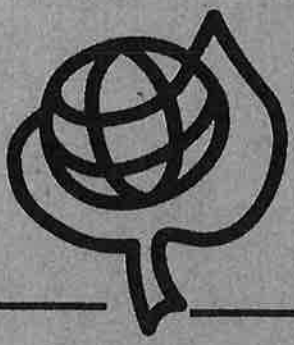

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Breeding for Resistance to Insects and Mites

editor:

Nicholas Birch

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Organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles
West Palaearctic Regional Section / Section Régionale Ouest Paléarctique

IOBC / WPRS

Working Group "Breeding for Plant Resistance to Pests and Diseases"

Proceedings of the meeting

Breeding for Resistance to Insects and Mites

at

Rostanga, Sweden

8-12 December 2001

Editor:

Nicholas Birch

IOBC wprs Bulletin
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Preface

Background to the WG:

The five main areas currently active in our WG are:

1. **Mechanisms of host plant resistance and techniques**. This area is actively researched, for pests of vegetable and forage / oil brassicas, avocado, cucumber, celery and cotton. This is mainly fundamental or basic research underpinning more applied activities. The group interested in resistance to brassica pests (cabbage / turnip root flies, aphids, *Pieris* spp.) is particularly strong and has been actively collaborating for 10+ years, resulting in several high quality joint publications and grants (e.g. British Council / Swiss National Science Foundation, EU).
2. **Sources of resistance, breeding and testing.** This area is more applied, with several examples of commercial exploitation in lettuce, carrot, roses, blackcurrants, raspberries and brassicas. Seed and biotechnology companies are actively involved. Some difficulties in open discussions when company / academic Intellectual Property is involved.
3. **Durability of resistance / pest biotypes.** This area is expanding due to increased interests in transgenic resistance, where particularly high cost investments are involved. Lessons are being learned from conventional breeding (e.g. raspberry aphid, blackcurrant mite, lettuce aphids , apple ahids, Russian wheat aphid). Recent involvement of genetic and mathematical modelling (e.g. Bt-based resistance in cotton, maize, potato).
4. **Collaborative project groups.** After past successes with carrot and lettuce breeding, activities in this area have fallen, probably due to lack of funding and long term nature of the research before returns on investment are achieved. One EU proposal on multiple pest resistance in vegetable brassicas is currently being re-submitted, involving several WG members.
5. **Developing new areas and links**. The WG is particularly keen to attract active participation from biotechnology and plant breeding companies. Links have been made with the IOBC Global WG on transgenic crops and IPM (biosafety, durability, complementarity) and with the IOBC WG on inducible resistance (parallel mechanisms of pest and disease resistance) .

6. **Identifying current knowledge gaps.** These include development of marker-assisted breeding for pest resistance, optimal use of pest resistance genes (conventional and GM) within an IPM framework, new sources of durable resistance genes, biosafety (e.g. non-target effects), consumer acceptance, socio-economic assessment of durable pest resistance (e.g. enabling reduced pesticide usage).

The 9th meeting of the Breeding for Resistance to Insects and Mites Working WG was held in Rostanga, Sweden on 8-12 December 2001. This meeting was attended by 8 plant breeders (both private and public sector) 10 entomologists, 2 chemical ecologists, 2 plant biotechnologists and 1 mathematical modeller. Although attendance was slightly lower than

previous WG workshops time of year, location and reduced availability of travel funding were probably contributing factors) it was very successful in stimulating discussion between these scientific disciplines.

General discussions at the start of the workshop:

We discussed two main options for increasing the size and scope of the WG:

1. The first option is to invite plant pathologists and stored products entomologists to the next WG workshop (planned for 16-19 September 2004 in Poland). Our WG members had mixed views on this option; some preferred a smaller sized WG focussed only on insects and mites whilst others preferred a broader group. Since many of the experimental and breeding approaches are similar for insects, mites and fungal pathogens we concluded that we should encourage the wider participation of stored product entomologists, IPM specialists, chemical ecologists and pathologists in attending our next workshop in 2004. We agreed to alter the name of the WG to "Breeding for Plant Resistance to Pests and Diseases"

2. The second option (deferred until after the 2004 workshop) involves merging our WG with the newer and larger WG on Induced Resistance. Most of our members were opposed to the merger because they felt they would lose some focus and identity. As convenor of the Breeding for Resistance to Insects and Mites WG I proposed to attend at least one of the Induced Resistance WG meetings so we could discuss future options for joint meetings and more interactions between the two Working Groups. The convenor of this WG plans to attend the next meeting of the WG "Induced resistance in plants against insects and diseases" in November 2004, to discuss future interactions of the two current WGs.

- A more recent development in 2002 has been our involvement in the Global IOBC's WG on GM Crop Biosafety (I serve both as a link between WPRS and Global and as a member of the steering committee of the GMO project). This has already resulted in closer links between the two WGs (the common link is resistance to pests – either conventionally bred or GM) and for new opportunities to collaborate between WPRS and Global IOBC organisations. The first workshop in Kenya on ecological impacts of pest-resistant *Bt* maize (November 2002, attended by Nick Birch) was very successful and already a second workshop is being planned in Brazil for 2003.

- The WPRS Breeding for Resistance to Insects and Mites WG encourages increased interaction with other WPRS and Global WG's (e.g. pathology, stored products, induced resistance, GMOs, pheromones and semiochemicals, IPM) and we welcome suggestions and invitations for joint meetings and collaborations.

Please email Dr Nick Birch (N.Birch@scri.sari.ac.uk) with ideas for future interactions and collaborations.

Contents

WG 'Pheromones and other semio-chemicals in integrated production'

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Programme

9th meeting of the IOBC/WPRS working group on Breeding for Resistance to Insects and Mites

Röstånga gästgivaregård, Röstånga, Sweden
8-12 December 2001

Session timetable

Monday 10 December

09.00-09.10 *Opening address*
-Anders Nilsson, Head of Breeding and Research at Svalöf Weibull AB
-Nick Birch, Convenor of the Working Group

09.10-10.30 *Breeding aspects – conventional and molecular approaches*
Industry Aspects on breeding for resistance to insects
-Anders Nilsson
It's not all roses. Matching host plant resistance tests and pest damage observations in (semi-) commercial tests
-Susanne Sütterlin

11.00-12.30 *Efficiency of resistance genes – pest adaptations*
Insect biotype development due to plant host resistance. A literature study
-Aad van Arend
Challenges to durable aphid resistance in raspberry
-Nick Birch

13.30-15.00 *Mechanisms of host plant resistance*
Mechanism of resistance in Mi tomato to potato aphid
-Fred Tjallingii
Triticale resistance factors to grain aphid
-A. Wojcicka, Bogumil Leszczynski and K. Salak-Warzecha
Poster: Plant defense responses to phloem-feeding insects in Nigeria
-Ani Uchenna

15.30-17.00 *Mechanisms of host plant resistance continues*
Resistance to insect oviposition due to chemicals from the plant surface
-Sylvie Derridj
Poster: Apple tree resistance against Cydia pomonella *oviposition*
-Nadia Lombarkia and S. Derridj
Abstract only: Evaluation of okra genotypes for field resistance to the leafhopper
-Lokesh and Ram Singh

Tuesday 11 December

09.00-10.00 *Breeding aspects – conventional and molecular approaches continues*
Corn with Bt resistance developed at Syngenta
-Paul Tenning

10.30-12.00 *Efficiency of resistance genes – tri-trophic interactions*
A tri-trophic model to explore insect community response to the introduction of a pest-resistant GM crop
-Jon Hillier
Written summary report of a publication: Transgenic crops in an agro-ecological context: Multitrophic effects of insect-resistant plants
-Astrid T. Groot and Marcel Dicke

13.00-14.00 *Efficiency of resistance genes – genotype x environmental interactions*
Does plant nutrient content influence the performonce of Macrosiphum euphorbiae*?*
-Johanna Jansson

14.30-16.30 *Future activities of the group*
Project groups
Venue of the next meeting

Breeding for Plant Resistance to Pests and Diseases
IOBC wprs Bulletin Vol. 27 (7) 2004
pp.1 - 3

Resistance-breaking raspberry aphid biotypes: Constraints to sustainable control through plant breeding and Integrated Crop Management.

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Abstract: Breeding red raspberry for resistance to the large raspberry aphid (*Amphorophora idaei)* using single major genes or polygenic minor genes has proved successful in controlling this virus vector aphid for a period of more than thirty years. Currently about 90% of raspberry U.K. plantations, valued at more than £28 million, contain raspberry varieties with one or more of these *A. idaei* resistance genes. However, surveys in 1990-1993 found that more than more than 75% of the U.K. *A. idaei* population consisted of biotypes with the ability to break the most widely used resistance gene, A_1. Since then growers in England (but not yet in Scotland) have reported intermittent breakdown of the formerly strongest resistance gene, A_{10} Genetic analysis, based on rDNA IGS DNA patterns, has shown that *A. idaei* populations in the UK are genetically very variable within and between the 5 known *A. idaei* biotypes. Alate migrations of parthenogenetic females in summer and males in autumn means that resistance-breaking genes are readily exchanged between populations. It is therefore predicted that the A_{10} gene will be overcome throughout the U.K. within the next few years. We have recently found (end of 2001 growing season) *A. idaei* on an A_{10}-containing variety (Glen Rosa) for the first time in Scotland. Small numbers were detected on an experimental field maintained free from pesticides for 10 years at SCRI. Virus incidence is increasing in parallel with breakdown of the aphid resistance genes in U.K. raspberry plantations. Other resistance genes are not readily available within the genus *Rubus*. Anti-aphid genes from other plants (lectins) have been genetically engineered into crops. Initial risk:benefit assessment of one candidate aphid resistance transgene was presented, together with future prospects for introducing other sources of aphid resistance. The compatibility of aphid resistance genes in *Rubus* with the most abundant natural enemies of aphids is currently being studied at SCRI.

Keywords: *Rubus idaeus*, *Amphorophora idaei* biotypes, aphid resistance genes, lectins, natural enemies, risk:benefit assessment, tri-trophic interactions .

Introduction

The large raspberry aphid, *Amphorophora idaei* Börner, is the most important vector in the U.K. and Europe of four viruses causing decline in vigour, yield and fruit quality of red raspberries. Pesticides control aphid numbers but are ineffective in preventing the spread of viruses. For more than 30 years the aphid and associated viruses have been effectively controlled using several genes for resistance to the aphid (Birch *et al.*, 1994; Jones *et al.*, 2000).. The use of resistance genes has inevitably created selection pressure on *A. idaei* populations to overcome specific genes, leading to 5 resistance-breaking aphid biotypes in the U.K. (Birch and Jones 1988; Birch *et al.*, 1994; 1997). This intra-specific genetic diversity is

maintained by sexual reproduction each autumn, with clonal (asexual) expansion of the surviving aphid genotypes during each summer (Birch *et al.*, 1994).

Materials and methods

Methods for screening raspberry cultivars for aphid resistance and for detecting *A. idaei* biotypes are published elsewhere (Birch and Jones, 1988; Jones *et al.*, 2000). Methods for analysis of genetic variability within and between populations of *A. idaei* are published in Birch *et al.*, 1994. Methods for risk:benefit analysis of introduced resistance genes on target pests and on non-target natural enemies of aphids are published in Birch *et al.*, 1999.

Results and discussion

Natural resistance genes from Rubus spp.
Both glasshouse and field-based methods for screening aphid-resistant progeny plants from crosses work well, provided the environmental conditions are suitable (well lit, temperature between 15 and 20 °C) and control plants (susceptible and resistant parent plants) are included. The threshold for scoring resistance is generally very low (0-1 adults, < 3 nymphs, after 10 days). Bioassays and chemical analyses (Birch and Jones, 1988; Shepherd *et al.*, 1999) showed that the chemical factor(s) in A_1 and A_{10}-based resistance were complex and located on the leaf surface, causing aphids to reject plants within 24 hours, after initial landing and probing. Aphid biotypes which could overcome A_1-based resistance were detected in large numbers (> 70 of samples) in both England and Scotland during the 1980s and 1990s (Birch *et al.*, 1994), whereas the A_{10}-breaking biotype has only been detected more recently in parts of England, but is not yet established in Scotland (Birch *et al.*, 1997). A wide range of aphid genotypes were detected in U.K. populations of *A. idaei* using rDNA IGS markers (Birch *et al.*, 1994), highlighting the capacity for this aphid to readily exchange genes between populations each year during the sexual cycle.

Transgenic resistance genes from other plants (lectins).
Whilst several plant lectins were shown to be effective in reducing aphid populations by up to 50% under contained conditions, this degree of resistance was insufficient on its own, particularly against virus vector species. Bioassays to check the compatibility of aphid resistance based on the snowdrop lectin (GNA), expressed in experimental lines of transgenic potato, showed adverse effects on a beneficial aphid predator species, the 2-spot ladybird *Adalia bipunctata* L. (Birch *et al.*, 1999). Ladybirds fed aphids from GNA-expressing plants were adversely affected in terms of their fecundity (egg fertility and hatch rates) as well as suffering a 50% reduction in female adult ladybird longevity. Thus, tri-trophic biosafety testing (resistant plant, target pest and non-target predators / parasitoids) was shown to be important in the risk:benefit assessment of novel aphid resistance genes. The lectin genes tested to date were considered to be unsuitable candidates for insertion into *Rubus* to protect against *A. idaei*, because of their lack of efficacy and their potential toxicity to non-target organisms, including humans (Birch *et al.*, 1999; Fenton *et al.*, 1999).

