Union Internationale des Sciences Biologiques

ORGANISATION INTERNATIONALE DE LUTTE BIOLOGIQUE ET INTEGREE CONTRE LES ANIMAUX ET LES PLANTES NUISIBLES

SECTION REGIONALE OUEST PALEARCTIQUE

GROUPE DE TRAVAIL "LUTTE INTEGREE EN AGRUMICULTURE"

WORKING GROUP "INTEGRATED CONTROL IN CITRUS FRUIT CROPS"

TEL-AVIV(ISRAEL)

10.03.88

ISBN 92 9067 017 7

BULLETIN SROP
WPRS BULLETIN

1988/XI/6

International Union of Biological Sciences

INTERNATIONAL ORGANIZATION FOR BIOLOGICAL AND INTEGRATED CONTROL OF NOXIOUS ANIMALS AND PLANTS

WEST PALAEARCTIC REGIONAL SECTION

IOBC/WPRS WORKING GROUP " INTEGRATED CONTROL
IN CITRUS FRUIT CROPS"

REPORT OF THE MEETING AT TEL-AVIV (ISRAEL)
ON 10 MARCH 1988

OILB/SROP GROUPE DE TRAVAIL " LUTTE INTEGREE
EN AGRUMICULTURE"

RAPPORT DE LA REUNION A TEL-AVIV (ISRAEL)
A 10 MARS 1988

WPRS BULLETIN 1988/XI/6
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INTRODUCTION

After the complete reorganization of our Working Group, in appropriate and specific meetings, long and short term research priorities were established. Study and research programmes on the same or different problems have been initiated in many countries.

Of course, not all the studies decided upon have made progress, because of the extreme difficulty in getting people involved in cooperative work outside of their current research projects, unless specific funding is offered.

Many of the studies or basic activities reported here are only partially consequent upon cooperation between different countries, being mainly just the results of current work in the course of specifically financed research programmes.

In this respect, it would be helpful if our Organization could reach an understanding with the Commission of the European Communities to collaborate more closely in their operations and so realize joint projects supported by Community funds.

Regarding the implementation of the results achieved, biological agents and biotechnical means have been employed in many cases, but almost exclusively by scientific institutions. Often, technical and economic difficulties impede citrus farmers from utilizing much of the information available from research laboratories.

In today's symposium, some general considerations will be presented on the present state of integrated control in citriculture, as well as those specific and fundamental to proper pest management.

Investigations into biocoenoses, time and space studies of key pest populationdynamics, research on entomophagous species, monitoring of biological agents utilizable in practical control programmes, and the data obtained from biotechnological trials to contain important pests and plant diseases, all represent the fruits of labour effected by the Members of our Working Group.

Among the questions still remaining to be answered is, for example, that of the value to attribute to economic thresholds. All the factors causing their variability have to be taken into account, as well as researches into the most selective ways of using pesticides, and their possible secondary effects on all the other organisms living in and outside the system under consideration.

I would now like to say that full credit must go to all the participants for promoting the exchange of ideas, interests and experiences, as is the spirit of our organization, in an endeavour to coordinate efforts in order to get faster and better results and to avoid overlapping.

I would also like to express my gratitude to everyone who has helped to realize this meeting; and, in particular to Prof. Mendel, the Congress President; to dr. Goren, the Programme Chairman; and to Prof. Rosen of the University of Rehovot, Chairman of Section 5, for his sterling efforts in maintaining such close contact.

Romolo Prota

Convenor

LIST OF PARTICIPANTS MEETING TEL-AVIV, 10 MARCH 1988

CATARA, P.- Istituto di Patologia vegetale, Via Valdisavoia 5, Catania, Italia.

DAVINO, M.- Istituto di Patologia vegetale, Via Valdisavoia 5, Catania, Italia.

DE CICCO, V.- Dipartim. di Patologia vegetale, Via Amendola 165/a, Bari,Italia.

DELRIO, G.- Istituto di Entomologia agraria, Via E. De Nicola, Sassari, Italia.

DI SILVESTRO, I.- A.I.D. Research Centre, Catania, Italia.

GARRIDO, A.- Instituto Valenciano de Investigaciones agraria, Apartado oficial, Moncada (Valencia), Espana.

LIOTTA, G.- Istituto di Entomologia agraria, Via delle Scienze 13, Palermo, Italia.

MAGNANO DI SAN LIO, G.- Istituto di Patologia vegetale, Via Amendola 165/a, Bari, Italia.

NUCIFORA, A.- Istituto di Entomologia agraria, Via Valdisavoia, Catania, Italia.

QUILICI, S.- Institut de Recherches Agronomiques Tropicales, Ile de la Réunion, St. Dénis, France.

RAGUSA, S.- Istituto di Entomologia agraria, Via delle Scienze 13, Palermo, Italia.

ROSEN, D.- Dept. of Entomology, Faculty of Agriculture, The Hebrew University, P.O.Box 12, Rehovot, Israel.

RUSSO, A.- Istituto Difesa Piante, Università, Reggio Calabria, Italia.

SALERNO,- Dipartimento di Patologia vegetale, Via Amendola 165/a, Bari, Italia.

THANASSOULOPOULOS, C.C.- Regional Direction of Agriculture, Zaimi 21, Patras, Greece.

TROPEA GARZIA, G.- Istituto di Entomologia agraria, Via Valdisavoia 5, Catania, Italia.

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INTEGRATED CONTROL OF FUNGAL AND BACTERIAL DISEASE OF CITRUS: PRESENT STATUS AND FUTURE DEVELOPMENT.

M. Salerno
Dipartimento di Patologia vegetale, Università degli Studi, Bari, Italy.

Summary - After a short introduction on peculiarity of integrated control in plant pathology, the present status of application of integrated control towards the most important fungal and bacterial diseases of Citrus in Italy and in the Mediterranean basin is discussed. At end, future developments of integrated disease control are discussed.

As an introduction I would like to point out that the principles underlying integrated control have frequently been applied in plant pathology, even though unconsciously. As a matter of fact, standard interventions against plant diseases have always been diversified, as they include cultural practices, varietal resistance, particular nutritional situations, etc.; fungicides and other chemicals usually are used only when necessary. However, it is a fact that in plant pathology, biological control procedures, although applied in many istances, often are not well understood in their mechanism. In addition, supervised control, which is to be considered one of the first steps of integrated control, is not of common use in plant pathology, because the intervention thresholds for many diseases are hard to determine and somewhat aleatory. Anyway, the advent of systemic, curative fungicides is quickly changing the picture, although plant pathologists are still rather far from the notable results obtained with supervised control against artropods. Anyway, integrated control - that may be defined as the armonious and, if possible, synergic use of every available means suitable for controlling a plant adversity, taking into account the conservancy of agro-ecosystem and the analysis of cost/benefit - with the necessary fittings proper of different phytopatological disciplines, is applicable against every plant adversity, either disease, parasitic or not, or animal and weed infestation.

Because treating here all fungal and bacterial diseases of citrus is far beyond the scope of this presentation, the present status of control strategies against the most serious diseases in Italy and the Mediterranean basin will be reviewed, namely: Mal secco, caused by Phoma tracheiphila (Petri) Kanc.et Ghik., diseases due to Phytophthora spp. and,among bacterial diseases, the only that is dangerous to our citriculture, induced by Pseudomonas syringae pv.syringae van Hall.

Mal secco. This vascular disease is at present the most serious disease of Italian and Mediterranean citriculture, occurring in nearly all lemon (**Citrus limon** (L.) Burm.), citron

(C. medica L.) and bergamot (C. bergamia Risso) orchards. The commonly used sour orange (C. aurantium L.) rootstock is also susceptible to the disease. With the exception of Spain, Morocco and possible Egypt, all countries bordering the Mediterranean Sea are affected by the disease to some extent. The lemon industry, because of its outstanding economic importance, is particularly plagued by the disease.

So far chemical control is rather uncommon, as results have not always been satisfactory, not even after applying the new systemic fungicides several times each year (4). A poor understanding of some aspects of the disease epidemiology may be partly responsible for the poor results. Consequently, at present, mal secco control is based mainly on the use of improved cultural practices (3) and the use of resistant lemon cultivars.

Cutting of infected twigs and branches is fundamental to the control of mal secco. Sanitation cutting must be performed repeatedly, as soon as symptoms become evident during the late spring-early summer. To low the inoculum cutting must be also performed at the beginning of autumn, shortly before infection time, when pycnidia are differentiating. It is also very important to remove the stumps of affected trees to prevent pycnidia developing on stump sprouts. Tree pruning may exert an evident influence on the incidence and severity of mal secco. A light pruning "hardens" the trees and makes them less susceptible to the disease. Rootstock suckers should be removed, because they became infected readily, and these infections may lead to sudden death of the tree.

Soil cultivation in late autumn and winter is dangerous because it increases the chances for root infections. Nontillage has also proved to be detrimental because it allows development of the susceptible rootstock roots near the soil surface and increases the chances for root injuries and infections.

