependent on substitution-based practices while the future must surely be based on the integration of sustainable organic production systems. Neither market access, fruit quality or productivity issues have undermined grower confidence in organic fruit production but any erosion of current premiums would see some organic growers questioning the viability and future of organic apple production.

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