Conclusions

Aphid resistance genes have been very successfully deployed in *Rubus idaeus* for more than 30 years in Europe. Not surprisingly, raspberry aphids have counter-adapted over this time and we are now at the point where our last major resistance gene (A_{10}) has been overcome in

much of England and at least one isolated case in Scotland (Birch *et al.*, unpublished data). With the benefit of hindsight we can look back and learn important lessons concerning the choice and deployment of aphid resistance genes. We may still be able to combine some of our existing genes from *Rubus* (e.g. minor gene and major gene-based resistance), particularly with the help of molecular markers. Alternatively, we may find and introduce novel aphid resistance genes from other plant genera or even other organisms, via biotechnological routes. Whichever way we proceed, it is important that we think carefully about risk:benefit ratios and how any new aphid-resistant cultivars fit into the wider view of sustainability and durability within an Integrated Pest Management (IPM) framework. Mathematical models are now also being developed at SCRI as tools to predict the optimal deployment of pest resistance genes over space (fields, regions) and time (seasons).

Acknowledgements

Studies on raspberry aphid biotype interactions with resistance genes and on risk:benefit assessment are funded at SCRI by the Scottish Executive Environment and Rural Affairs Department (SEERAD). Mathematical modelling research on optimal resistance gene deployment is funded by the Department of Environment, Food and Rural Affairs (DEFRA).

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Breeding for Plant Resistance to Pests and Diseases
IOBC wprs Bulletin Vol. 27 (7) 2004
pp.5 - 15

Insect Biotype Development due to Plant Host Resistance. A literature study

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Abstract: After 15 years of breeding, the first lettuce, Lactuca sativa, variety with resistance to the lettuce leaf aphid Nasonovia ribisnigri was released in 1996. Many varieties with this insect resistance are currently available. Due to this fact (selection pressure) and the almost unique relation with the necessary host, the insect may transform into a resistance breaking biotype. Comparable insect-host relations with different resistance genes are accessed for possible similarities to provide the aphid-host relation as a gene-for-gene interaction.
On the other hand, the closest comparable aphid-host relation, aphid resistance to lettuce root aphid Pemphigus bursarius, still survives already for over 40 years. The sequential release of single resistance genes, combined with careful monitoring and testing of insect population biotypes found on different hosts, is the most effective gene deployment strategy for insect resistance in a host. The use of chemicals, refugees crops and the reduction of the alatae phase will slow down the development of the insect towards a resistance breaking biotype.

Keywords: *Lactuca sativa*, lettuce, leaf aphid, *Nasonovia ribisnigri*, host resistance, insect resistance, insect pests, pest resistance, biotypes, greenbug aphid, *Schizaphis graminum*, lettuce root aphid, *Pemphigus bursarius*, resistance strategies.

Introduction

It took lettuce breeders of Leen de Mos, now Nunhems, 15 years of breeding to develop the *Nasonovia ribisnigri* lettuce aphid resistant lettuce variety Dynamite (Van der Arend, 1999). Since the release of this variety in 1999 many more Nasonovia resistant lettuce varieties were introduced. The breeding company Nunhems seeds of Haelen the Netherlands is now selling the Nasonovia resistant Butterhead varieties; Dynamite, Comina, Sylvesta, Fiorella, Caterina, Nun 4000, Nun 4500 and Nun 4501, the Crisphead; Barcelona, Brest and Nun 0021, the Salad Bowl types Smile; Belize and Veredes, the Red Salad Bowl type; Nun 7803, the Batavia; Leny, and the Lollo types Nun 8000, Nun 8001, Nun 8800, Nun 8801. Other companies are also introducing Nasonovia resistant varieties of Crisphead, Butterhead, Salad Bowl and Lollo types.

Lettuce breeding uses mainly the antibiosis test by allowing aphids a choice to feed on plants of different lines. The differences between susceptible and resistant plants are clearly visible because the rate of aphid increase differs. A certain internal characteristics of a resistant plant cause adverse effects on the insects that feed on it. The aphid migrates to an other plant. Sometimes the differences between plants are also visible due to non-tolerance when the plant reacts through growth reaction and reduction. The plant is in that case not able to repair the insect injury so that plant development is reduced as a result of supporting an insect population living on it. With antixenosis the insect avoids the plant because it is an undesirable host due to certain plant characteristics (e.g. surface texture). The success of host plant resistance strategy will be challenged by the occurrence of resistance-breaking biotypes.

Dilemma

What is the chance that the resistance will be broken by the development of a new insect biotype?

Biotype refers here to a population of insects that is capable of damaging plant varieties that are resistant to other populations of the same species.

Insects biotypes in different crops

Greenbug in Sorghum and wheat

Five biotypes of the greenbug aphid, *Schizaphis graminum*, have been identified on wheat and 4 on sorghum since greenbugs were identified as pests of small grains. The biotypes are called B, C, E, F, G, H, I, J, K.

In sorghum the occurrence of resistant-breaking biotypes of this greenbug is challenging the breeding for plant resistances. Biotypes recognised as seriously damaging to sorghum hybrids are C, E, I and K. In 1995, biotype K, which damages sorghum resistant to biotype I, was first identified in a biotype I greenbug colony being reared in the greenhouse. Field samples of greenbugs collected from wheat and sorghum in Kansas and Oklahoma, USA, from 1996 to 1998 were identified as biotypes. These samples indicated that biotype I was the dominant biotype on both crops. Biotype E and K were present in about 21 and 12% of the samples collected from sorghum in 1998, respectively. Studies conducted at several constant temperatures indicated that biotypes C, E, I and K had similar reproductive capacity and survival at 22 and 27 deg C (Kindler, 2001).

When several sources of greenbug resistance were compared differences were noticed. The plant resistance index (PRI), which combined values from the 3 resistance mechanisms, antibiosis, antixenosis and tolerance, was greatest for rye accession PI 240675. (Curvetto, 1998)

Durable resistance to greenbug, *S.graminum*, in wheat is a goal of wheat improvement teams, and one that has been complicated by the regular occurrence of damaging biotypes. Simulation modelling studies suggest that pyramiding resistance genes, i.e. combining more than one resistance gene in a single cultivar or hybrid, may provide more durable resistance than sequential releases of single genes. The theory was examined by three pyramiding resistance genes in wheat and testing the series of greenbug biotypes E, F, G, H and I. It was found that pyramiding provided no additional protection over that conferred by the single resistance genes. Based on this result it was concluded that the sequential release of single resistance genes, combined with careful monitoring of greenbug population biotypes, is the most effective gene deployment strategy for greenbug resistance in wheat (Porter, 2000).
Several aphid and plant measurements (e.g. total number of aphids produced per plant, aphid selection preferences and plant damage ratings) were recorded for each plant entry to reveal the components of resistance (i.e. antibiosis, antixenosis and tolerance) present in several resistance genes in seedlings. Results indicated that different levels of combined resistance components exists. (Webster, 2000)

The dominant theory suggests that the planting of resistant cultivars of cereal grains may drive biotype development in the greenbug aphid. Different native grasses were investigated because they may also have a potentially important role in driving the development, and in harbouring unknown biotypes. Greenbug biotypes F adults cultured on Canada bluegrass produced significantly more nymphs than the other biotypes and inflicted a significantly higher damage rating. These results suggest that native grasses drive the development of

greenbug biotype F. It also suggests that a more detailed survey of other native species would reveal similar results. (Kindler, 1999)

Russian wheat aphid.

Although oviparae of the Russian wheat aphid *Diuraphis noxia* have been identified, males have not been found and there is no evidence that a sexual cycle occurs in the United States. Therefore, North American populations overwinter as parthenogenetic morphs (Kiriac, 1990). It has been shown that Russian wheat aphid *Diuraphis noxia* populations from other parts of the world exhibited considerable biotypic variation. Thus, with the threat of future introductions of more virulent biotypes, the Russian wheat aphid should be periodically monitored for biotypic variation, before and after the deployment of resistant cultivars (Shufran, 1997).

Differences in damage on wheat between Hungarian and South African wheat aphids suggest genetic differences between these populations. The result support the idea that resistant plant germplasm has geographical limits because of variation in agro-ecosystems. (Zsuzsa, 2001)

Hessian fly in wheat

Twenty-three Hessian Fly, *Mayetiola destructor*, populations collected in the south-eastern, midwestern and north-western United States from 1995 to 1999 were evaluated for biotype composition based on response to Hessian fly resistance genes H3, H5, H6 and H7H8 in wheat, *Triticum aestivum*. Biotypes GP, B, D, E, F, G, H, J, K, L, M, N and O were identified and the frequencies per state are revealed. Several populations were also tested against the H13 resistance gene to Hessian fly biotype L and two Purdue wheat lines with unidentified genes for resistance. Continued monitoring of biotype frequency in Hessian fly populations is required for optimal deployment and management of resistance genes in all wheat production areas (Ratcliffe, 2001).

The most practical method of controlling *M.destructor* has been the use of resistant cultivars. In the USA, 27 genes for resistance, designated H1 to H27, which are effective against this pest have been identified in *Triticum* species and *Secale cereale*. Because of the highly specific gene-for-gene relationship between wheat and *M.destructor*, biotypes of the fly have evolved as a result of selection pressure exerted by large scale growing of resistant cultivars. The evolution of new biotypes exerts continued pressure in entomologists and breeders to find and use new sources of resistance. A highly significant correlation was observed with cluster analysis between the genetic and geographic distances among the populations. It provided genetic support for dispersal of the fly from its presumed origin in West Asia to Morocco (Naber, 2000).

Gall midge in Rice.

The gall midge, *Orseolia oryzae*, is a major dipteran pest of rice affecting most rice growing regions in Asia, Southeast Asia and Africa. Chemical and other cultural methods for control of this pest are neither very effective nor environmentally safe. The gall midge problem is further compounded by the fact that there are many biotypes of this insect and new biotypes are continuously evolving. However, resistance to this pest is found in the rice germplasm. Resistance is generally governed by single dominant genes (sometimes allelic), and a number of non-allelic resistance genes that confer resistance to different biotypes have been identified. Genetic studies have revealed that there is a gene-for-gene interaction between the different biotypes of gall midge and the various resistance genes found in rice. PCR-based molecular markers have been developed to speed up the identification process. Similarly, molecular

markers have been developed for two gall midge resistance genes (Gm2 and Gm4t) in rice. (Sardesai, 2001). Biotypes 1, 2 and 5 are avirulent to hosts bearing the Gm2 resistance gene, whereas biotype 4 is virulent on Gm2. Based on the sequence of an identified AFLP marker, that is only specially amplified in biotypes 1, 2, and 5, SCAR primers were designed and used in combination with other developed SCAR primers to distinguish effectively all five biotypes in a multiplex PCR-based assay. (Behura, 2000)

Amplified fragment length polymorphism (AFLP) analysis was used to access the biodiversity of the Asian rice gall midge *O.oryzae*. Larvae and pupae were collected at 15 locations in five Asian countries. AFLP analysis provided insight into the relations and origins of gall midge biotypes. Some biotypes developed through selection others through mutations. (Katiyar, 2000)

Brown Planthopper in rice

The brown planthopper, *Nilaparvata lugens*, is one of the most serious pests of rice. Comparison of the chromosomal locations and reactions to brown planthopper biotypes indicated that the two resistance genes in a highly resistant line B5 that derived its resistance genes from the wild rice, *Oryza officinalis*, are different from at least nine of the ten previously identified brown planthopper resistance genes. (Huang, 2001) The virulence of *N.lugens* to a resistant variety of rice is suggested to be under polygenetic control.

Insect populations have a wide range of genetic variability that maximises their fitness in the presence of genetic diversity of host plants. The wide spread planting of one rice variety (monocrop) that has been common place since the "Green Revolution" has significantly decreased the genetic diversity of rice plants. As a result some rice insect species have overcome the resistance of certain rice varieties. The first brown planthopper resistant variety was released in 1974 by IRRI (Heinrichs, 2001).

Leaf midge on the black currant

At least two biotypes of the black currant leaf midge, *Dasineura tetentsi* are distinguished by the ability to gall and survive on the resistant black currant genotype cultivar Storklas. Larvae of the avirulent strain suffered high mortality or remained in the first instar on the resistant cultivar. (Hellqvist, 2001)

Large raspberry aphid on raspberry

The introduction into commerce of raspberry cultivars with major gene resistance to the large raspberry aphid, *Amphorophora idaei*, an important pest and virus vector on red raspberry in Europe, has been very effective for more than 40 years both in decreasing pest numbers and greatly restricting infection with the viruses it transmits. However, biotypes of the aphid able to overcome these genes have developed in the field in recent years (Jones, 2000). Clear RFLP differences between laboratory reared clones of 3 standard biotypes were found, but analysis of field populations gave more complex RFLP patterns which were not biotype-specific. The results indicate considerable genetic diversity within the common biotypes of this aphid (Birch, 1995).

Grape phylloxera on grapes

Nine phylloxera (*Daktulosphaira vitifoliae* Fitch (*Viteus vitifoliae*)) populations were collected from different grape varieties and locations. Some populations developed much better on one of the 2 used grape rootstocks. Other populations developed much better on the other one. The data was consistent with the concept that these pest biotypes were host-based races (Martinez-Peniche, 1999). Performance bioassays recorded over a 3-day period

indicated that the California biotypes A and B of grape phylloxera exhibit differential host choice. Rootstock AXR#1 was antixenotic to biotype A, and rootstock 5C was antixenotic to both biotypes. Biotype A showed significant preference for Cabernet Sauvignon. Both biotypes were unable to survive or develop on rootstock 5C, suggesting the presence of antibiotic resistance. For both AXR#1 and 5C rootstocks and both phylloxera biotypes A and B, the antibiotic mechanism was considerably stronger than the antixenotic mechanism (Omer, 1999).

Rosy apple aphid on apple

Survey of resistance variability of the resistant apple cultivar Florina to *Dysaphis plantaginea* were carried out in 6 European countries in 1995 and 1998. On more than 54 ha, three sources of breaking-resistance were found on three different sites. The capacity for breaking resistance was confirmed for one population, line M, under controlled conditions. The line M induces susceptible symptoms, tolerance is reduced. The gradient in fecundity fits well with a lower antibiosis effect (Rat-Morris, 1998).