Protection against adverse meteorological events, such as wind, frost, or hail, is of great value for lowering the incidence of mal secco infections. In fact, any adverse meterological condition which causes injuries, increase the chances for new infections. In windy areas, windbreaks are absolutly necessary. Covering nursery plants with a plastic net appears highly desirable for mal secco control. Moreover, such a covering seem to be efficacious against the disease in the orchards as well (2).

Mineral fertilization plays an important role in affecting the predisposition of plants to mal secco. Nitrogen, applied at a rate optimal for yield, not only makes the plants more susceptible, but favours a more rapid development of the disease. The use of manure with a low nitrogen content, alone or, still better, with the addition of phosphorus and potassium fertilizers, slows disease progress and facilitates the surgical treatments.

Finally, the choice of cultivar is also important in the control of mal secco. Although the different resistant lemon cultivars and clones are commercially not a good ones, nowdays they are largely replacing the excellent but very susceptible

cultivars, as Italian 'Femminello'.

In my opinion, if all the above control practices were applied by lemon growers, chemical treatment could give better results, particularly in nurseries and young groves.

Root rot. Root rot caused by **Phytophthora** spp. is a problem where soils and irrigation methods are unfit and pruning is careless, particularly in old trees. A poor bud union also can weaken the roots making them more susceptible to rot.

Therefore, beside the use of a resistant rootstock, it is very important to choice suitable soils, discarding those heavy, not setted and poorly drained. The excessive deepness of planting also weakens the roots, reducing the original defences.

In conclusion, pruning of the tree canopy, soil drainage, and improved irrigation methods, are the most important control measures. If a tree has been removed and a new tree is to be planted soon, the site should be treated with a soil fumigant as methyl bromide or vapam, otherwise a delay of 1-2 years is recommended before replanting. To this regard the new systemic fungicides, as Efosite Al and the Acylalanines give confidence to the future of chemical control.

Foot rot. Foot rot, caused by the same **Phytophthorae** responsible for root rot, is fairly common in rainy years and in compact soils.

In addition to using a resistant rootstock, the disease is prevented by taking the following precautions: (i) graft buds of susceptible cultivars high on the rootstock seedling; (ii) avoid deep planting, particularly on heavy and poorly drained soils; (iii) prevent water standing in contact with the crown; (iv) avoid contact of organic manure and weeds with the crown region. In those areas where the disease is more frequent, the trunk-crown should be areated by removing the soil and, during the rainy season, painting it with a high concentration of a copper compound or an acylalanine fungicide. Also, the growers should examine plant crowns periodically for early symptoms of gummosis, and promptly apply surgical treatments or paint them with highly concentrate suspension of an acylalanine fungicide, as matalaxyl.

Brown rot. Lemon and other citrus fruits sometimes become infected by the same fungi that cause root and foot rot, as well as by other species of the same genus **Phytophthora**.

Serious outbreaks usually develop only in years when the autumn and winter are very wet. The soil conditions are also important. For istance, soil cultivation in autumn and winter, performed sometimes in lemon orchards to increase fruit size, and nontillage carried out by weedkillers, are conducive to heavy attacks by these soil-inhabiting pathogens.

As disease outbreaks are erratic, growers do not apply fungicide sprays routinely but usually leave a weed covering to reduce splashing of soil-borne zoospores onto low-hanging fruits. Where brown rot is of frequent occurrence, it is advisable to spray the low part of the tree and the soil under

the canopy and outward for one meter beyond the drip area with a copper fungicide. This protective spray should be applied to the whole tree in case of orchards located on terraced land or where the spray is also needed for mal secco and bacterial blast control.

Blast and black pit. This disease, caused by **Pseudomonas syringae** pv. **syringae** van Hall, is present in almost all the citrus growing regions of the world, except where moisture and the right temperature condition for infection (not exceding 20 C) do not occur simultaneously (7). There are two types of manifestation of the disease: blast (leaf and twig lesions) and black pit (fruit lesions).

In the Mediterranean region infections leading to development of blast occur during autumn and winter, following mechanical injuries, especially by wind. The bacteria invade the wings of leaf petioles of leaves that are less than one year old.

Black pit occurs principally on lemon fruits, almost exclusively on the last picking of the winter crop. The infections leading to development of black pit are promoted by mechanical injuries, particularly those caused by hailstorms.

To lessen blast damage it is advisable to prevent the development of an untimely flush of susceptible growth in the autumn. Windbreaks are also very useful. The severity of blast varies considerably from year to year and from one locality to another. Only with severe attacks and in those areas where damage is very serious and not easily controlled by cultural practices, is chemical control deemed necessary. On the basis of our results (5) a single application of 1% Bordeaux mixture or other copper spray at the end of October or beginning of November, is recommended.

As this quick look of the status of citrus disease control indicates, a diversification, more than integration, of control operations exists, both, out of necessity, as effective chemical treatments are often lacking, and for a certain cultural attitude of growers. In fact, during the past centuries, they have refined and modified cultural practices, adapting them to a reality inclusive of cryptogamic diseases. With this in mind it is possible to assert that some of these cultural practices still deserve investigations to determine their exact phytopathological meaning. In any case, the introduction of new cultural techniques has created new situations, very often misunderstood by farmers in their phytopathological implications. Therefore, farmers should have to be educated, both, to a better phytopathological use of the old cultural practices, and to a proper use of new ones. But this education is rather hard to practice, in particular in zones of old citriculture, where farmers no longer young are still active.

The problem now mentioned, the education of farmers, is one of the most difficult to overcome, in order to succed with integrated control. A constant, accurate and specialized

technical assistance seems essential, at least towards a number of growers, representative and well distributed in the different citrus areas. To this regard I would like to mention that a project of integrated control in Emilia-Romagna, a foremost fruit growing region of Italy, has produced a notable dragging effect on those farmers who were not directly involved (1). Anyway, a more direct involvement of farmers would peraphs be useful, for instance, by establishing an "elite class" of fruits, possible identified by a quality mark, or else, obliging farmers, before selling, to demonstrate by analytical results the good quality of their crops with reference to residues. However, this specific problem should not be left without touching upon the necessity, surely not so pressing but nevertheless rather useful, of educating also the consumer, who shoud privilege fruits with no residues even though they may show possible small blemish or aesthetic deficiency. In any case, besides all the above, what allows me to look with confidence to the future of integrated control, are the notable economies that usually have been registered where integrated control has been practiced (1, 6).

So, what are the prospects for the future of integrated control against plant disease in general and citrus diseases in particular? Surely they are exciting even if very taxing. It is necessary, in fact, a financial, administrative and organizatory effort, but mainly research must progress in the right direction. To this regard it is important to keep in mind that integrated control is a philosophy, whose concepts are applicable with success only when the necessary basic knowledge of pathogen, host, and environment, as well as their reciprocal interelationships is available. In this context, attention must be paid to analysis of both , efficacy of each single control means and integration among them, as well as cost/benefit ratio, including the indirect benefits, often overlooked by farmers, as those on the environment.

Unfortunately these investigations and studies are rather hard to perform, either because they are within the capacity of few, in well equipped laboratories, or because many financial means as well as a continuous engagement are necessary. In addition, it must be remembered that the majority of founds are supplied by governmental agencies for applied investigations, aiming to obtain very pratical results, the only ones easily understood by the mass of farmers.

Coming to specific subject regarding the future of integrated control against citrus diseases, the following reflections seem possible:

- In my opinion the genetic improvement of disease resistance will be the backbone of integrated control, in particular using modern biotechnology;
- The choice of sites for new orchards are also of paramount importance, even if the matter is rather difficult. However, it is a fact that a lot of wrong has been done in the past, planting citrus in unsuitable pedo-climatic zones, where diseases have a higher incidence;
- Biological control, in its two categories of microbic antagonism (mainly against root and foot rots) and of induced

resistance (mainly against mal secco), very probably will have an incisive part in the framework of integrated control;
- Cultural practices, as tillage, irrigation, manuring, pruning, spacing, all adequately studied, will gain more and more importance;
- Chemical treatments, currently of little relevance for controlling some fungal diseases of citrus, in the future could become much more important, acquiring the character of supervised chemical control, completely integrated with other interventions, particularly if new fungicides more active and specific than present ones will be formulated;
- Finally, use of plant material certified for both, trueness to variety and health, as well as effective quarantine services, are also important factors for a successful integrated control in citriculture.

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POPULATION FLUCTUATIONS OF SCIRTOTHRIPS AURANTII FAURE IN CITRUS ORCHARDS IN REUNION ISLAND

S. QUILICI, P. GESLIN and B. TRAHAIS
CIRAD/IRFA, Station de Bassin Martin
B.P. 180 - 97455 SAINT PIERRE CEDEX

Summary

A study was conducted in 1985-86 in order to determine the population fluctuations of Scirtothrips aurantii FAURE in a citrus orchard in the south of Reunion Island. Populations were monitored by visual control and trapping with Saturn yellow sticky traps so as to define the best monitoring method. Thrips populations began to increase at the end of the flowering period and maintained high levels until the end of the austral summer period where they began to decrease. No captures were registered from march until the beginning of the next increase of september.