Woolly aphid on apple rootstock

Several apple cultivars have been resistant to woolly apple aphid, *Eriosoma lanigerum* for more than 100 years. Variety Northern Spy, with its single dominant resistance gene *Er*, was used extensively as a parent in breeding programs to obtain resistant apple rootstocks. Several resistance-breaking biotypes have been reported in Australia. South Africa and in the USA (Young, 1982).

White fly in tomato

The host preference of the Q-biotype of the whitefly *Bemisia tabaci* was determined by comparing tomato cultivars bearing the Mi-1.2 gene providing resistance to nematode *Meloidogyne spp.* and to the potato aphid *Macrosiphum euphorbiae* and cultivars not bearing this gene. In a choice assay, *B.tabaci* females laid a significantly lower number of eggs on the cultivars that carried the Mi gene. Q-biotypes were found to produce higher daily infestation rates on most of the tomato cultivars. The Q-biotype infested less Mi plants and more non-Mi plants than the B-biotype. Q-biotype females produced significantly fewer pupas than the B-biotype females on both groups of plants. These results suggest the existence of an antixenosis and antibiosis based resistance to the Q-biotype of *B.tabaci* in Mi-bearing commercial tomato cultivars. (Nombela, 2001)

Acyrthosiphon aphid on Alfalfa

Symptoms of susceptibility to *Acyrthosiphon kondoi* (Sjinji) in previously resistant alfalfa's were observed in 1991 in Oklahoma. The aphids, collected in 1991 and 1992, proved to be much more virulent on resistant cultivars than those collected before 1991 (Zarrabi, 1995).

Potato aphid and 'green' peach aphid on several hosts including lettuce

Different colour biotypes of the potato aphid *Macrosiphum euphorbiae* (Thomas) and 'green' peach aphid *Myzus persicae* are known, feeding on many hosts, p.e. tomato, pepper and lettuce. It is expected that the existing of darker coloured biotypes in colder periods are related to better light-absorption. No differences in level of reproduction were found between the two colour clones of *M.euphorbiae* (Reinink, 1989).

Several clones of *M.persicae* showed very different levels of aggressiveness on lettuce. Differences between lettuce lines in aphid reproduction increased with increasing aggressiveness of the aphid clone, which means that aggressive clones are most effective for

selection purposes. No evidence was found for clone-specific plant genotype reactions, meaning that lines resistant to one clone will also be resistant to other clones of *M.persicae*, although not necessarily at the same level (Reinink, 1989).

Lettuce root aphid.

Pemphigus bursarius, lettuce root aphid, exhibits a host-alternating lifecycle, overwintering as eggs on the primary host plant (poplar) before migrating in summer to the secondary host plant, mainly annual *Compositae* including lettuce. A proportion of the population does not produce return migrants (sexuparae) in the autumn but remains in the soil and overwinter as asexual apterae, even after the annual plants have died in early winter. Both temperature and photoperiod are important in the morph determination. Apterae remaining in the soil in the autumn, overwinter successfully in large numbers and are able to reinfest directly the root systems of newly plant lettuce that is grown in the same field in the following growing season. Overwintered asexual populations also produce alates in July, which are able to colonise other lettuce plants, indicating that they were not sexuparae. Hence *P.bursarius* can avoid the ecological dead-end that would occur through local path extinction. Clones can therefore persist indefinitely as both asexual apterae and alatae without the need to return to the poplar and undergo the sexual phase of the life cycle. (Philips, 1999).

Striking varietal differences in susceptibility to attack by the lettuce root aphid were first found in lettuces grown at Wellesbourne in 1955. Subsequent work has confirmed that several varieties show differences in resistance. Immigrant winged forms of *P.bursarius* showed no preference for colonising any particular variety of lettuce and it seems that resistance to attack results from antibiosis. (Dunn, 1960)

Lettuce leaf aphid

The lettuce leaf aphid *Nasonovia ribisnigri* has a comparable lifecycle as the lettuce root aphid *Pemphigus bursarius*. It has a sexual phase in winter on the primary host *Ribes* (gooseberry, red and white currants) and an asexual phase in summer on the secondary hosts lettuce and chicory and various wild plants, nipplewort (*Lampsana*), hawkweed (*Hieracium*), *Crepis*, other latex *Compositae* and *Scrophularia*. The fundatrix, emerging from winter egg, feed on currant and gooseberry leaves (primary hosts) and by parthenogenesis and viviparity produces foundation colonies from which, in May and June, appear the winged aphids which migrate to the *Compositae* (secoodary hosts). The aphids then establish colonies, comprising individuals from several successive generations, wingless or winged forms, which colonise neighbouring plants. In autumn, the sexuparous individuals appear, male and female, which migrate back to the primary hosts. Each mated female lays a winter egg on the primary host. In warm regions, overwintering may also occur on the secondary host (http://www.inra.fr).

Resistance, governed by the same single dominant gene Nr, to this aphid was found in 1978 in several *Lactuca virosa* accessions. Different coloured biotype are known but no clone- specific plant genotype reactions are to be expected (Reinink, 1989).

Results

Table 1. Biotype developments in several insect-host relations.

Insect	Name	Host	# Resistance Genes/type	# Breaking biotypes
Greenbug aphid	*Schizaphis graminum*	Wheat & Sorgum	Many/dominant	Many
Russian wheat aphid	*Diuraphis noxia*	Wheat	Several/	Several
Hessian fly	*Mayetiola destructor*	Wheat	27/divers	Many
Gall midge	*Orseolia oryzae*	Rice	5/divers	5
Brown planthopper	*Nilaparvata lugens*	Rice	10/divers	Many
Leaf midge	*Dasineura tetentsi*	Black current	1/dominant	1
Large raspberry aphid	*Amphorophora idaei*	Raspberry	3/dominant	2
Phylloxera	*Daktulospaira vitifoliae*	Grapes	2	Several
Rosy apple aphid	*Dysaphis plantaginea*	Apple	1/dominant	1
Woolly apple aphid	*Eriosoma lanigerum*	Apple	1/dominant	Several
White fly	*Bemisia tabaci*	Tomato	1/dominant	1
Acyrthosiphon aphid	*Acyrthosiphon kondoi*	Alfalfa	1/dominant ca partial	1
Potato aphid	*Macrosiphum euphorbiae*	Lettuce (others)	Partial	Several
Green peach aphid	*Myzus persicae*	Lettuce (others)	Partial	Several
Lettuce root aphid	*Pemphigus bursarius*	Lettuce	>2/dominant	0
Lettuce leaf aphid	*Nasonovia ribisnigri*	Lettuce	1/dominant	0

Conclusions

General

- 15 Insect biotype developments are evaluated in this paper, described by many authors.
- Chemical and other cultural methods for control of the insect pest are neither very effective nor environmentally safe.

Insect population

- Monitoring the insect population biotypes.
- Variation in host plant performance among populations of a phytophagus insect pest is a potential threat to the durability of host plant resistance. Aggressive biotypes may overcome the protective properties of formerly resistant cultivars.
- Insect populations from different parts of the world exhibit considerable biotypic variation.
- Pest biotypes are host-based races.
- Biotypes of the insects are evolving as a result of selection pressure exerted by large scale growing of resistant cultivars
- The wide spread planting of one variety (monocrop) is decreasing the genetic diversity of a crop. As a result some insect species have overcome the resistance of certain varieties.
- Wild susceptible relative plants may also have a potentially important role in driving the development, and in harbouring unknown biotypes.
- Obligatory sexual reproduction limits the development of possible resistant breaking lines.
- Aggressive clones, resulting in increased reproduction, are most effective for selection purposes.

Genetic resistance
- If single genes govern the resistance to different biotypes a gene-for-gene interaction may be active.
- The sequential release of single resistance genes is equally save compared to pyramiding resistance genes.
- The wide spread use of one resistance gene is decreasing the genetic diversity of a host. As a result some insect species will break the resistance gene.
- Resistant plant germplasm has geographical limits because of variation in agro-ecosystems of insect populations.
- Resistance genes that kills the insect are more selective towards a resistance breaking biotype.
- Resistance due to Antibiosis (as in lettuce) will put high pressure on biotype development.
- Resistance due to Antixenosis will put little pressure on biotype development.
- Different levels of combined resistance components exist in different lines.
- Tolerance will put no pressure on biotype development.

Discussion & Strategic proposals

General
- Resistance breaking biotypes are to be expected.
- Chemicals give extra protection next to genetical, cultural, mechanical, biological and seasonal protection.
- Use crop rotation to break the pest life cycle.
- Remove or destruction of plant debris, weeds or other sources of pest infestation.

Insect population
- Monitoring the insect population for biotypic variation, before and after the deployment of resistant cultivars.
- Biotype testing will show variation between the insect populations especially populations collected from wide growing areas.
- Aggressive clones are most effective for selection purposes.
- Monitoring the resistant crops for resistance breaking biotypes.
- Use of susceptible cultivars for at least 20% of the growing area to offer refugees to the main avirulent biotype in the insect population. On these refugees the insect should be left alone or only treated with chemical insecticides.
- Use of multiline cultivars or tolerant cultivars that minimises biotype selection is possible in some crops but not in lettuce.
- Inspect the wild plant relatives of the host for possible new biotypes.
- Reduction of males or females in the mating population.
- Stimulating insect survival through sexual phase without a cloning alatae phase, will be less stimulating for new biotype development.

Genetic resistance
- Genebank testing to find new sources with probably new resistance genes.
- Gene rotating where varieties with different resistance genes are used in different cropping seasons to minimise selection pressure on given resistance genes.
- Geographical deployment by planting varieties with different resistance genes in adjacent cropping areas.

- Use of cultivars with different insect resistance genes.
- Stimulate migration of the insect by using deterrent genes.
- Use of cultivars with different types insect resistance genes.
- Develop horizontal resistance, a type of resistance that is expressed equally against all biotypes by combining several resistance components.
- Use of tolerant varieties. The consumer must tolerate insects in the vegetable plant product. This is not acceptable for lettuce. It may be acceptable in e.g. potatoes.

How to handle *Nasonovia ribisnigri* resistance breaking biotypes.

Nasonovia ribisnigri and *Pemphigus bursarius* can avoid the ecological dead-end that would occur through local path extinction. Clones can indefinitely overwinter as both asexual apterae and alatae without the need to return to the winter host and undergo the sexual phase of the lifecycle.

Immigrant winged forms of *Pemphigus bursarius* and *Nasonovia ribisnigri* show no preference for colonising any particular variety of lettuce and it seems that resistance to attack results from antibiosis after landing on the secondary host.

The leaf aphid *Nasonovia ribisnigri* **depends on lettuce*** to survive and a resistance breakdown can certainly not be ruled out. *N.ribisnigri* has long cycles with **many*** cloning **parthenogenetic*** phases and aphids are not killed by the resistance gene but forced to migrate to susceptible plants (migration is the only diminishing biotype development factor). When **more area*** is grown with Nasonovia resistance lettuce varieties possessing the gene Nr, with **antibiosis*** as the mode of resistance, biotype development is stimulated (*).

Therefore it is to be expected that Nasonovia biotypes will develop (Baenziger, 2001).

Several means should be used to nurse the resistance gene and keep it effective as long as possible.

a) Chemical control. Growers should always use chemicals in a Nasonovia resistant crop twice. The first time when plants start heading and the second time 10 days before harvesting. In this way 2 objectives are reached. 1) Possible new biotypes of Nasonovia are killed and 2) The harvested lettuce head will be clean of aphids. Not using any chemicals means an attack on the endurance of the resistance gene.
b) Monitoring. Attention should be paid to growers that use Nasonovia resistant varieties. Special care should be taken when complaints emerge towards aphids found in a resistant variety. Is Nasonovia the attacking aphid? Is the lettuce variety/plant Nasonovia resistant?
c) Resistance breeding. If a new biotype of *N.ribisnigri* may occur the breeding program has to start searching as soon as possible for a new resistance source (gene).

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A tri-trophic model to explore insect community response to the introduction of a pest-resistant GM crop

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Abstract: Concerns about the environmental impact of pest-resistant GM crops have necessitated novel methods of risk assessment. In addition, accessibility to more powerful computers in recent years has allowed the employment of computationally intensive individual-based modelling methods. At SCRI we have developed a tri-trophic, individual-based, mathematical model with which we can explore the possible impacts of pest-resistant GM crops on their associated arthropod community. Individual insects in the model have common traits but are parameterised differently to allow for different strategies regarding resource foraging and acquisition, dispersal, temperature responses, and reproductive strategies. Different pest-resistant GM crops act in different ways on target and non-target pests (e.g. lectins vs. *Bts*). Such differences at the plant-pest interface and consequences for the pest-natural enemy interface can be incorporated in to the model, to explore their effects on system properties, such as crop yield (efficacy), arthropod diversity (community structure), and sustainability of deployment (pest counter-adaptation).

Key words: individual based mathematical model, insect community structure, sustainability, pest-resistant GM crops, Integrated Crop Management.

Introduction

Over the last few years numerous studies have highlighted the potential for harmful ecological effects which may be associated with the introduction of transgenic insecticidal crops. For example, Birch *et al* 1999, showed that, although aphids (*Myzus persicae*) which consumed GNA potato transformed to express a snowdrop lectin experienced reduced fecundity and a longer pre-reproductive period, the consumption of the GNA potato lead to knock-on effects at the herbivore-predator interface: When 2 spot ladybirds were fed aphids which had consumed this crop, they themselves experienced reduced fecundity, egg hatch, adult lifespan, and longer pre-reproductive period.
Many similar studies have been made on *Bt* crops, e.g. Schuler *et al* 1999, Hilbeck *et al* 1998.

True ecological risk is hard to assess. Lab-based experiments are usually tightly controlled so as to eliminate unwanted variation. In the real ecological world communities can be very complex, consisting of a number of interacting species over numerous trophic levels, and be subject to unmanaged environmental fluctuations (weather, migration events, etc).

Mathematical modelling

Mathematical modelling has often been used in the past as a means of generating hypotheses for lab and field testing, and informing on the potential of ecological risk. Simple (strategic) mathematical models (e.g. Volterra 1926) are amenable to analysis via standard mathematical techniques but can be subject to the same criticisms as may be made of lab-based studies; to

enable analysis the models must necessarily be simple and this often means that such factors as environmental, or spatial variability have to be ignored. Similarly, as the number of components of a model grows the analysis quickly becomes more complicated so such models are often restricted to food webs as opposed to food chains. This raises questions about the robustness of predictions raised from such models.

As an example, a well-known tri-trophic model can be re-analysed to consider the possibility of local extinctions of herbivores and predators in a tri-trophic food chain when parameters concerning food quality in the system were altered, such as is the case for the GNA potato - *Myzus persicae* - 2 spot ladybird system. The model is given below

$$(1)\quad \begin{aligned} \frac{dM}{dt} &= \theta M(1-M/K) - fMA \\ \frac{dA}{dt} &= \phi_A fMA - gAP - \mu_A A \\ \frac{dP}{dt} &= \phi_P gAP - \mu_P P \end{aligned}$$

where M is crop biomass, A is pest biomass, and P is predator biomass, and f, g, ϕ_A, ϕ_P, μ_A, and μ_P are factors relating to energy exchange between trophic levels and respiration.

If food qualities ϕ_A and ϕ_P are defined as conversion coefficients we obtain expressions which inform on the likely local persistence or extinction of a species; namely that the ϕ_A and ϕ_P must be above certain thresholds which are functions of other system parameters.

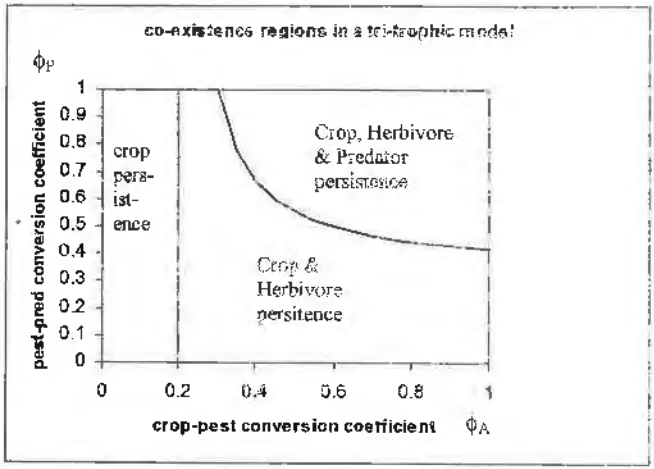

Figure 1: Persistence regions in Model (1).

One of the criticisms levelled at this simple tri-trophic food chain model is that it is a mean-field approximation which ignores the variance at the individual level. Such variance is the main driver for pest adaptation since individuals which can cope better with the insecticidal crop consequently have higher fitness, and will displace the relatively less fit

phenotypes. Classical models such as the above have been modified to give a bi-trophic model for pest adaptation in which the herbivore population consists of 3 subpopulations in terms of food quality (of crop to pest) and respiration/mortality rates (Hillier & Birch 2002).

$$
(2)\quad
\begin{aligned}
\frac{dM}{dt} &= \theta M(1-M/K) - fM(A+B+C)\\
\frac{dA}{dt} &= \phi_A fMA - \mu_A A + \eta\left(\frac{B^2}{16} - \frac{AC}{4}\right)\\
\frac{dB}{dt} &= \phi_B fMB - \mu_B B + \eta\left(-\frac{B^2}{8} + \frac{AC}{2}\right)\\
\frac{dC}{dt} &= \phi_C fMC - \mu_C C + \eta\left(\frac{B^2}{16} - \frac{AC}{4}\right)
\end{aligned}
$$

where A, B, and C represent the different pest phenotypes and η is concerned with the rate of reproduction. Via standard methods for analysing such systems the model yields predictions for the rate of increase of the resistant and partially resistant phenotypes.

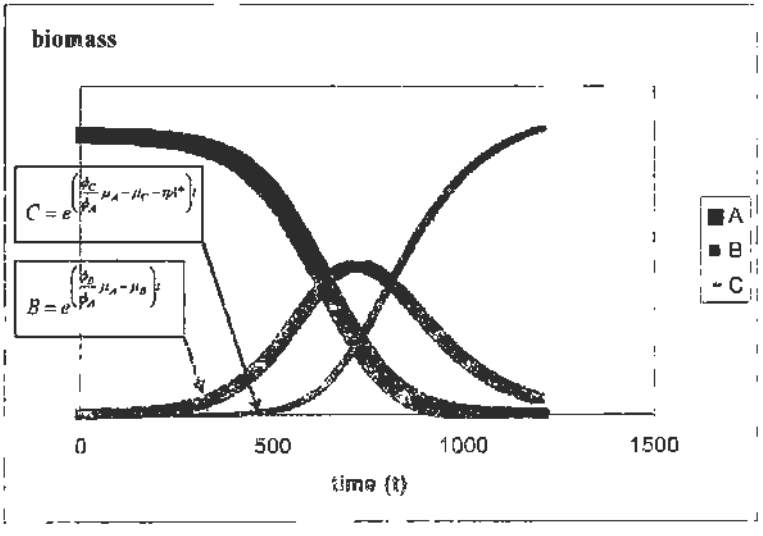

Figure 2: Increase of resistant and partially resistance phenotypes in Model (2).

The robustness of the results of such models is often questioned because of the numerous assumptions that are implied in their formulation. The above models are spatially implicit, and they do not include both a predator level and within species variation. To combine the two models above into tri-trophic model considering pest adaptation would add considerable complexity to the analysis. If we similarly tried to add in extra species, along with explicit

spatial heterogeneity the model would soon become intractable to the standard methods of mathematical analysis.

Since the 1980s, widespread accessibility to high power computing facilities has allowed fast development of the new field of individual (or agent) based modelling (see Grimm 1999 for a recent review of animal and plant models). Recently, workers at SCRI and the University of Abertay Dundee have developed an individual-based physiological competition for models for plants (Pachepsky *et al* 2001, Bown *et al* 2001). Plants in the model were defined in terms of 12 traits which determined their energy allocation and reproductive strategies, and fitness, in terms of persistence in the presence of competition from other individuals was found to be dependent on 3 key parameters determining fecundity, time to reproduction, and mortality rate.

This approach has recently been extended by workers at SCRI and The University of Abertay in Dundee to a tri-trophic model in which individual arthropods are defined in terms of a number of traits concerning resource foraging and acquisition, resource assimilation, dispersal, development, and reproductive strategy.

Discussion

Strategic models can provide baseline conditions which we seek to verify with the more complex individual based models. Indeed, the 2 types of model in tandem provide quite a nice approach: Without the individual-based models we cannot trust the criteria of the above strategic models, but on the other hand, the strategic models are very useful in providing criteria and conditions to test in the individual-based model with regard to certain system properties of interest. In this way the simple models generate hypotheses to be tested with the individual-based model, and by successively including more and more components in the simulations we can see at what point the predictions of the simple models break down. In a similar step, the predictions that are validated or generated by the individual based model can often be tested in experiments by successively relaxing experimental constraints and observing the accuracy of the model predictions.

Acknowledgments

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Breeding for Plant Resistance to Pests and Diseases
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pp.23 - 27

Surface waxes - possible triticale resistance factor to grain aphid

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Abstract: The grain aphid, *S. avenae* considerably less accepted triticale cultivars that are heavy covered with surface waxes. GLC-MS analysis of dichloromethane extracts from flag leaves and ears of waxy and wax-less triticale showed clearly different profile of the surface lipid chemicals. Epicuticular waxes both types of the triticale were comprised of alkanes, aldehydes, fatty acids and esters, instead β-diketones and lauric acid derivatives were present only on surface of the waxy cultivars. Relationship between content and composition of the surface chemicals and acceptance of the studied triticale cultivars by the grain aphid is discussed.

Key words: grain aphid, *Sitobion avenae*, triticale resistance, surface waxes

Introduction

Epicuticular waxes play an important role in the first contact of migrant aphids with colonised host-plants. The surface waxes are responsible for plant texture and colour of the aerial plant organs that can appear "light-green" (nonglaucosness) or "dark-green", "blue-grey" and even "white" (glaucosness) according to nature and content of the epicuticular lipid layer. Glaucosness, due to a superficial deposit of light caused by scattering crystallites of wax, is generally thought to be resulted from amount and chemical composition of the epicuticular waxes. The ubiquitous wax components of the cereals are alkanes, aldehydes, alcohols, acids, esters, β-diketones and hydroxy β-diketones. The glaucousness or non-glaucousness of the cereals is usually strongly related to presence or absence of β-diketones in the surface waxes (Lowe *et. al.*, 1985, Bianchi & Figini, 1986).

It is well documented that stimuli detected by herbivorous insects at the plant surface are important cues for host plant selection, particularly during the first contact with a new host-plant (Städler, 1986; Woodhead & Chapman, 1986). Thus the surface compounds often play an important role as mediators of insect-plant interactions (Eigenbrode *et al.*, 1995; Eigenbrode, 1996, Storer *et al.*, 1996). For the aphids, phytochemicals detected at the plant surface are especially important, since their initial plant selection clearly depends on colour and texture of plants (Niraz *et al.*, 1985, Montlor, 1990, Leszczynski, 1999). In the present paper we report on possible importance of the surface wax chemicals in triticale resistance to the grain aphid, *Sitobion avenae* (Fabricius).

Material and methods

Plant material

Selected winter triticale cultivars: waxy covered (RAH 122), and wax-less (RAH 325) were used in the field experiments. The tested triticale cultivars were obtained from the Institute of Plant Breeding and Acclimatisation, Radzików/Blonie. The experiments were performed on flag leaves and ears of the triticale cultivars.

Field experiments
The field observations were carried out on small experimental plots (0.5 m x 0.5 m), during 1999-2001. Abundance of *S. avenae* on the studied triticale was evaluated according to Wratten *et al.* (1979) and Lykouressis (1984). The number of aphids was counted weakly on ten plants selected at random and the experiments were done in three independent replicates. The field observations were carried out from the beginning of the host plant selection by the grain aphid until the triticale maturity (G.S. 47-80; in Tottman & Broad scale) (Tottman & Broad, 1987). The aphid performance on the studied triticale cultivars was expressed as cumulative aphid index per stem and an average percentage of the infested plants.

Extraction of surface waxes
The surface waxes were extracted from flag leaves and ears of the triticale cultivars, at florescence stage (G.S. 65). Dipping the studied triticale organs into cold dichloromethane for approximately 30 s carried out the extraction. Then the extracts were filtered and treated with BSTFA - PIRYDYNE reagent (v/v 2:3) at temperature 70 ^{0}C for 60 min. The separation mixture was composed of one part of the wax extracts and ten parts of BSTFA-PIRYDYNE reagent.

GC-MS analysis
Chemicals occurred in the surface wax layer of the triticale cultivars were analysed by combined gas chromatography - mass spectrometry (SHIMADZU GC-MS, QP 5050A, equipped with Zebron ZB-5 column (30 m x 0.25 mm). The sample was introduced to the gas chromatograph *via* an injector and the GC separation was performed at temperature - programmed from an initial 280 ^{0}C min to 340 ^{0}C (a rise of the temperature 9. 8 ^{0}C per min). Qualitative analysis of the epicuticular waxes chemicals were done using their mass spectra, which were matched by computer search with the Mass Spectral Library (MS - Windows CLASS - 5000).

Statistics
The differences among the cultivars in tested resistance towards *S. avenae*, were tested with one-way ANOVA, followed by Duncan's test.

Results and discussion

Field observations indicated that waxy-covered triticale (RAH 122 cultivar) significantly affected biology of the grain aphid *S. avenae*. Plants of this cultivar were worse accepted by the grain aphid than plants of the wax-less cultivar RAH 325. The grain aphid was less abundant and formed smaller colony on the waxy cultivar. As a result significant differences in cumulative aphid index value and the average percentage of infested plants of the both studied triticale were found (Tab. 1).

Results of the GC-MS analysis, showed clear differences in profile of the surface chemicals of the waxy and wax-less triticale. Dichloromethane extracts of the surface chemicals from winter triticale cultivar heavy covered with the waxes contained following classes of the chemical constituents: aldehydes, acids, esters and β-diketones. The major difference in the profile of surface chemicals extracted from the wax-less cultivar RAH 325 was absence of the β-diketones in the plant extracts from flag leaves and ears (Tab. 2).

Table 1. Abundance of the grain aphid on waxy and wax-less triticale.

Studied parameters	Year of observations					
	1999		2000		2001	
	RAH 122	RAH 325/95	RAH 122	RAH 325/95	RAH 122	RAH 325/95
Cumulative aphid index	2,67d	7,72bc	4.90cd	11,87ab	3,33d	14,17a
Percentage of infested plants	10,00d	22,22bc	16,11cd	32,78a	13,33cd	27,78ab

The values in the same rows followed by different letters are significantly different at 0.05 (Duncan's test)

Table 2. Major classes of surface waxes compounds extracted from flag leaves and ears of the studied winter triticale.

Wax Components	Flag Leaf		Ear	
	RAH 122	RAH 325	RAH 122	RAH 325
ACIDS	+	+	+	+
ALDEHYDES	+	+	+	+
ESTERS	+	+	+	+
β-DIKETONES	+	-	+	-

+ present; - absent

To our knowledge it is for the first time reported that a winter triticale is completely deprived from β-diketone compounds. Furthermore, such a total inhibition of β-diketones biosynthesis is passed on and maintained in the both studied organs of the winter triticale.

When the individuals were studied some differences in content and presence of the surface chemicals were also found (Tab. 3). For example, the pentacosane ($C_{25}H_{52}$) was found only in the extracts from ears of the RAH 325 cultivar, instead the trimethylsilyl ester of hexacosanoic acid ($C_{29}H_{60}O_2Si$) showed an opposite occurrence. The hentriacontane ($C_{31}H_{64}$) was detected in surface lipids only extracted from flag leaves of the both cultivars. The trimethylsilyl ester of octanoic acid ($C_{11}H_{24}O_2Si$) and trimethylsilyl ester of tetradecanoic acid ($C_{17}H_{36}O_2Si$) were found in extracts of the ears of the both types of the studied triticale, but only in flag leaves of the waxy cultivar.