1. Introduction

Though most cultivated areas of Reunion Island are still devoted to sugarcane, fruit crops have developed regularly during the past ten years with the aim of answering the needs of the local market and exploiting some exportations openings. After the implementation of a successful biological control against the two psyllid vectors of citrus greening (1), the citrus plantations could increase and, in 1986, organized citrus orchards represent an acreage of 210 ha and a production of about 1700 t.

Together with rust mite and fruitflies, citrus thrips Scirtothrips aurantii FAURE, represents today one of the local key-pests of citrus. It causes blemishes of the fruit that generally take the aspect of a stem-end ring of scarred tissues that depreciates the commercial value of the crop. Other blemishes can also occur that extend along the longitudinal axis of the fruit, which allows to distinguish these injuries from wind blemishes (2). Young fruits are especially sensible just after petal fall until they reach approximately a diameter of 4 cm. Until now, the control of this pest in the island has been based on a preventive chemical treatment program of 2 or 4 dimethoate + endosulfan sprays (45 + 30 g a.i./hl) at 15 days intervals, during the critical period that extends from petal fall (september) to november-december. S. aurantii is also a major pest in South Africa where studies have been conducted on its bioecology (3, 4, 5) or control (6,7, 8).

In a first step towards a better management of this pest, the purpose of our study has been to determine the population fluctuations of the thrips during the year in a citrus orchard situated in the south of the island. We present here the preliminary results of this study, obtained in 1985-86.

2. Materials and methods

The study has been conducted on the CIRAD/IRFA Station of Bassin Martin, situated at an altitude of 300 m in the leeward coast, south of the island. Schematically, the climatic conditions prevailing on the station are as follows : rainfall : 1397 mm/year ;

mean T° : 19-25° C ; mean maximum T° : 23-29° C ; mean minimum T° : 15-21°C (on a 7 years period). The parcel used consists of nine monovarietal blocks, about 35 twelve years old trees each, including the four varieties : Tahiti lime (2 blocks), tangerine Clementine (2 blocks), tangelo Orlando (2 blocks) and orange Pineapple (3 blocks).

To study the population fluctuations of the thrips, two complementary methods have been used :

- Fluorescent yellow sticky traps : they consist of metallic rectangular boards (21 x 14 cm) covered with a self-adhesive yellow tape. The color chosen was "Saturn Yellow 3485" (manufactured by "3 M Corporation"). During studies on psyllid populations with such traps (9), we found this material to be quite effective in trapping S. aurantii, what has been confirmed in a precise study by SAMWAYS (10).

The yellow tape central part is covered with a transparent sheet of PVC of the same dimensions spread with Bird Tanglefoot® , clipped over the yellow tape and renewed weekly. The trap is placed on a metallic stem situated 50 cm from a tree, at a height of 1,70 m, its yellow face directed towards the tree.

A network of nine traps (one per block) has been settled from the end of november 1985 until the end of 1986. Captures are recorded weekly by counting the thrips under the stereomicroscope with the help of a grid with unit squares of 4 cm2 for a total surface of 216 cm2.

- A visual control of thrips populations has been done weekly from petal fall, at the end of september 1985 until harvest in 1986. Twenty fruits per tree (5 in each of the 4 orientations) are selected randomly at mid-height on ten per cent of the trees in each block. For each of the inspected fruit, the total number of thrips visible (larvae and adults) are recorded. To express the results, we defined six infestation classes corresponding respectively to 1, 2, 3, 4, 5 and more than 5 thrips instars/fruit.

During the visual control, the appearance and the development of thrips blemishes on the growing fruits were recorded on the same sample. For this, we defined four classes of injury corresponding to blemishes that extend to less than 1/4 (class 1), from 1/4 to 1/2 (class 2), from 1/2 to 3/4 (class 3) and to more than 3/4 (class 4) of the fruit surface.

During the study period, all blocks received a spraying program against the different key-pests ; sprays against thrips were decided considering the population trends observed during the critical period.

3. Results and discussion

A few thrips species can be found in citrus orchards in Reunion Island whose precise inventory is under completion. However, preliminary observations indicated that S. aurantii could be distinguished under the binocular (x 40) from the other species caught on sticky traps.

Fig. 1 shows the results of trapping from november 1985 to december 1986. Captures of each trap have been cumulated on 15 days intervals and means calculated for each of the citrus varieties. We observe well defined seasonal variations in the captures. The beginning of the trapping period probably corresponds to the end of the pullulation period of 1985. In most blocks, captures decrease and reach the zero level towards the beginning of march 1986. Nearly

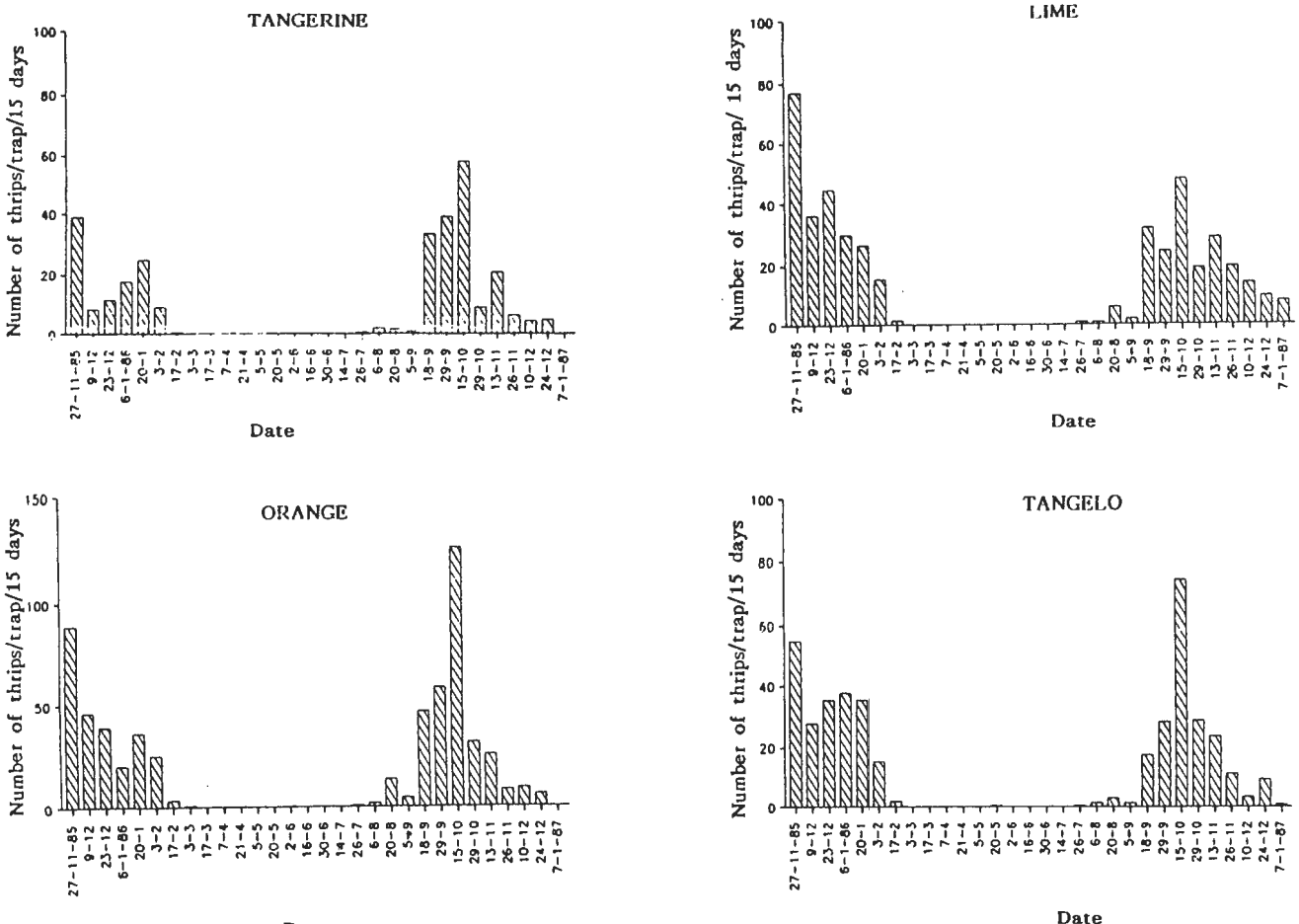

Fig. 1 - Captures of thrips (Scirtothrips aurantii FAURE) on yellow sticky traps.