Table 3. Presence of the identified compounds extracted from surface waxes of the flag leaves and ears of the studied winter triticale.

Surface Wax Compounds	*Flag Leaf*		*Ear*	
	RAH 122	RAH 325	RAH 122	RAH 325
Propanoic acid ($C_3H_6O_2$)	+	+	+	+
Nonanal ($C_9H_{18}O$)	+	+	+	+
Trimethylsilyl ester of octanoic acid ($C_{11}H_{24}O_2Si$)	+	-	+	+
Trimethylsilyl ester of tetradecanoic acid ($C_{17}H_{36}O_2Si$)	+	-	+	+
Trimethylsilyl ester of hexadecanoic acid ($C_{19}H_{40}O_2Si$)	+	+	+	+
Trimethylsilyl ester of octanoic acid ($C_{21}H_{44}O_2Si$)	+	+	+	+
Eicosane-2-methyl ($C_{21}H_{44}$)	+	+	+	+
Trimethylsilyl ester of eicosanoic acid ($C_{23}H_{48}O_2Si$)	+	+	+	+
Tricosane ($C_{23}H_{48}$)	+	+	+	+
Pentacosane ($C_{25}H_{52}$)	-	-	-	+
Trimethylsilyl ester of docosanoic acid ($C_{25}H_{52}O_2Si$)	+	+	+	+
Hexacosane ($C_{26}H_{54}$)	+	+	+	+
Trimethylsilyl ester of tetracosanoic acid ($C_{27}H_{56}O_2Si$)	+	+	+	+
Octacosane ($C_{28}H_{58}$)	+	+	+	+
Trimethylsilyl ester of hexacosanoic acid ($C_{29}H_{60}O_2Si$)	+	+	+	-
Triacontane ($C_{30}H_{62}$)	+	+	+	+
Hentriacontane ($C_{31}H_{64}$)	+	+	-	-
14,16-Hentriacontanedione ($C_{31}H_{60}O_2$)	+	-	+	-
Lauric acid 2-(hexadecyloxy)-3-(octadecyloxy) propyl ester ($C_{49}H_{98}O_4$)	+	-	+	-

+ present; - absent

The most important differences, strictly related to the grain aphid performance on the studied triticale, considered 14,16-hentriacontanedione ($C_{31}H_{60}O_2$) and lauric acid 2-(hexadecyloxy)-3-(octadecyloxy) propyl ester ($C_{49}H_{98}O_4$). These compounds were found only in the surface waxes extracted from the waxy triticale cultivar RAH 122 (Tab. 3). The obtained results suggest that presence of the surface waxes and differences in their chemical composition may well be responsible for triticale resistance to the grain aphid. On the other hand non-glaucosness was suggested as an aphid-resistance character (Thompson, 1963; Starks & Weibel, 1981). Lowe et al. (1985) noticed that wheat resistance to *S. avenae* may be related to epicuticular waxes lacking diketones. Since these compounds strongly absorb ultraviolet light, their absence in the surface lipid layer may result in visual deterrence of the immigrant-winged aphids. However, the results presented here confirm data obtained during our previous study, which showed that the grain aphid preferred light-green wheats, only slightly covered with waxes than dark blue-green cultivars heavy covered with these compounds (Niraz, et al., 1985). Thus the presented results suggest particularly important role of the β-diketones and fatty acid in resistauce of the waxy triticale to the grain aphid. However, further study considered possible role of triticale surface chemicals in resistance to the grain aphid is needed.

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Breeding for Plant Resistance to Pests and Diseases
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Apple tree oviposition resistance against the codling moth, *Cydia pomonella* L. (*Lepidoptera, Tortricidae*) and leaf surface metabolites.

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Abstract: The study of the apple tree cultivar *X65-11* resistant against *Cydia pomonella (C.p)* allowed us to localise the resistance at the oviposition stage of the insect. Several approaches were followed: i: counting eggs on trees in orchards where the insect had the choice between two cultivars X65-11 resistant and *P5R50A4* susceptible ; ii: analysing the effect of metabolites collected on leaf surfaces of the two cultivars on oviposition in artificial conditions. Eggs laid on X65-11 were 5 to 6 times less than on *P5R50A* throughout the first and second moth flights. X65-11leaf surface composition shows lower amounts of metabolites and particularly in fructose, sorbitol and *myo*-inositol which we already demonstrated as influencing site acceptance and stimulating oviposition. In artificial no choice conditions, the activity blends of six metabolites found on both cultivar leaf surfaces reproduced the resistance observed in orchard. This is a new aspect of resistance due to the absence of oviposition stimulants.

Key words: *Cydia pomonella*, oviposition, resistance, apple tree, leaf surface, *X65-11*, *P5R50A4*.

Introduction

Cydia pomonella L. (*C.p.*) is the most important pest occurring in apple orchards. Intensive stand treatments with insecticides induced insect resistance (Sauphanor & Delorme, 1996), kill beneficial insects and increase the amount of pesticides in the environment. Biological control tactics are effective only on low populations (Deschanel & Florac, 1996; Baudry & *al*., 1996). An alternate integrated insect management procedure could be developed through the use of pest resistant cultivars.

We already demonstrated that there are water-soluble metabolites (soluble carbohydrates: glucose, fructose, sucrose and sugar alcools: sorbitol, quebrachitol, *myo*-inositol) on the leave and fruit surfaces of the apple tree. The majority of eggs are laid on the spur leaves near the fruits. Fructose, sorbitol and *myo*-inositol influence *C.p.* site acceptance and oviposition (Lombarkia & Derridj 2002). Our aim in this study is to look at an apple cultivar X65-11 bred for *C.p.* resistance and evaluate the incidence on *C.p.* oviposition and the relationships with the leaf surface metabolites already known.

Material and methods

Oviposition resistance in orchard

Study was conducted in 2000 in an unsprayed apple tree orchard at Gotheron (France) where *C. p.* populations are high. We selected three standard *P5R50A4* trees and two *X65-11* grown consecutively within the same row. Trees were respectively 17 and 10 years old and 3-4 m

high. Live and hatched eggs were recorded at the maximum of 1st and 2nd moth oviposition periods. 51 branches (30 to 50 cm long) were sampled per tree at each oviposition period

Leaf surface spraying for collect of metabolites and analyse

Metabolites were collected on both cultivars in an orchard in Angers (France) where *C. p.* populations were low and leaves rather intact. During the oviposition periods at twilight, collecting process consisted in spraying ultra-pure water on the lower spur leaf sides where there was the highest number of eggs. 4 replicates per cultivar consisting each of all leaves of one spur. The collect was performed immediately after cutting. Leaf surfaces were sprayed at 10 cm distance with a flow of nitrogen gas, and pressure of 17-L min^{-1}. Collection of internal leaf fluid was avoided by sealing the wounded leaf part in liquid parafilm (Fiala *& al* , 1990). Collection was followed by filtration of the samples through a 0.25µm filter to remove epiphytic micro-organisms. Chemical analysis of metabolites were carried out on silylated derivated products by gas chromatography coupled to the flame ionization detector (FID) Delsi Nermag D N 200 apparatus.

Bioassay

Glucose, fructose, sucrose, sorbitol, quebrachitol and *myo*-inositol, mixed as blends representing respectively X65-11 and P5R50A4 leaf surface composition were tested for *C. p.* oviposition.

The codling moths used in the bioassays came from an INRA mass rearing in Magneraud (France) kept for 6 years and infused every year with wild insects. The pupae were put up in transparent plastic Perspex cage (47 x 27 x 27 cm) for emergence in the same conditions as above. Preparation of insects and bioassays were carried on in a climatic chamber under a photoperiod of L 16: D 8, at 80 ± 10% r.h. and 23 ± 2°C. 24 h after emergence two males and a single female were transferred to a cylindrical plastic box (10 cm of diameter and 8 cm high) for mating. Females, which had been laying eggs in these boxes for two days, were used in the oviposition bioassays. Oviposition responses of gravid females were examined in no-choice conditions. Each isolated gravid female (without male) was confined in a small cylindrical cage of 11 cm in diameter and height, which was lined at the top, bottom and wall with dried nylon clothes impregnated with a metabolite blend. 3 replicates of 10 insects were followed on three different days. The impregnated nylon clothes were given to the females one-hour before the start of scotophase. Oviposition was observed after 63 min (60 min of light and 3 min of darkness) of contact with the substrate. On control (nylon cloth impregnated with ultra pure water) 50% of females oviposit after 63 min of contact.

Preparation of solutions and impregnation of artificial substrate

Chemical tests were screened with ultra-pure water solution of both 6 metabolite blends. They came from commercial sources of synthetic SIGMA products (Ultra): sucrose (S 7903), D-sorbitol (S7547), *myo*-inositol (I 5125), L-Quebrachitol (Q 3629), anhydrous cell culture test: D (+) glucose G 7021, D (-) fructose (F 0127). Concentrations of the solutions in which the nylon clothes were soaked were calculated to obtain on the nylon surface quantities collected on leaves diluted 100 times.

The oviposition substrate consisted of a white 200 cm square nylon cloth and 0.5µm mesh was soaked in the test solution and then dried horizontaly under the hood at ambient temperature during 30 minutes.

Statistical analysis:
Comparisons of the numbers of eggs laid per female and quantities of metabolites collected on the leaf surfaces on both cultivars were compared by Student t-test. Comparison of the percentages of females laying eggs in the different treatments were compared by the χ^2 test. For both tests the level of significance chosen was 0.05.

Results

Oviposition resistance in orchard
The numbers of eggs in orchard were 5 to 6 times less on *X 65-11* than on *P5R50A4* at both moth oviposition flights (Figure 1 (A) and (B)).

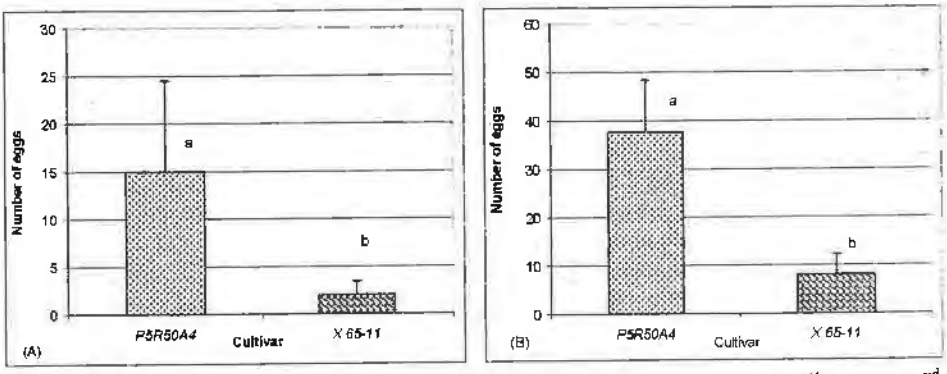

Figure 1. Numbers of *Cydia pomonella* eggs per tree and standard errors, at the 1st (A) and 2nd moth flight (B).

Leaf surface metabolites

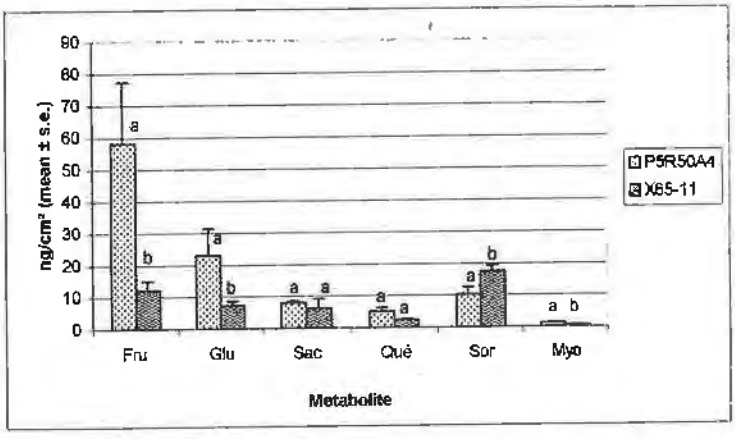

Figure 2: Amounts of metabolites collected on the lower side of spur leaves of the 2 cultivars during the 2nd moth flight. (Fru: Fructose, Glu: Glucose, Sue: Sucrose, Que: Quebrachitol, Sor: Sorbitol, Myo: *Myo*-inositol).

X65-11 shows lower quantities of fructose, glucose and *myo*-inositol than the susceptible cultivar. Fructose and sorbitol are already known as influencing the *C.p.* oviposition.

When the six metabolite blends of each cultivar are reproduced on artificial substrates the number of females ovipositing and the numbers of eggs laid per female ovipositing is smaller on X65-11 blend. (Figure 3). The results are in similar ways as the observations in orchards. These metabolites are representative of the resistance activity against oviposition of *C.p.*.

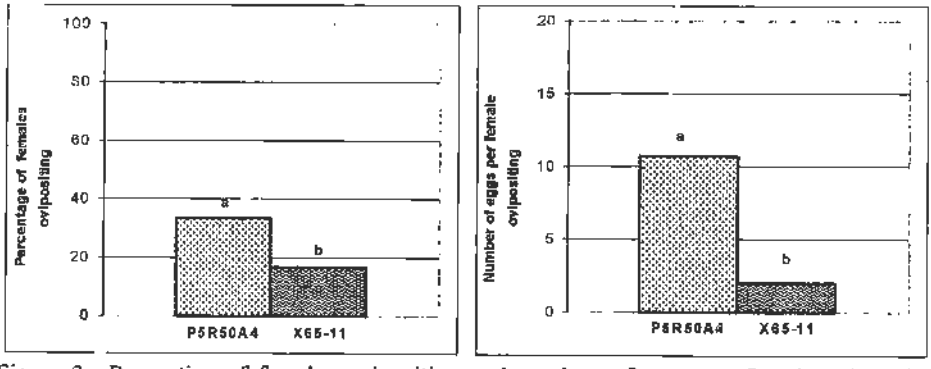

Figure 3 : Proportion of females ovipositing and numbers of eggs per female ovipositing within 63 min on artificial substrates impregnated with six metabolite blends of both cultivar X65-11 and P5R50A4.