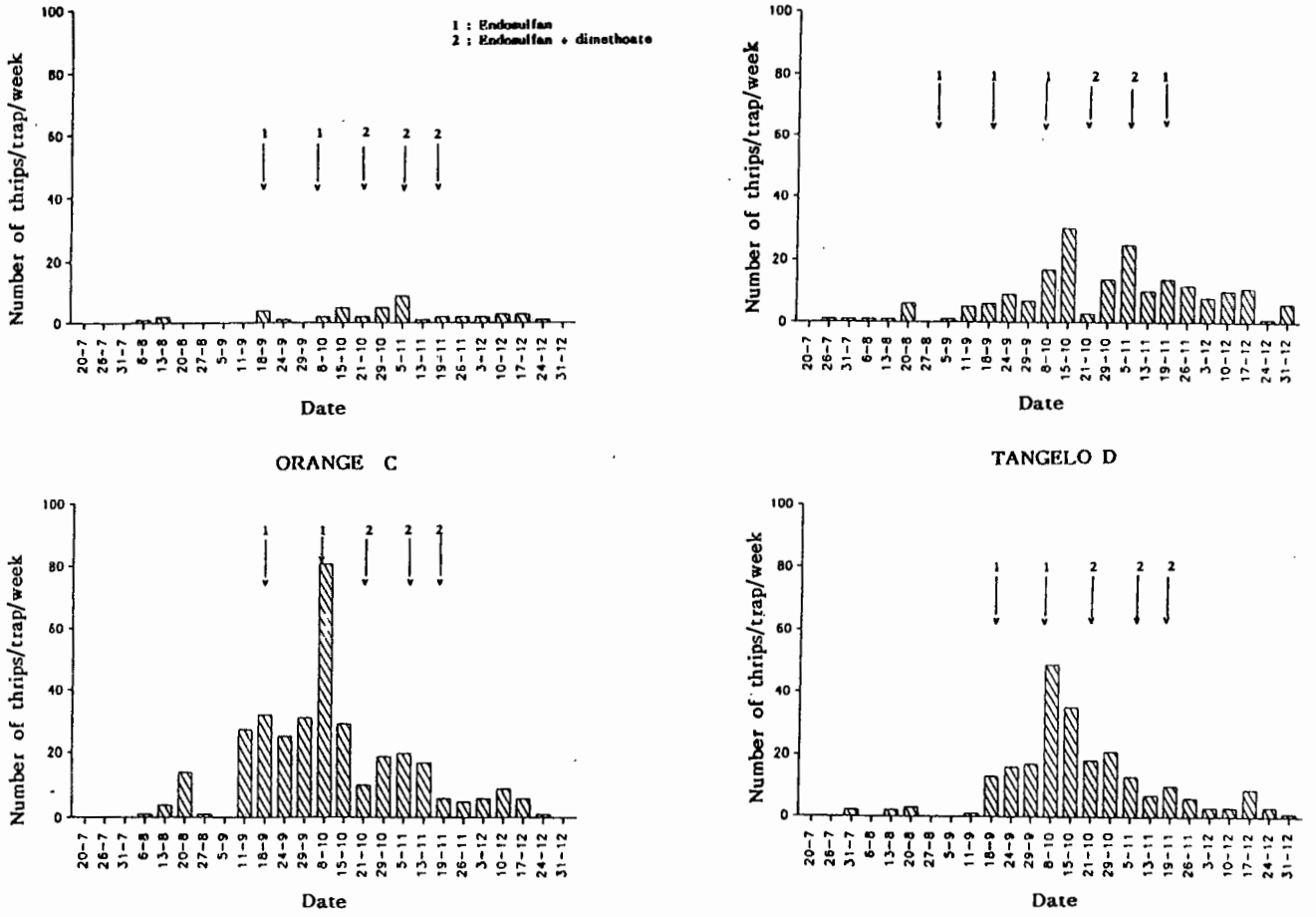

Fig. 2 - Weekly captures of thrips (Scirtothrips aurantii FAURE) on yellow sticky traps and chemical treatments Bassin Martin - 1986

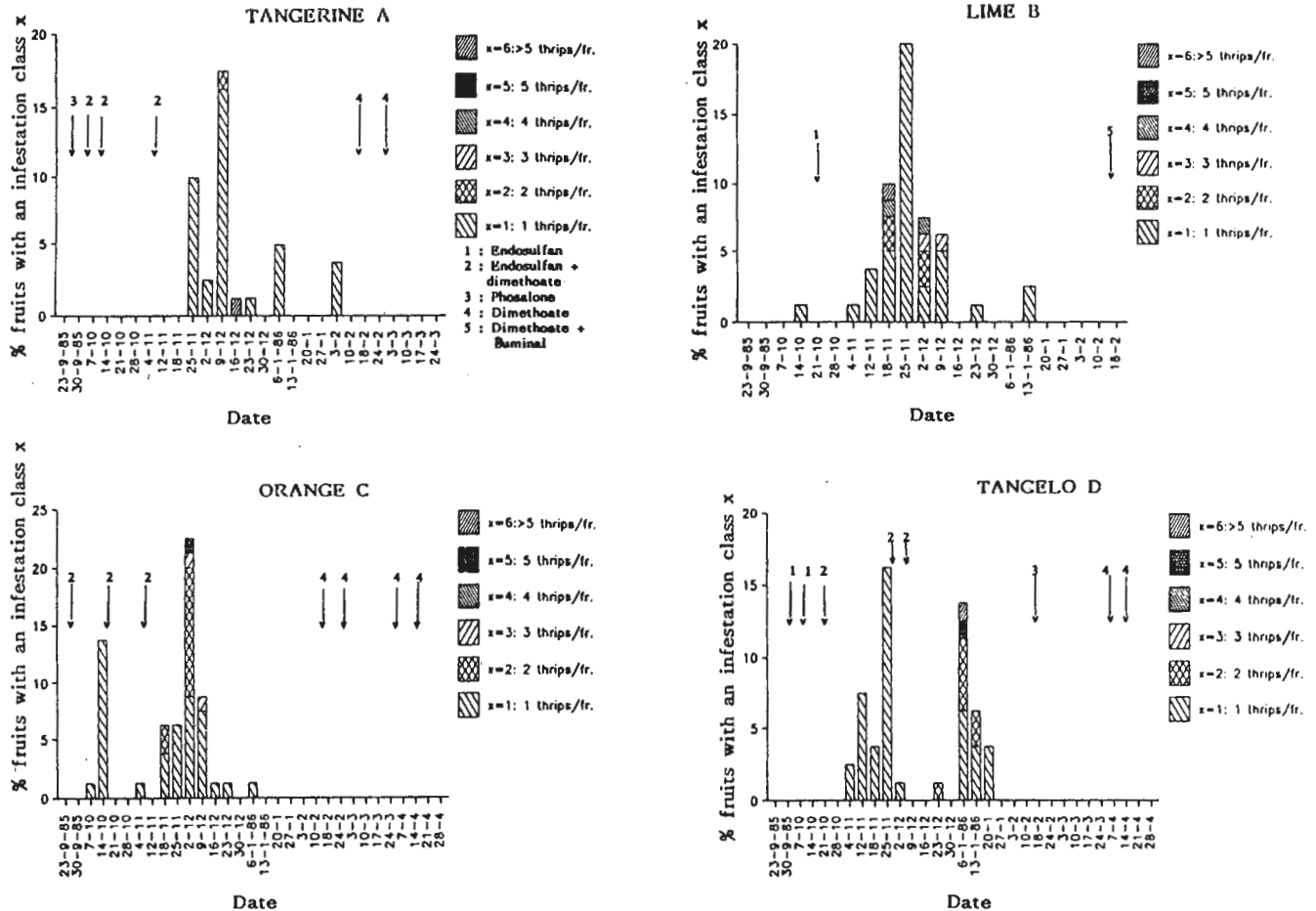

Fig. 3 - Visual control of thrips : evolution of the relative frequencies of the fruit infestation classes
Bassin Martin - 1985-86

no captures are then recorded until the beginning of august when populations increase again during the flowering period.

Comparing the four varieties, we find that the mean trapped effectives are of comparable importance on lime, tangerine and tangelo with maxima of 60-80 thrips/trap/15 days. In orange blocks, maxima are higher and reach 120 thrips/trap/15 days.

Fig. 2 represents the weekly captures on four of the blocks, for the 1986 pullulation period and shows the influence of the chemical treatment program on the trapping results. Dimethoate treatments are generally followed by a marked though not complete reduction in the numbers of thrips captured possibly indicating that a certain level of resistance to this insecticide may have developped in the local populations. During the pullulation period, the peaks of captures recorded show that very high populations were present compared with those observed in south african conditions (10).

The results of visual control are presented in Fig. 3. We note a high frequency of the lowest infestation classes, which can be attributed partly to the insect behaviour. Thrips most often hide and feed beneath the sepals of the young fruits and it is therefore probable that part of the population actually present on the fruit is missed at the control. Depending on the blocks, the maximum infestation level reaches 15 to 40 % of the sampled fruits. The highest infestation levels are again observed on oranges. From the beginning of february until harvest, no more thrips are observed on the fruits, which can be attributed partly to the dimethoate treatments against fruitflies that begin before the end of february on oranges and tangerines. However, results of trapping (Fig. 1) show that low captures are still recorded in february ; so it is probable that by this period of the year, young fruits have also reached a stadium of growth where they are less or no more attractive to the thrips. Studies under progress should allow us to precise the respective influence of treatments, phenology of citrus climatic conditions of austral winter and the possible role of alternative host-plants on the population trends recorded.

The observations of damage levels showed that most blemished fruits presented of slight level of injury (class 1). At the period of harvest, the percentage of these slightly damaged fruits could reach respectively 10, 25 and 30 % in some blocks of tangerines, tangelos and oranges.

3. Conclusions

The preliminary results of this pluriannual study, obtained with two complementary methods of visual control and trapping, indicate the main tendencies on the seasonal population fluctuations of S. aurantii in the conditions of a lowland orchard of the western coast of Reunion Island.

Further studies should permit to precise the relation between the results obtained by these two methods with the aim of establishing an intervention threshold again S. aurantii.

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BIOTECHNICAL CONTROL MEANS ADOPTED AGAINST *CERATITIS CAPITATA* WIED. IN CLEMENTINE GROVES

Ortu, S. and R. Prota
Istituto di Entomologia agraria, Università degli Studi di Sassari, Italy

Abstract - Prevention of high production losses in Sardinian clementine groves particularly sensitive to the Mediterranean fruit fly, required repeated treatments with insecticides during the ripening period. To reduce chemical control, biotechnical methods were tested using two types of trap both baited with trimedlure dispenser and hydrolysed protein: one of transparent plexiglas coated with "glue" and the other of wood soaked in Deltamethrin (0.25% a.i.). The first type of trap, exposed from September to December, showed average captures per trap of 478.4 adults (368.2 males and 110.2 females). Efficiency of the second type of trap in the field was confirmed by comparing the insects' oviposition. In the grove protected with mass-trapping the percentage of fruit punctured was 13.3% (plexiglas trap) and 26.6% (wood trap) compared with 38.1% in the unprotected control grove.
Traps activated by trimedlure dispenser proved to be the most efficient and to have a more extensive attraction capacity than those baited with Trimedlure mixed in the glue.

Ceratitis capitata Wied.(medfly) represents the most damaging pest for fruit cultivations of Summer-Autumn maturity in Sardinia (5). The considerable ability to shift, the large number of annual generations and the high fecundity of the species allow it an almost constant presence in the citrus fruit cultivations of the island, with high density from July to December. The difficulties that arise for an appropriate pest control, which at present is carried out with exclusive and repeated application of larvicidal products (Dimethoate) around harvesting, necessitate the use of alternate techniques in order to reduce the harmful residues in the produce destinated for food, and at the same time preserve the useful insect population.

With this in mind, research has been intensified over recent years on substances which attract Tephritids (10, 2, 9) and on the preparation of traps suitable both for the monitoring and direct control of populations (6, 3).

The massive use of trimedlure traps has permitted, in so-

me cases, an efficient control of infestations (1), even though it appears more practical to look towards means that act on both sexes considering the proven polygamy of males (4). On the basis of this knowledge it was intended to verify the efficiency of olfactory traps, baited with sexual attractants and food, for the direct control of Ceratitis in a citrus grove.

Materials and Methods

Studies were carried out in a central eastern area of Sardinia (Siniscola) where Ceratitis capitata finds particulary favourable climatic and breeding conditions for development.

During the second half of 1986, the medfly captures in the sticky traps, baited with trimedlure dispenser (supplied by the Biological Control System Ltd. Mid Glamorgan, U.K.) wich was left unchanged during the entire season, were compared with captures from the traps in wich the trimedlure was renewed weekly.

The experimental field for the studies on the mass-trapping control was prepared on 150 adult Clementine plants which result as being amongst the most prone to attack by Ceratitis capitata of the citrus cultivated in our area, suffering losses that effected up to 80% of the production (7).

Two types of trap were used for the protection of the fruit: one made of transparent thin sheets of plexiglas smeared with glue (Temo) and the other of wooden boards soaked for 12 hours in a solution of Deltamethrin at 0.25% of a.i. able to kill the insect on contact. The traps were baited with a trimedlure dispenser and with a cotton wick soaked weekly in protein hydrolysate poisoned with Deltamethrin. The traps were exposed in the field from the month of September, one per plant, at eye level, in the south-west of the foliage.

The adult Ceratitis capitata population eliminated in the cultivation was evaluated by a weekly count of the specimens captured in the plexiglas traps. The killing efficiency of the wooden traps on the other hand was periodically observed in the laboratory using a stock of artificially reared medflies.

Control of infestation was carried out by examining samples of mature fruit with a binocular microscope at harvesting.

Results and Discussion

1 - Population Density and Trap Efficiency

The dynamics of the medfly population were preliminarly surveyed for 9 years by means of adult captures in white plexiglas sticky traps baited with trimedlure.

A considerable variability in the population density of

Ceratitis capitata, marked by a progressive drop from 1979 to 1981 and a notable recovery in the following years, was observed in the citrus cultivations under examination (Fig. 1).

The traps baited with trimedlure dispenser showed considerable efficiency for over four months and therefore resulted as being able to cover the entire period of insect

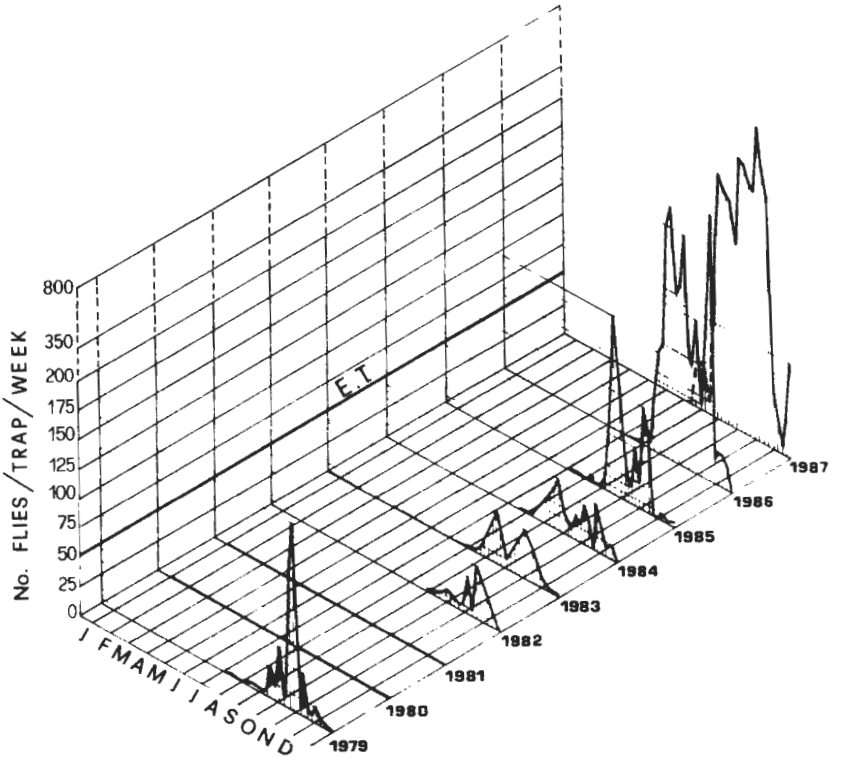

Fig. 1. Trend of Ceratitis capitata captures (Siniscola).

presence. In fact the captures of these traps from August to December were constantly higher than those baited with trimedlure mixed with glue (altogether 682.1 flies/trap as against 452.6).

2 - Mass-trapping

Ceratitis capitata captures in the field subjected to mass-trapping began during the first week of exposure of the traps in September. The highest captures were registered in

November with 122 adults/trap/week. At the end of the experiment the average total captures resulted as 478.4 adults/trap of which 368.2 were males (Fig. 2).

The killing efficiency of the wooden traps was periodically tested in the laboratory. A few seconds contact by Ceratitis capitata determined death in all individuals within 5-20 minutes, even after a period of 11 weeks exposure of the traps in the field.

Fruit observation showed a higher number of ovipositions in the fruit of the nontreated control field in comparison with those protected either by wooden traps or by plexiglas traps (Tab. 1).