Discussion

Similar results with metabolite blend cultivars in artificial conditions and on trees could mean that events which happen before alighting on the plant surface are not very important compared to the stimulation by contact with plant surface. This corroborates the work of Finch & Collier (2000) which show that part of events prior to the oviposition decision by contact stimulation with the plant is rather reduced except the plant colour.

Which is noticeable in these results is that metabolites which are stimulant are in very small quantities (ng/cm^2), and found outside the plant cuticle. They function as kairomones in eliciting an oviposition response of gravid females. Sugar alcohols which stimulate site acceptance and characterise *Rosaceae* explain the relative specificity of *C.p.* for this plant family. Fructose which is in higher concentrations on spur leaves and which stimulate oviposition could explain the site selection within the tree and then the adequacy for the progeny to fetch the fruit on which they feed.

Researches on resistance of apple were generally focused on antibiosis against larvae and little is known about resistance against oviposition. Goonewardene and Howard (1989) reported that *E31-10 = X65-11* (*Malus domestica* Bokh.) was resistant to *C. p.* larvae damage in field and laboratory, to apple scab (*Venturia inaequalis* (cke.) Wint.) and also to European red mite (*Panonychus ulmi*) in greenhouse tests and in the field. The novelties concerning the resistance of X65-11 are: i) It is an antixenosis resistance based on contact with plant surface; ii) Non toxic metabolites are concerned; iii) The resistance is due to the absence of stimuli; iii) The resistance is expressed very clearly at both moth flights. Whatever the efficiency of

this resistance it has to be integrated with other control measures to avoid any selective pressure.

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Evaluation of okra genotypes for field resistance to the leafhopper

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Abstract: Leafhopper *Amrasca biguttula biguttula* (Ishida) (Cicadellidae: Homoptera) is an important pest of okra in India and several other countries. Forty genotypes (advance lines/cultivars resistant to yellow vein mosaic) were evaluated in the field for resistance to the leafhopper on the basis of nymph abundance and leafhooper injury index during 2000 and 2001 seasons. Three resistant (HRB 128-1-1, HRB 105-2-2 (GS) and HRB 118-2-1), two moderately resistant (HRB 108-2-1 and ST2) and two susceptible (HRB 107-4-1 and HRB 121-1-1) genotypes along with standard cultivar (Varsha Uphaar) were identified for elaborate investigations. Resistant genotypes had significantly ($P< 0.05$) fewer nymphs (14.2 to 20.5 nymphs/leaf) compared to susceptible (29.5 to 44.6 nymphs/leaf) genotypes during peak period of leafhopper infestation. Nymph duration was longer (8.2 to 9.8 days) and nymph survival lower (69 to 72%) in resistant genotypes compared to susceptible genotypes which manifested shorter nymph duration (7.1to 7.4 days) and higher nymph survival (86 to 93%). Identified resistant genotypes expressed both antibiosis and antixenosis mechanisms of resistance. Hair density and length, total sugars, tannins, total phenol and potassium contents were higher in resistant genotypes.

Key words: okra, leafhopper, resistance, genotype, phytochemicals

Introduction

The leafhopper *Amrasca biguttula biguttula* (Ishida) is an important pest of okra, Abelmoschus esculentus (L.) Moench in Southeast Asian countries (Atwal, 1976; Hooda et al.,1997). The characteristic symptom of leafhopper attack is phytotoxemia (hopper burn) caused by de-sapping of leaves by nymphs and adults (Uthamasamy, 1985). In severe attacks, plants are stunted and unable to produce flowers and fruits. The use of okra cultivars resistant to attack by leafhopper seems to be a more sustainable control measure. The aim of this study was to identify sources of resistance and factors associated with resistance in advance breeding lines of okra. The selected lines were tolerant to yellow vein mosaic.

Material and methods

Field experiments were conducted during 2000 and 2001 at Hisar, the research farm of CCS Haryana Agricultural University. Forty genotypes were grown in the research farm in randomized block design, replicated thrice with each plot having 3 rows of 2.5 m each. Distance between rows was 45 cm with an interplant distance of 30 cm. Genotypes were sown on 17 July in 2000 and 28 Jnne in 2001. Based on field evaluations, 8 genotypes ranging from resistant to susceptible were retested for elaborate studies in 2001. Sowing was done on 28 June in 5 replicates. Each replicate consisted of a plot having 3 rows of 2.5 m each. All agronomic practices were adopted to raise the okra genotypes, except insecticide applications.

The counting of leafhopper was initiated 40 days after sowing. In each plot ten plants were randomly selected and the nymphs were counted on upper 3 fully expanded leaves. Thus a total of 30 leaves per replicate of each genotype were examined. Scoring for leaf injury

index commenced from 4 weeks after initiating the counting of leafhopper population. Visual scoring for injury was done according to five-grade method of Mahal (1978) and leafhopper injury index was calculated from the formula adopted by Hooda *et al.* (1992).

Leafhopper development, survival and oviposition
Leafhopper development and survival were studied on the leaves of 8 genotypes through leaf cage method (Singh & Taneja, 1989) while oviposition preference was studied as per the method of Singh and Agarwal (1988).

Leaf pubescence
The second or third expanded leaf from shoot tops of the same age as those used in the leaf cage studies was plucked from each test plant. The leaves were processed in 95% ethanol to record hair density and length on the ventral surface as described by Singh and Taneja (1989).

Phytochemical analysis
Healthy leaves of each test genotypes were used to estimate total sugars (Dubois *et al.*, 1956), protein (AOAC, 1985), total phenol (Swain & Hillis, 1959), tannin (Burns, 1971) and potassium (Richards, 1958). All estimations were based on dry weight of leaves from 3 independent samples. Phytochemical analysis was restricted to chemicals reported to affect leafhopper incidence (Singh, 1988).

Statistical analysis
The data were subjected to analysis of variance after appropriate transformations in randomized block design (Snedecor & Cochran, 1968).

Results and discussion

Resistant genotypes (HRB 128-1-1, HRB 105-2-2 (GS), HRB 118-2-1) supported significantly ($P< 0.05$) fewer nymph population than the moderately resistant and susceptible genotypes (Table 1). Leafhopper injury indices for resistant genotypes (2.70- 2.91) also confirmed the higher resistance of these genotypes compared to susceptible (3.40-3.99) genotypes. By adopting similar methods previous workers (Bindra & Mahal, 1979; Teli & Dalaya, 1981; Singh, 1988) have also identified resistant genotypes against leafhopper.

Nymph duration and survival, and oviposition
The period from first instar to adult emergence varied from 7.1 to 9.8 days on different genotypes (Table 2). Nymphs took 1.1 to 2.4 days more to emerge as adults on leaves of resistant genotypes than on susceptible genotypes. Survival of nymphs also varied significantly on resistant and susceptible genotypes. On resistant genotypes 69 to 76% nymphs became adults, while on snsceptible genotypes, 86 to 93% nymphs became adults. Longer nymphal duration and lower survival on resistant genotypes suggest antibiosis resistance generally controlled by morphological and /or biochemical factors in the plants (Singh, 1988; Hooda *et al.*, 1997). Resistant genotypes in present studies also supported less number of eggs (156.8-174.4 eggs/leaf) compared to susceptible genotypes with 256.0 to 280.4 eggs/leaf, thereby indicating oviposition antixenosis in resistant lines.

Table 1. Field resistance of selected okra genotypes against leafhopper during 2001.

Genotype	Number of nymphs/leaf				Leafhopper injury index		
	26.8.01	2.9.01	9.9.01	16.9.01	27.8.01	10.9.01	24.9.01
HRB 128-1-1	10.5	10.3	16.4	11.8	1.16	2.00	2.70
HRB 118-2-1	11.2	12.9	14.2	8.5	1.24	1.84	2.43
HRB 105-2-2 (GS)	13.7	13.4	20.5	13.5	1.33	2.09	2.91
HRB 108-2-1	16.3	20.9	29.5	16.4	1.32	2.40	3.17
ST2	15.5	21.3	28.6	16.0	1.45	2.28	3.05
Varsha Uphaar	15.4	20.3	28.1	21.7	1.31	2.38	3.14
HRB 107-4-1	15.2	23.5	29.5	19.0	1.39	2.69	3.40
HRB 121-1-1	22.2	36.5	44.6	23.2	1.73	3.09	3.99
SEm±	1.49	1.94	2.97	2.16	0.07	0.13	0.16
CD (P<0.05)	4.34	5.66	8.64	6.29	0.21	0.38	0.47

Table 2. Nymph duration and survival, and oviposition of leafhopper in leaf cages on selected okra genotypes.

Genotype	Rating[a]	Nymph duration (days)	Nymph survival (%)	No. of eggs/leaf
HRB 128-1-1	R	9.8	72 (58.15)[b]	174.4 (13.13)[c]
HRB 118-2-1	R	8.2	76 (60.88)	156.8 (12.33)
HRB 105-2-2 (GS)	R	8.8	69 (56.33)	167.9 (12.84)
HRB 108-2-1	MR	8.0	80 (63.57)	227.7 (14.72)
ST2	MR	8.1	77 (60.09)	243.4 (15.56)
Varsha Uphaar	MR	8.1	83 (66.42)	205.0 (14.18)
HRB 107-4-1	S	7.1	86 (69.19)	280.4 (16.07)
HRB 121-1-1	S	7.4	93 (75.48)	256.0 (16.00)
SEm±		0.42	(3.64)	(0.59)
CD (P<0.05)		0.95	(7.35)	(1.67)

[a]R, resistant; MR, moderately resistant; S, susceptible
[b]Figures in parenthesis are angular transformation
[c]Figures in parenthesis are square root transformation

Effect of leaf pubescence and phytochemicals

Leafhoppers generally feed on the ventral leaf surface where hair density in resistant genotypes (8.0-10.3/microscopic field at 60X) was significantly (P<0.05) higher than in susceptible genotypes (Table 3). Hairs were also generally longer on resistant genotypes than on susceptible genotypes. In earlier studies, trichome density and length in okra (Uthamasamy, 1985; Singh, 1988) were reported to affect leafhopper abundance. However, some okra genotypes (Singh, 1988) were resistant to leafhopper in spite of having low trichome density and length. The basis of resistance in such genotypes was attributed to phytochemicals (Singh, 1988). In present studies resistant lines manifested higher total sugars (3.5-4.6%), tannins (0.18-0.21%), total phenols (0.54-0.62%) and potassium (1.3-1.5%) than susceptible genotypes (Table 3). Hooda *et al.* (1997) also reported higher contents of these phytochemicals in leafhopper resistant cultivars of okra.

Table 3. Leaf pubescence and quantitative estimation of leaf phytochemicals of some selected genotypes of okra.

Genotype	Leaf pubescence		Phytochemicals				
	Hair density	Hair length (mm)	Protein (%)	Potassium (%)	Total sugars (%)	Tannin (%)	Total phenol (%)
HRB 128-1-1	8.0	0.77	15.7	1.5	4.6	0.21	0.57
HRB 118-2-1	9.1	0.62	14.0	1.3	4.2	0.18	0.62
HRB 105-2-2 (GS)	10.3	0.87	15.3	1.4	3.5	0.20	0.54
HRB 108-2-1	8.5	0.80	17.4	1.3	3.7	0.15	0.45
ST2	6.3	0.76	18.0	1.3	3.0	0.16	0.56
Varsha Uphaar	5.1	0.73	17.8	1.4	3.7	0.18	0.47
HRB 107-4-1	3.5	0.66	19.4	1.3	3.1	0.15	0.46
HRB 121-1-1	4.0	0.71	17.5	1.3	3.5	0.13	0.50
SEm±	0.59	0.02	0.52	0.05	0.30	0.02	0.04
CD (P<0.05)	1.71	0.05	1.58	0.12	0.67	0.05	0.08

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It is not all Roses: Matching Host Plant Resistance Tests and Pest Damage Observation in a (Semi-) Commercial Glasshouse

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Abstract: In rose resistance tests to *Frankliniella occidentalis*, silver damage, as a type of feeding damage by thrips, showed the most consistent picture, when ranking 16 rose cultivars according to thrips damage on the leaves in a series of experiments. The relationship between this feeding damage and the thrips population in the flowers is discussed. We characterised the relationship between adults and larvae of the thrips population per cultivar. Feeding damage by *F. occidentalis* on flowers was grouped into four damage-classes and the relationship of these classes with the thrips population was studied on all cultivars.

On 'semi-commercial' glasshouse scale the same cultivars were studied for thrips feeding damage to flowers in a marketable stage. The same four feeding damage-classes as used for the test series were maintained when gathering data at glasshouse scale. In addition, the thrips population in the flower buds was determined and connected with the damage data.

Linking both series of experiments, resistance tests and glasshouse experiments, will be discussed.

Key words: host plant resistance, rose cultivars, *Frankliniella occidentalis*, feeding damage, tests, glasshouse, semi-commercial scale

Mechanism of Resistance in *Mi* Tomato to the Potato Aphid: an EPG study.

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Abstract. Nematode resistance in tomato by the *Mi* gene from a Peruvian tomato species has appeared to include resistance to the potato aphid, *Macrosiphum euphorbiae* as well. Susceptible tomato plants (cv 'Moneymaker') and an *Mi* containing cultivar with the same genetic background (Motelle; INRA, France) were compared in an electrical penetration graph (EPG) study. Adult, apterous potato aphids were wired and allowed to probe young tomato plants for 4 h, about 15 replicates per cultivar. *Mi* tomatoes showed less phloem ingestion, especially. A typical alternation between phloem salivation and ingestion was observed but this occurred on both tomatoes. The mechanism seems an additional effect to the alternation causing factor. Its nature is discussed.