The number of eggs noted per puncture varied considerably passing from 2 to 13, although in many cases no eggs could

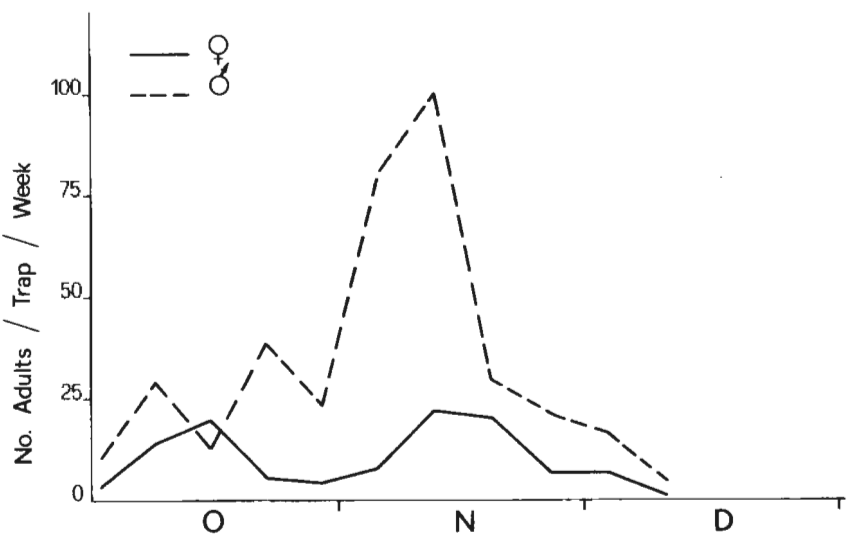

Fig. 2. Trend of Ceratitis capitata captures in sticky traps in the mass-trapping citrus grove (Siniscola, 1986)

be seen. The depth of oviposition resulted as rather variable. In some fruits females managed to penetrate the thickness of the peel laying their eggs directly into the endocarp at a depth of about 2 mm.

Larval development was only completed, howewer, in a limited number of cases (Tab.1).

The various percentages of larval development found in the observed sample do not seem to depend on the number of ovipositions found but probably on the various depths at which the eggs are laid. In fact it is possible that eggs

laid in the deepest layers of the peel or even directly into the endocarp, can avoid the fruits natural resistance factors and so complete development.

Table 1 - Ceratitis capitata infestation in clementine protected with different types of trap (Siniscola, Sardinia, 1986)

	Punctured fruits (%)	Punctures per fruit (n.)	Wormed fruits (%)
Non treated	38.1	2.4 ± 2.2	1.2
Insecticide treated traps	26.6	1.9 ± 3.7	0.4
Sticky traps	13.3	2.1 ± 2.0	1.0

Conclusions

The experiment carried out in Sardinia using different types of trap baited with olfactory substances, showed the possibility of their advantageous use in the population control of Ceratitis capitata for the protection of citrus cultivations.

The use of such means for control in the considered agroecosystem in fact results as being particularly favoured by the known temporary resistance of the fruit to attack of the fly (11). This makes it possible to intervene during a period in which the fruit does not undergo damage from early stages of the insect, even if exposed to ovipositions. It is certainly necessary to improve the types of traps to be used as a means of control. They must however, necessarily answer the requirements of simplicity , practicality and economy. The need to safeguard the useful insect population was kept in mind when choosing the traps, avoiding the yellow chromotropic type that, as is known, do not permit selective captures (6, 8). The transparent sticky traps were shown to be sufficiently selective but certainly less pratical in comparison with the wooden ones. In fact they have a higher weekly labour cost due to washing, glueing and replacement in the field. From this point of view the wooden traps appear to be more suitable, even though their efficiency must be further verified especially in different climatic conditions and environments.

The attractant effect of the trimedlure dispenser was shown to be persistant enough to cover the entire period of

Tephritid presence in the citrus grove. The persistence of the poisoned protein hydrolysate, on the other hand, was conditioned by seasonal climatic conditions. In fact rain on some occasions washed out the cotton wick making the weekly substitution indispensable.

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ATTRACTION OF CERATITIS CAPITATA TO SEX PHEROMONES, TRIMEDLURE, AMMONIUM AND PROTEIN BAIT TRAPS

Delrio, G. and S. Ortu
Istituto di Entomologia agraria, Università di Sassari, Italy

Abstract - Attraction tests were conducted in a Sardinian citrus grove during 1986-87 using neutral sticky traps baited with sex pheromones, parapheromones and food lures. Three substances previously identified in the pheromone mixtures of male medfly, methyl (E)-6-nonenoate, linalool and 3,4 dihydro-2H-pyrrole, showed no attraction. Trimedlure was confirmed as the strongest attractant for males, whereas undiluted ammonium acetate and protein hydrolysate (Buminal) attracted a very high proportion of females without any difference in efficiency of attraction. The combination of ammonium acetate and protein bait showed a stronger attraction than when used alone. By adding ammonium acetate and protein to the trimedlure baited traps no increase in total captures was observed, but it increased the proportion of females. Total female captures were further increased by separating the lures into two traps (one with trimedlure and the other with food lures) suspended in the same tree 1 m apart.

The Mediterranean fruit fly, Ceratitis capitata (Wied.) is a serious pest in many citrus areas of the world. The detection, monitoring and control of this species is dependent upon the use of attractants employed in traps or formulated for use in lure and kill bait sprays.

The most potent lure for medfly is the commercially available trimedlure which attracts almost exclusively the males. Due to its high selectivity trimedlure is used worldwide as a standard attractant for the survey and detection in various types of trap, the captures in these traps however, do not truly reflect the actual population fluctuations, because the efficiency is influenced too much by climatic conditions (2). Controlled-release dispensers have recently been developed that may extend the field life of traps and stabilize the rate of emission of trimedlure (8). There is no comparable attractant for the female C. capitata although ammonium compounds and protein hydrolysates are used to attract and bait both sexes in McPhail traps. These substances however are not selective and furthermore their attraction efficiency can be impaired by a humid climate (2, 3, 4).

Study of the sexual pheromone secreted in male glands in the last abdominal segment has not yet brought about the development of a potent and selective attractant for females. Pheromone mixtures trapped by aereation techniques from mature male medfly are of remarkable complexity and over ten components have been isolated and identified (1, 7). One of these components (methyl (E)-6-nonenoate) was found to be attractant in laboratory and field tests trials

(2, 10). A cyclic imine, 3,4-dihydro-2H-pyrrole and the monoterpene alcohol linalool were also found to be attractive in laboratory bioassays (1, 6), but their field efficiency is still not known.

The development of a supertrap, that combines known and available attractants, to be used in control programmes for C. capitata with the mass-trapping technique, was dealt with in an IOBC programme. The results obtained were not however very satisfactory and brought to light the need for deeper studies into the interaction between attractants (2).

The aim of this work was to evaluate the attractancy of some of the pheromone compounds on the natural medfly populations in citrus groves and also to study the attraction efficiency of the male lure and of ammonia-releasing substances, in various combinations, on both sexes.

Materials and Methods

The components of the pheromone mixture of C. capitata tested were: 1) methyl (E)-6-nonenoate (obtained from Istituto Donegani, Novara, Italy); 2) 3,4-dihydro-2H-pyrrole (prepared in acetic ether solution of 60% as described by Jakoby and Fredericks, 1959); 3) linalool (Fluka AG - Buchs SG, Switzerland). Chemicals were applied to cotton wicks inserted in perforated polyethylene vials (15 mm diameter by 50 mm long) in 200 µl dosages. The attractants used were: 1) trimedlure, in Biolure controlled-release dispensers (Biological Control Systems Ltd., Mid Glamorgan, UK); 2) undiluted protein hydrolysate (Buminal, Bayer, FRG), 4 ml in polyethylene vials with six 2 mm holes at the top; 3) dry ammonium acetate, 4 g in the above polyethylene vials.

Traps were made of transparent plexiglas, 2 mm thick, cut into 20 x 15 cm boards. The board was coated thinly on both sides with the insect adhesive Temo (Kollant S.p.A., Italy).

Two tests were conducted in an orange grove, with the trees planted at 5 x 5 m, near a stone fruit orchard at Siniscola, Sardinia, Italy. In test 1, in autumn 1986, traps baited with the pheromones were hung vertically on the trees about 2 m above the ground and about 20 m apart in a randomized complete-block design (one replicate per block; 10 blocks). Each week the traps and pheromones were substituted and the flies adhering to the traps were removed, sexed and counted. In test 2, during autumn 1987, there were 5 treatments: 1) trap baited with trimedlure; 2) trap baited with protein hydrolysate; 3) trap baited with ammonium acetate; 4) trap baited with trimedlure plus protein plus ammonium acetate; 5) two traps baited respectively one with trimedlure and the other with protein plus ammonium acetate, suspended in the same tree at a distance of about 1 m. Each trap system was hung on the outer part of an orange tree, 20 m from the next. The traps were arranged in a completely randomized block design with ten replicates for each case.

Data were transformed by the square root so as to normalize the catch distribution for analysis of variance. The means were separated by Duncan's multiple range test

(P = 0.01), and the weighted means were back-calculated from the means of the square root for presentation in the tables.

Results and Discussion

Sex pheromones

All compounds of the pheromone mixture of the male C. capitata tested at dosages of 200 mg gave insect captures which were not significantly different from those trapped with the unbaited traps (Tab. 1). These substances were also tested, during October 1986 at various other dosages (50, 100, 500 mg), with no replications, however they were found to be completely inefficient.