Key words: tomato, aphid resistance, phloem, Macrosiphum euphorbiae, EPG

Introduction.

Aphid resistance from non-host plant species has been introduced into crop plants by 'non-molecular', traditional plant breeding techniques in a number of cases. We earlier investigated resistance to the aphid *Nasonovia ribisnigri* from *Lactuca virosa* bred into, a number of lettuce *Latuca sativa* L. cultivars (Van Helden & Tjallingii, 1993). Now we studied the resistance effect of the *Mi* gene in a commercial tomato (*Lycopersicon esculenta* (Miller)). This resistance was developed as nematode resistance that appeared to include resistance to the aphid *Macrosiphon euphorbiae* (Thomas) as well. Recently, results from 24h experiments using electrical recording (ACsystem) of plant penetration by *M. euphorbiae* have been published (Kaloshian *et al.*, 2000). It was claimed that the resistance was caused by limiting phloem sap ingestion.

The aim of this study is to use the electrical penetration graph (EPG, DC system) to get more details about the behavioural impact of the *Mi* resistance. Within phloem phase, the DC system allows distinction between salivation into and ingestion from a sieve elements once punctured by the aphid's stylets, whereas the AC system does not allow such (Tjallingii, 1988; 2000; Reese et al., 2000). Moreover we recorded 4 (or 8) hours only to avoid wire effects of the probing results.

Material and Methods.

Plants used in the experiments were young plants with 4-6 true leaves of cv. 'Motelle" (INRA, France), which is a homozygous *MiMi* plant supposed to be resistant to *M. euphorbiae* and cv. 'Moneymaker', as a near isogenic susceptible line. Some data of an earlier (preliminary) experiment will be used, in which we used a heterozygous *Mimi* line. All seeds were kindly provided by a Dutch seed company (De Ruiter Seeds).

Aphids used originated from a *M. euphorbiae* culture in the IPO (now PRI-WUR, Wageningen), which is the same source as used by Kaloshian *et al.* (2000). The aphids were reared on potato plants in the greenhouse at 20-22 °C under long day conditions, 16h light per day. After collecting apterous virginiparous adults with a soft brush from the plants they were each attached to a thin (20 µm) gold wire electrode of about 2 cm long using water based silver glue. These aphids were each connected to an amplifier input about 20-40 minutes later. Sixteen aphids were recorded simultaneously using two Giga-8 EPG systems (Lab. of Entomology, Wageningen University), one for 8 susceptible and one for 8 resistant recordings. Each aphid was put on the abaxial side of the one but youngest, fully developed leaf of separate plant. EPGs were recorded for 4 hours and in a preliminary experiment for 8 hours in which we used heterozygous (*Mimi*) plant material. Data were written to a computer hard disk at 100 Hz A/D conversion rate using STYLET 3.7 software (Lab. of Entomology, Wageningen University). The analysis part of the same software was used for the analysis of the waveforms.

Data processing and statistics of sequential (time, waveforms durations and numbers before or after certain events) and non-sequential EPG parameters (total numbers and summed durations) was accomplished (Van Helden & Tjallingii, 2000). In the preliminary (*Mimi*) experiment the 8h parameter values were calculated for 4h and 8h, both. Differences between cultivars were statistically tested by the non-parametric Mann-Whitney test for two independent samples (SSPS package).

Results

Hardly any non-phloem differences were found between cultivars. Only more total time was spent in stylet path activity (i.e. waveforms A, B and C lumped) on resistant and susceptible plants (Table 1, p=0.07). Neither numbers of probes nor their total durations differed between the two cultivars. The total number of probes shorter than 3 minutes, also their number before the first phloem phase was similar. These are early ended probes (Fig. 1) that are considered to go not much deeper than the epidermis. Often they include an intracellular puncture (pd, Fig.1).

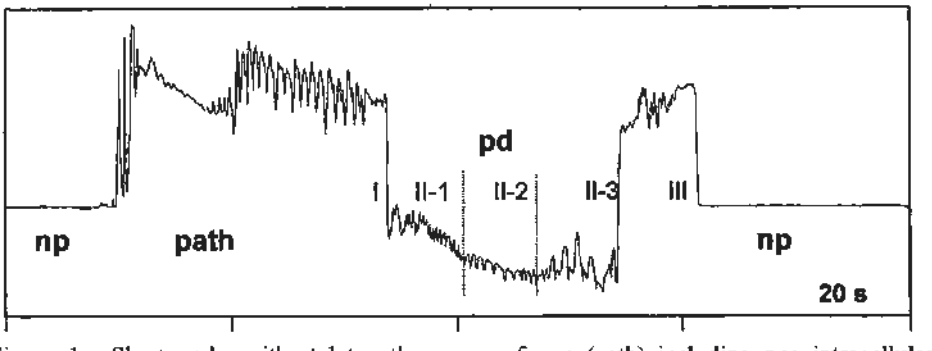

Figure 1. Short probe with stylet pathway waveforms (path) including one intracellular puncture, reflected by the period of dropped electrical potential (pd). In the intracellular second phase (II) 3 sub-phases occur, during the last of which (II-3) the aphid is thought to suck up a sap sample from the epidermis cell. In 3 minutes no deeper cells can be reached than epidermal or the first cell layer of the mesophyll. On basis of the sample's chemical information the probe might have been ended by the aphid.

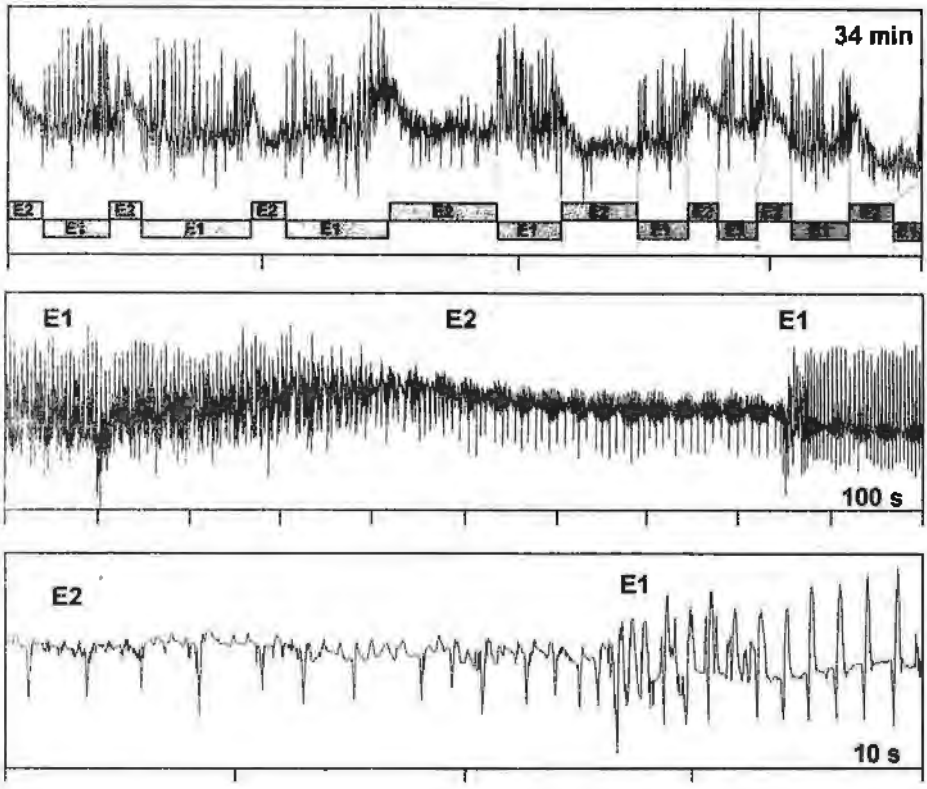

Figure 2. Alternating phloem salivation (E1) and ingestion (E2) periods occurred on both, resistant and susceptible tomato. *Top*, overview of alternating periods separated by dotted lines; *Middel*, period with a typical smooth E1-E2 transient and abrupt E2-E1 transient; *bottom*, detail of E2-E1 transient.

The time spent before the first phloem phase (activities whilst stylets in a sieve element) was equal, both, from plant access (in experiment, Table 1) as well as from the beginning of the probe including the first phloem phase (in probe).

After the first phloem phase (Table 1), the total number of phloem phases was not different but their total duration was slightly longer ($p=0.09$) on susceptible plants. The 'acceptance' of the phloem, time until sustained phloem ingestion (> 10min, Table 1) was a little delayed on *Mi* plants ($p=0.08$). Within the phloem phases, the differences are much clearer. Separate fractions of phloem salivation (E1 waveform) and ingestion (E2 waveform) could clearly be distinguished (Fig. 2, bottom). First, it appeared that the mean number of salivation fractions was higher than the number of phloem phases (Table. 1), which means that many phloem phases had more than one E1 fraction, especially on susceptible tomato. As the number of phloem phases with just one E1 period (single phloem salivation) shows, this was much higher in *Mi* plants. The other way around, the number of ingestion fractions was much lower than the number of phloem phases which means that only a small number of phloem phases include ingestion, on resistant plants especially.

On both plants there was a frequent alternation of E1 and E2 fractions once an E2 fraction was shown (Fig. 2, top). Transients from E1 to E2 were gradual, whereas E2-E1 transients were more abrupt (Fig. 2, middle and bottom).

Table 1. Mean values per aphid and standard errors of EPG parameter values from 4h recordings on susceptible (Moneymaker) and *Mi* plants (Motelle). *=p<0.10, **=p<0.05 in non-parametric the Mann-Whitney U test. Parameters calculated on numbers of aphids (N) or aphids showing the item (n).

		Moneymaker (*mimi*) *N=16*				**Motelle (*MiMi*)** *N=17*		
Parameter	(unit)	AVG	*SE*	(n)	sign.	AVG	*SE*	(n)
total numbers (mean of #)								
probes	#	9.5	*1.4*			10.1	*1.6*	
phloem phases	#	3.8	*0.6*			3.9	*0.5*	
single phloem salivation	#	1.4	0.3		**	3.4	0.6	
salivation fractions	#	7.0	*1.2*			4.8	*0.7*	
ingestion fractions	#	3.9	*1.1*		**	1.0	*0.6*	
summed durations (mean of sums)								
probes	*min*	202	*13*			213	*5*	
path	*min*	95	*9*		*	122	*11*	
phloem phase	*min*	96	*13*		*	64	*12*	
salivation fractions	*min*	39	*8*			42	*9*	
ingestion fractions	*min*	61	*11*		**	22	*9*	
F & G waveforms	*min*	11	*5*			26	*9*	
% probing (mean %)	%	85%	*5%*			90%	*2%*	
probes < 3min								
total number	#	6.4	*1.2*			6.8	*1.4*	
before 1st phloem phase	#	4.6	*1.0*			2.2	*0.6*	
time until first (mean)								
phloem phase in exp.	*min*	65	*13*			59	*14*	
phloem phase in probe	*min*	21	*3*			24	*3*	
phloem ingestion (>10min)	*min*	142	*21*	(15)	*	195	*16*	(9)
maximum period (mean of max.)								
phloem phase period	*min*	76	*12*	(15)	*	45	*11*	(16)
salivation fraction	*min*	19	*4*	(15)		22	*7*	(16)
ingestion fraction	*min*	51	*12*	(15)		38	*12*	(9)
period (mean of means)								
phloem phase	*min*	40	*11*	(15)		24	*6*	(16)
salivation fraction	*min*	6	*1*	(15)		9	*2*	(16)
ingestion fraction	*min*	33	*11*	(15)		30	*11*	(9)

The total numbers of phloem phases was equal between plants, and total time spent in phloem phase (i.e. all E1 and E2 summed, Table 1) was only slightly reduced on *Mi* plants (p=0.09), although the maximum duration of a phloem phase was significantly reduced. Also,

phloem salivation parameters did not differ. The main difference effect of the *Mi* gene was a decrease in ingestion. Especially the number of ingestion fractions and the total time spent in phloem ingestion were significantly reduced on resistant plants. The maximal ingestion fraction, was also reduced but due to the huge variation and the low number of aphids contributing (Table 2) this was statistically not significant (p=0.48). The mean (mean of means per aphid) period of phloem phase and the mean fraction of phloem salivation and phloem ingestion, however, did not differ between the cultivars. This was different in the preliminary experiment with heterozygous plants where the mean phloem phase was 83min for susceptible and 16min for the *Mimi* plants, which is significant reduction (p=006). Opposite to the homozygous plants (Table 1) the *Mimi* plants had a higher number of phloem phases with salivation (E1) only (1.9 vs 0. 9 in susc.; p=006), which reduced the mean phloem phase since they are much shorter than E1/E2 phloem phases. Consequently they contributed little to the mean phloem phase.

The time until the first phloem phase from start of the experiment was similar between the cultivars (Table 1). Also, within the probe with the first phloem phase, the time until the phloem activities was equal, 21 and 24min respectively, which indicates that no exceptional long pathway times were needed before aphids showed phloem activities. On resistant plants, some delay was shown before aphids attained sustained (> 10min) phloem ingestion (in exp.).

Finally, Table 2 shows the fraction of aphids showing any phloem activity (E), phloem ingestion (E2), and sustained phloem ingestion (E2>10min), respectively. Considerable fewer aphids showed phloem ingestion, especially sustained ingestion on the resistant cultivar. No apparent differences were shown between heterozygous and homozygous *Mi* plants.

Table 2. Percentages of aphids (4h data) showing different phloem activities on susceptible and resistant cultivars in the experiment with homozygous (*MiMi*) and heterozygous (*Mimi*) plants.

Experiment	% aphids with	E	E2	E2>10min
	susceptible	94%	94%	63%
Homozygous	*MiMi*	94%	53%	35%
(prel. exp)	susceptible	88%	71%	76%
Heterozygous	*Mimi*	94%	44%	44%

Conclusions and discussion

Our results strongly support the conclusion that the *Mi* resistance is located in the phloem, as earlier suggested by Kaloshian *et al.* (2000). Nevertheless, the tendency of decreased amounts of probes shorter than 3 minutes in *Mi* plants indicates that the epidermal cells may be somewhat more attractive than in susceptible plants. During many of these shorter probes, often not longer than 30-60s, a potential drop (pd) before stylet withdrawal indicated that a cell was punctured and a sap sample was ingested (Martin *et al.* 1997 and tasted by the epipharygeal gustatory organ (Wensler & Filshi, 1969).

No fewer aphids showed phloem phases - periods of phloem penetration - on *Mi* plants and the number of phloem phases was similar to aphids on the susceptible control plants.

Although the total and mean duration of the phloem phases was somewhat shorter on *Mi* plants, this was significant at a low level only ($0.05<p<0.10$) and does not support the earlier EPG observations with the AC system (Kaloshian *et al.*, 2000). The mean phloem phase duration in their study of about 3 minutes only on *Mi* plants might be due to their extremely long experimental time: 24 hours, vs. 4 hours in our experiments. Experiments with wired aphids on resistant plants should not be continued longer than needed to answer the question concerned here: "where is the resistance located?". Once the aphids have decided to rejection op the plant they cannot escape when tethered (Tjallingii, 1986). So, in fact all probing activities shown after that decision do bias the total results. Mostly this will lead to an underestimation of the resistance: more probing than in the free, not wired situation on that plant. In the Kaloshian's *et al.* study the phloem phases might have become shorter and shorter on *Mi* plants, presumably leading to the extremely short mean value of the duration. Although the mean duration of phloem phases in the preliminary experiments was even shorter on *Mimi* plants (26 vs 70min=res. vs. sus.) than on the *MiMi* (45 vs. 76 min, Table 1) this still was much longer than the ca. 3min in the Kaloshin's *et al.* study (2000).

The advantage of the DC EPGs is that one can distinguish between fractions of phloem salivation and ingestion during a phloem phase (Reese et al., 2000; Tjallingii, 1988, 1990). Normally, phloem ingestion is always preceded by a period of phloem salivation of about one or two minutes. Then ingestion is mostly sustained continuously on susceptible plants.

In contrast to the small differences in the mean number and duration of phloem phases and the salivation fractions, there was a significant reduction in the number and total duration of ingestion fractions. Moreover, the number of single phloem salivation periods increased, thus the stylets were more often pulled out before switching to ingestion on *Mi* plants. However, the mean and the maximal duration of ingestion periods was similar on both plants. It seemed that ingestion was mostly restricted to about 30 min after which the aphids either withdrew from the phloem or switched back to phloem salivation. This alternation between phloem ingestion and salivation was a predominant feature of aphids on both, *Mi* and susceptible tomatoes. Such a sustained alternation of the two phloem activities is not very common in most other aphid-host combinations and therefore, these data suggest that the possible mechanism of the *Mi* resistance is something additional to the alternation causing factor that already exists in Moneymaker. As was shown, fewer aphids on *Mi* plants showed sustained ingestion (>10min), thus a feeding deterrent in the phloem sap can be an option. Moreover, we experienced that *M. euphorbiae* developed on rather poor on this susceptible tomato cultivar. Presumably we need studies on *M. euphorbiae* with other host plants and tomato cultivars as well as other aphids on *Mi* plants to confirm this.

Jiang *et al.* (2001) compared *Bemisia tabaci* probing on the same tomato cultivars using EPGs. They found early (epidermis and outer mesophyll) resistance factors in Motelle, as indicated by a much longer time with more probes until first phloem phase on this cultivar. Apparently, the probing behaviour of *B. tabaci* differs here from *M. euphorbiae*.

One might speculate on the mechanism behind the alternating phloem waveforms. Possibly, the E1 salivation does suppress or avoid the phloem wound reactions, in which coagulating proteins seem to play an important role (Knoblauch and Van Bel, 1998. The tomato may have phloem proteins in which clogging is more difficult to suppress or avoid. It seems unlikely from this study, however, that the *Mi* gene has a direct relationship with this mechanism. Also, the relation with the nematode resistance remains uncertain.

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Breeding for Plant Resistance to Pests and Diseases
IOBC wprs Bulletin Vol. 27 (7) 2004
pp.51 - 53

Reports from other IOBC/WPRS WG convenors with linking research interests

WG "Integrated Control in Protected Crops, Temperate Climate"

Aims of the WG and potential to interact with other WGs

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Number of members ca. 150 from 27 countries

Introduction

Practical application of biological control of pests in protected crops is still increasing. In the past, this control measure was only used in vegetables, but 10-12 years ago biocontrol was introduced in ornamentals and has since then become increasingly important in these cultures. In the most progressive countries biocontrol of pests is applied on up to one third of the area with ornamentals. This figure is quite astonishing considering that many previously regarded biocontrol in ornamentals as a complete utopia due to the extremely low damage threshold of these cultures and the zero tolerance status for many pests. Our WG has played an important role in stimulating this development worldwide through coordinated fundamental and applied research, intensive advisory and public relations work.

Aim

The general goal of our WG is to promote the research, development, implementation, and training of Integrated Pest Management (IPM) systems in protected crops, as well as promoting cooperation between scientists, advisors and beneficial producers working in this field. Our group has realised large-scale practical use of biological control through intensive advisory and public relations work.

- Our group design commercially applicable IPM programmes based on biological control of pests and diseases in combination with host-plant resistance and other non-chemical control methods.
- It initiates, coordinates, and evaluates fundamental and applied research for the development of biological and integrated control programmes.
- It develops scientific criteria for the selection of natural enemies, assists in the development of mass production methods for natural enemies, and devises quality control methods for natural enemies.
- It contributes to national and international courses where IPM and biological control is taught.

The priorities are to develop biological control of pests and diseases in ornamentals and to devise quality control methods for all natural enemies.

Activities

Meetings

Our WG meets every 3rd year. The 12th full meeting of our WG will take place in Turku, Finland in 2005. People working with host plant resistance to pests are highly welcome.

Establishment of list server

Our WG has a list server, "GoodBugs-L" (http://www.agrsci.dk/plb/iobc/goodbugs-l.htm) – an open e-mail based discussion list service on the Internet. GoodBugs-L is a forum for discussion and exchange of information concerning different aspects of biological and integrated pest control – including host plant resistance – in protected crops. GoodBugs-L strengthens the links between group members for the benefit of biological control in glasshouses worldwide.

Web site

Our WG has a web site (http://www.agrsci.dk/plb/iobc/iobc_home.htm), which at present contains a short description of the group and a list of members with names, addresses, email-addresses etc. In addition, links are provided to our Newsletters, STING, as well as to GoodBugs-L. The site serves as place for announcements of future meetings, calls for registration, instructions to authors etc.

The Newsletter STING

Every year at least one issue of STING, the newsletter of our WG, is made. The newsletter brings information on e.g. relevant upcoming meetings and courses, summaries of workshops (our group and others), notes on e.g. new pests, notes on new books, etc. Next issue around July 2002. STING is not restricted to our WG members – anyone can subscribe (free). To be put on the mailing list for STING, please contact me.

Future potential for interactions with the WG "Breeding for Plant Resistance to Pests and Diseases"

Although the main focus of our WG is biological control of pests, many group members have a strong interest in other aspects regarding IPM in protected crops, as well. Many members have in the course of time undertaken investigations to elucidate the influence of plant species and varieties on the biology not only of pests but also of natural enemies and thus on the outcome of biocontrol. The influence of plant characteristics on natural enemies may be direct through e.g. interference with searching behaviour, or it may be indirect through influences on the pest insects and mites. Practical implementation in commercial greenhouses of plants with increased host plant resistance is welcomed as an important addition to IPM programs – an addition that could reduce the vigour of pest populations and increase the chances of successful biocontrol. It is however, important that the characteristics of plants with increased host plant resistance are evaluated for influence on the beneficials that are likely to be used in the crop – not only beneficials aimed directly at the pest(s) for which the host plant resistance has been targeted, but also other beneficials released for control of other pests.

On behalf of our WG I highly recommend that people from the WG "Breeding for Host Plant Resistance to Insects and Mites" acquaint themselves with our WG e.g. through our Newsletter STING and by attendance at our WG meeting.

Breeding for Plant Resistance to Pests and Diseases
IOBC wprs Bulletin Vol. 27 (7) 2004
pp.55 - 58

WG "Pheromones and other semio-chemicals in integrated production"

Integration of chemical ecology and plant breeding for sustainable insect management

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Abstract: Resistant plants and behaviour-modifying chemicals are important tools for environmentally safe control of insects. However, resistant plants and semiochemicals are species-specific to varying degree and do not cover all insects associated with a crop. The available methods should be developed as components of an integrated control programme, rather than as sole agents. In addition, synergies are expected from coordinating research on plant semiochemicals and plant breeding for resistance to insects. The identification of plant compounds mediating host-finding and oviposition in insects is a current focus in chemical ecology and the knowledge of plant semiochemicals will become increasingly important for plant breeding.

Key words: chemical ecology, plant resistance, semiochemicals, integrated production

Introduction

Crop protection relies primarily on synthetic chemical pesticides. This chemical approach to pest control is not sustainable and is therefore under increasing pressure. However, few reliable and economic biological techniques are availableto control the most important insects and fungal diseases. The attempted deregulation of neurotoxic insecticides can be achieved only if new, environmentally safe techniques are developed.

Behaviour-modifying chemicals, resistant plants and microbial pesticides can be used to control insects. However, biological methods are often rather species-specific, while most crops are infested by a guild of herbivorous insects, which varies between geographical regions. In addition, biological insect control methods, including plant-derived resistance factors, produce rather subtle effects compared to conventional insecticides, which are lethal upon contact. The available biological tools should therefore be developed as components of an integrated crop management programme, rather than as sole agents. Moreover, improved communication between different research fields will provide an important stimulus.

Insect sex pheromones

Insects use sex pheromone to communicate for mating. By permeating the atmosphere with synthetic pheromone, sexual communication and mating in can be prevented (mating disruption technique) (Ridgway et al. 1990, Witzgall and Arn 1997). The main applications of the mating disruption technique in Europe are against codling moth *Cydia pomonella* on >10.000 ha and the grape berry moths *Eupoecilia ambiguella* and *Lobesia botrana* on

>30.000 ha (Arn and Louis 1996, Waldner 1997, Kast 2001, Zingg 2001). This demonstrates the potential of the mating disruption technique for insect control. However, mating disruption is still not widely used (Witzgall 2001).

Pheromone-based control is species-specific, and only male behaviours are affected. The use of plant volatiles will allow to manipulate behaviour of gravid females and other species.

Direct use of plant semiochemicals

Volatile secondary metabolites are known to mediate insect attraction or repellence (Langenheim 1994) and they can be used to manipulate insect behaviours for population control (Ridgway et al. 1990). Plant volatiles can be used alone, or to enhance insect attraction to sex pheromones.

An important obstacle to the direct use of plant volatiles is the natural background of plant volatile compounds in ambient air. Orchard air, for example, contains numerous plant volatiles, in large amounts. This is illustrated by difficulties in measuring airborne pheromone concentrations in orchard air by chemical analysis (Bäckman 1997). Even when large amounts of synthetic pheromone are disseminated for mating disruption (up to 1 ng m^{-3}), it is hardly possible to use gas chromatography for concentration measurements. This can only be achieved by using the male antenna as a sensor (Koch et al. 1997). This reemphasizes that pheromones offer a quite unique change for insect control in the sense that the male sensory system is tuned to pick up tiny amounts of pheromone, and that natural pheromone is not accumulating in the atmosphere.

A recently discovered kairomon attractive to codling moth females is produced by pears and can therefore be used in apple orchards (Light et al. 2001). However, in many cases it will be difficult to directly use plant volatiles for insect control.

Plant semiochemicals and plant breeding

Chemistry and biochemistry play a significant role in plant-pathogen and plant-insect relationships. The knowledge of these interactions, and the chemicals mediating these interactions can make a most important contribution to plant breeding.

In most plant populations there are individuals which are resistant to fungal infestations or less susceptible to insect attack. Resistance is often related to inducible defence reactions in the plant. These plant responses include the production of pathogen-related (PR) proteins as well as non-protein secondary metabolites, including alkaloides, phenolics and terpenoids (Karban & Baldwin 1997, Agrawal et al. 1999). PR-proteins are a promising target for bioengineering, whereas secondary metabolites are the products of complex multi-enzyme pathways - their manipulation poses considerable technical difficulties and their biosynthesis is often associated with substantial metabolic cost (Bryngelsson et al. 1994, Hilder & Boulter 1999, McCaskill & Croteau 1998).

A realistic projection into a near future is probably that the knowledge of volatile secondary plant chemicals will be useful to explain resistance phenomena, rather than leading to the design of plant varieties with modified secondary metabolism through genetic engineering.

An example comes from apple fruit moth *Argyrethia conjugella*. Its principal host is rowan *Sorbus aucuparia*. However, flowering and fruit setting in rowan is strongly cyclic (Kobro et al. 2002) and females of *A. conjugella* invade apple orchards when too few rowan berries are available for egg-laying. Attraction of *Argyresthia conjugella* is obviously guided

by apple volatiles (Bäckman et al. 2002). Knowledge of the active compounds can be used to select more resistant, i.e. less attractive varities of apple.

Last not least, chemical ecology deals with non-volatile compounds on the the plant surface, which are important cues for egg-laying and feeding, after insects have landed on their host plants (Dethier 1982, Renwick 1989, Honda 1995). The chemistry of surface chemicals precludes direct use in most cases, but our knowledge of these chemicals will most certainly become increasingly important in plant breeding.

Integration of development and use of biological control methods

For future development, we must put stronger emphasis on a multidisciplinary approach. Clearly, there is a need for intensifying communication and collaboration, and for coordination of research activities between different fields, especially chemical ecology and plant breeding. In addition, the available methods should be developed as components of an integrated control programme, rather than as sole agents.

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