Table 1. Catches of medflies by plexiglas sticky traps baited with various components of the pheromone mixture (Siniscola, Sardinia; 1986; 10 replicates per type of lure).

Lure	No. of C. capitata captured per trap at indicated periods							
	1.X-14.X		15.X-28.X		5.XI-11.XI		12.XI-18.XI	
	♂	♀	♂	♀	♂	♀	♂	♀
None	20.4	20.2	45.1	9.9	3.5	89.6	11.3	29.8
Methyl (E)-6-nonenoate	22.1	18.4	39.6	12.5	-	-	-	-
3,4-dihydro-2H-pyrrole	20.3	23.2	42.3	10.6	-	-	-	-
Linalool	-	-	-	-	7.2	67.2	11.6	11.9

Methyl (E)-6-nonenoate resulted very attractive to male and female medfly in laboratory tests (7), but unattractive to females under field conditions in Hawaii, although at high doses (0.5-1 g) it was reported to be as attractive to males as trimedlure (10). The other two compounds of the pheromone mixture have only been bioassayed in the laboratory. An aqueous solution of the cyclic imine was found to be highly attractive to virgin female C. capitata (1), and the linalool proved a powerful attractant and arrestant for males and for virgin females in wind tunnel bioassays (6).

The inconsistent results that have been obtained up to now in the field pose certain questions on the natural functions and potential for exploitation as attractants of the C. capitata sex pheromones.

Attractants

Trimedlure was found to be the strongest attractant for male medfly, that made up 93% of the total captures in the traps baited with this parapheromone (Tab. 2).

The females were mostly attracted to the protein hydrolysate (Buminal) and to the ammonium acetate, with no

Table 2. Catches of medflies by plexiglas sticky traps baited with various attractants (Siniscola, Sardinia; 1987 10 replicates per type of trap).

Trap system	Mean No. of *Ceratitis capitata* captured per trap at indicated periods (*)									
	23.X-29.X		29.X-4.XI		5.XI-11.XI		12.XI-18.XI		19.XI-25.XI	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Trimedlure	969a	58c	530a	44c	684a	21c	213a	51bc	56ab	7b
Trimedlure + Protein hydrolysate + Ammonium acetate	807a	202b	386a	68c	299b	84b	154ab	35bc	30b	6b
Trimedlure (**)	626a	84bc	387a	17c	375b	20c	283a	15c	100a	4b
Protein hydrolysate + Ammonium acetate (**)	273b	575a	32b	333a	13c	197a	54bc	131a	6c	41a
Protein hydrolysate	32c	594a	52b	181b	16c	122b	10c	64bc	1c	6b
Ammonium acetate	56c	520a	21b	201b	8c	116b	11c	103ab	1c	12b

(*) Catches followed by the same letter within a column are not significantly different at 0.01 level.
(**) These two traps were placed in the same tree.

significant differences between the two substances. The traps baited with both ammonium salt and protein bait showed higher captures than when used alone. Females attracted to these food type lures made up 80-90% of the total captures (Tab. 2). In preliminary tests the attractiveness of Buminal was improved raising the pH of the commercial product from 6.1 to 7.1 with a consequently higher rate of ammonia release (9). This indicates the similar type of action of both protein and ammonium compounds, dependent mainly on the attraction that ammonia exerted on the flies.

By adding ammonium acetate and protein to the trimedlure baited traps no increase in total captures was observed, but it increased the proportion of females (altogether to about 20%). The absolute total of females captured in this multi-baited trap appeared inferior in comparison to those in the traps baited with only food lures, thus demonstrating a repellent effect of the male lure on the female medfly. A high level of both male and female captures was on the contrary maintained by separating the lures into two traps (one with trimedlure and the other with food lures) suspended in the same tree 1 m apart.

The persistence of attraction, whether for the trimedlure in the controlled-release dispenser (Biolure) or of the Buminal or of the ammonium acetate, was found to be longer than 3 months. At the end of this period of time the polyethylene vials still contained about 2/3 of the initial quantity of the last two attractants.

The strong attraction of undiluted Buminal and ammonium acetate in dry form exerted on C. capitata females has already been shown in previous tests in Sardinia (2, 3) and Israel (4). However, in this present experiment the traps captured a low number of males, probably due to the competition brought about by the high density of trimedlure baited traps present in the citrus grove. The repellent action of the male lures, strong enough to almost entirely counteract the attractive properties of the protein baits for some species of Tephritids (including C. capitata) has been observed in Australia (5). The reduced efficiency of female captures in traps that combine male attractants with food ones poses serious difficulties in the development of a supertrap for use in biothecnical control programmes for C. capitata. It is possible in fact, that the increased catches of male flies obtained would be more than offset by the greatly reduced kill of females. An interesting alternative could be found in the use of separate traps for males and for females, which when hang on the same plant, could bring about an increase in total captures, as shown also for another species of Tephritid, Dacus oleae Gmel. (11).

Conclusions

Research on the attractants for C. capitata has brought about the development of a long range trimedlure trap for detection. The major challenges for medfly trap design are presented by the need for intermediate power traps for use

in establishing economic thresholds and for the development of "supertraps" which would enable direct biotechnical control. These two types of trap must possess high female capture efficiency because the population dynamics of the females is directly connected to crop damage. Significant advances in medfly control can only be realized through the improvement of techniques in the use of food lures and when efficient sex attractants for females are available.

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CITRUS PESTS IN CALABRIA (SOUTHERN ITALY)

S.RAGUSA and A.RUSSO
Istituto di Difesa delle Piante, Piazza S.Francesco.
GALLINA DI REGGIO CALABRIA, ITALY
whiteflies, scales, mites

Abstract
A first contribution to the knowledge of Arthropodos injurious to citrus trees in Calabria is given. The species of whiteflies observed were: Aleurothrixus floccosus (Mask.), Dialeurodes citri (Ashm.) and Bemisia afer (Priesner and Hosny). As far as scales are concerned, we report the followings: Peryceria purchasi (Mask.), Planococcus citri (Risso), Pseudococcus longispinus Targ., Saissetia oleae (Oliv.), Saissetia coffeae (Walk.), Coccus hesperidum L., Ceroplastes rusci (L.), Ceroplastes sinensis D.G., Mytilococcus beckii (New.), Mytilococcus gloverii (Pack.), Parlatoria pergandei (Comst.), Chrysomphalus dictyospermi(Morg.), Aspidiotus nerii Bouchè and Aonidiella aurantii (Mask.). Brief data on their parasites are also reported. Finally we give information on phytophagous and predaceous mites.

1. Introduction

Because of its more than 37.000 cultivated Ha (9) and of the essences it produces, Calabria is considered one of the most important citrus regions in Italy. The most typical citrus tree is the bergamotto which is mainly found in the area of Reggio Calabria. This species has been utilized for a long time to extract an oil which is used in the perfume industry. However, researches regarding the arthropod fauna associated with citrus trees have been up to now sporadically carried out (4,6,7,8,11,12,13,14,16,18). Therefore a survey has been carried out in the past two years in order to have an organic view of the arthropods (mainly whiteflies, scales and mites) which form such biocenosis.

2. Materials and methods

Surveys were carried out in the most important citrus orchards of the Ionian belt near Reggio Calabria, in the plain of Gioia Tauro (Reggio Calabria), Lamezia Terme (Catanzaro) and Rossano (Cosenza). Two different citrus orchards were chosen for each area; there we collected 10 fruits and various branches whose total length measured 2

- Work supported partly by C.N.R. and Ministero della Pubblica Istruzione Funds (40%)

meters from 4-6 randomly chosen trees from the 4 different cardinal points. A part of the material collected was used to identify scales and whiteflies, to record their different postembryonic stages and the percentage of mortality and parasitism. The remaining part of the material was put into cages in order to obtain the emergence of entomophagous insects.
As far as phytophagous mites are concerned we took 20 fruits and young branchelets from the same above mentioned trees, and from these we took at random 20 leaves which were observed under a stereo-microscope in order to ascertain the presence of the mites. Moreover we also collected predaceous mites of the family Phytoseiidae, by shaking 20 branches of the tree.

3. Results and discussion

WHITEFLIES

During the survey we found the citrus whitefly, Dialeurodes citri (Ashm.),and the woolly whitefly, Aleurothrixus floccosus (Mask.). D.citri was found in Calabria for the first time at the end of the '60s (4) and has been sporadically noted in the citrus orchards in the plain of Lamezia Terme and Reggio Calabria . Viggiani (19), Liotta(10),Barbagallo, Longo,Rapisarda (1), do not consider such pest as a problem in this area, as it is controlled by the parasite Encarsia lahorensis (How.) very well. In fact such a parasite was introduced by Luppino (13) and it is now well spread in all the areas where the aleyrodid is present.

A.floccosus has been more recently introduced in Calabria (12). It is now present in all the citrus orchards in Calabria. Even if big and dangerous infestations of such species have been noted in all the checked areas, the damages caused by A.floccosus have been limited by the active presence of the parasite Cales noacki How..In fact the parasitism observed was as follows: 40% at Lamezia Terme, 60% at Rossano,90% at Reggio Calabria and 100% at Pizzo Calabro. It should be mentioned that a parasitism equal or higher than 40% is considered sufficient to control A.floccosus (2).

Bemisia afer (Priesner and Hosny) has not practical importance as it appeared in colonies of few specimens only in Nicastro (Catanzaro) and Reggio Calabria.

SCALES

14 species of scales, out the 21 associated with citrus trees in Italy, have been surveyed in Calabria.

Among Monophlebidae, we mention the cottony cushion scale, Peryceria purchasi (Mask.), which is present in all the areas where citrus trees are cultivated. The species is not considered harmful because it is controlled by Rodolia cardinalis Muls..

Among Pseudococcidae, the citrus mealybug, Planococcus citri (Risso), is a serious problem especially on oranges of the variety "moro" . Associated with this species we find indigenous parasitoids such as Leptomastidea abnormis (Grlt.) and Anagyrus pseudococci (Grlt.), and the coccinellid Cryptolaemus montrouzieri Muls., which is now established in Calabria. Unfortunately such entomophagous are not able to maintain P.citri below the economic threshold. For this reason it should be necessary, as already done before (14) to breed and to release exotic parasites such as Leptomastix dactylopii (How.).

However chemical control is often used.

The long tailed mealybug, Pseudococcus longispinus Targ., has been surveyed sporadically in quantities which were harmless for citrus.

As far as the black scale, Saissetia oleae (Oliv.), is concerned, chemical control is often necessary. In the areas taken into account, we surveyed the parasites Scutellista cyanea Mots., Metaphycus lounsbury (How.) and Metaphycus flavus (How.) and the predators Chilocorus bipustulatus (L.), Exochomus quadripustulatus (L.). Unfortunately also in this case the entomophagous are not able to control the population. Moreover we also noticed the fungus Verticillium lecanii (Zimm.) Viegas. This last one, however, has been found only in two specimens collected at Lamezia Terme.

The hemispherical scale, Saissetia coffeae (Walk.), has been surveyed sporadically only on oranges located in the town of Reggio Calabria.

As far as other lecanids are concerned, we found the fig wax scale, Ceroplastes rusci (L.), the chinese wax scale, Ceroplastes sinensis D.G., and the brown scale, Coccus hesperidum L.. The population of these species are usually at low levels because of the presence of parasites which have not been determined up to now. Such parasitism, as in the case of C.hesperidum, is between 85% and 90%.

The diaspidid chaff scale, Parlatoria pergandei (Comst.), is usually present in the three areas surveyed on almost 50 % of the samples, with a population which usually trespasses the economic threshold (3). For this reason the intervention is necessary.

The purple scale, Mytilococcus beckii (New.), is a common species on citrus trees. It has mainly been noticed on oranges and clementines, but it has been harmful only in one sample in the area of Reggio Calabria.

The glover scale, Mytilococcus gloverii (Pack.), already found at Taurianova (12), has been surveyed in Gioia Tauro. A further spread of this species might cause its inclusion among the species which need a particular attention both for the damages likely to be caused by this species which has already shown to be dangerous in Corsica (Benassy, personal communication) and for the probable absence of specific entomophagous.

The California red scale, Aonidiella aurantii (Mask.), has become the key phytophagous especially,after the culture of bergamotto has been progressively abandoned, because of the economic crisis of the essence market. In order to control such species it might be useful to increase the indigenous entomophagous, to introduce the exotic ones, and to use the pheromone traps to check the dynamics of males'flights, in the farm where the chemical control is usually used.

On the other hand, the dictiospermum scale, Chrysomphalus dictyospermi (Morg.), which was the main phytophagous of southern citrus orchards up to the '40 s(5) has almost disappeared.

The ivy scale, Aspidiotus nerii Bouchè, was surveyed at low densities in lemon orchards in Gioia Tauro, Reggio Calabria, Davoli Marina (Catanzaro), Nicastro (Catanzaro), and San Pietro (Cosenza).

MITES

During our survey we found mites belonging to the families Tetranychidae, Eriophyidae, Tydeidae, and Phytoseiidae.

As far as tetranychids are concerned, we found the two-spotted

mite, Tetranychus urticae Koch, and the citrus red mite, Panonychus citri (McGregor). The first one has been mentioned in Calabria since 1958 (8), and has been surveyed in almost all the citrus areas checked by us, though always at low levels. Also P.citri, mentioned in Calabria by Di Martino and Bono in 1974 (8), after an initial outbreak, has been maintained at low levels, usually lower than those caused by T.urticae. In order to control the two above mentioned species, when necessary, chemical spraying is used.

Among eriophyids, we found the citrus bud mite, Eriophyes sheldoni Ewing, which is always present in citrus orchards. This species is not harmful because winter sprays with mineral oils against scales decrease the mite population.

We also surveyed the tydeid mite Lorryia formosa Cooreman, which as it is well known, is a micophagous species, and is considered a sanitizing species on citrus trees (15). It should, however, be pointed out that in 1957 Smirnoff (from 15) has reported it as injurious to citrus trees in Morocco.

Associated with and preying upon the harmful species, we also collected on bergamottos, lemons and oranges the followings phytoseiid mites: Amblyseius stipulatus Athias-Henriot, Seiulus amaliae Ragusa and Swirski, Iphiseius degenerans (Berlese), Typhlodromus rhenanoides Athias-Henriot. The dominant species was A.stipulatus. Such species is also the dominant one collected in citrus orchards in Sicily (17). Unfortunately the presence of these species is not sufficient to control harmful mites.

To conclude, we should continue surveys on the species associated with citrus trees and on their dynamics of population. Moreover we should also deepen our knowledge on the side effects of pesticides in order to choose those which are harmless for useful arthropods.

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NOTES ON POPULATION DYNAMICS OF ARCHIPS ROSANUS(L.) AND CACOECIMORPHA PRONUBANA(HB.) IN SICILIAN CITRUS-GROVES

G.Siscaro & S.Longo
Istituto di Entomologia Agraria, Università di Catania, Italy
S. Ragusa
Istituto di Difesa delle Piante, Università di Reggio Calabria, Italy

SUMMARY

Archips rosanus(L.) and Cacoecimorpha pronubana(Hb.)(Lepidoptera, Tortricidae) are widespread in Sicilian citrus-groves and they are both considered as injurious to the spring vegetation. Investigations on flight dynamics carried out during 1987 by means of pheromones traps, correlated with morphological observations on male genitalia, showed that the adults of A.rosanus occur from the middle of May to the end of June, whereas the adults of C.pronubana are present throughout the year.
Samples of preimaginal stages collected on citrus sprouts and other plants growing in the same areas, confirmed that only A.rosanus live on Citrus plants, whereas C.pronubana larvae have been found only on weeds and Olive plants.
Studies on A.rosanus parasites have been carried out since 1970.
A complex of eleven Hymenopterous species has been found till now, nine of them are larval parasitoids, the remaining two parasitize the pupae.
The parasitization rate reached by the above mentioned complex of species ranged from 5 to 40%.

1.INTRODUCTION

Archips rosanus (L.) and Cacoecimorpha pronubana(Hubner)(Lepidoptera,Tortricidae) are polyphagous species occurring in citrus-groves in the Mediterranean Basin.

Although C.pronubana is of Mediterranean origin and holds remarkable importance on Morocco citrus-groves (5), it is not considered injurious to these orchards in Sicily (9) and in Algeria(8).

Infestations by A.rosanus have been recently recorded in Sicily (2) and in Campania (6); this species, which is widespread in the palaearctic area, presently occurs both in the main Italian citrus-groves(3) and in Greek ones(7).

The present studies on population dynamics of the two Tortricoid species began in Sicily with the aim of defining their bio-ethology in relation to the host plants, subsequently pointing out their role within the insect fauna of this agrosystem.

2.MATERIALS AND METHODS

The flight dynamics of the two species have been studied in Eastern Sicily, starting from 15th of April 1987, in a 2 Ha large citrus orchard by setting simultaneously 6 pheromone traps (Traptest Agrimont), 3 of which were primed with the synthetic pheromone of A.rosanus (Z-11-tetradecenil-acetate:0.9 mg and Z-11-tetradecenol:0,1 mg) and 3 primed with the pheromone of C.pronubana (Z-11-tetradecenil-acetate: 4,5 mg ,Z-11-tetradecenol:0,25 mg, Z-9-tetradecenol:0,25 mg and tetradecyl acetate:5 mg).

Catches by the traps were controlled about every ten days while, the dispensers were replaced at a monthly interval.

Owing to the macroscopic similarity between the males of the two species, the captured adults were removed from each trap and subjected to a suitable chemical treatment, with the aim of studying the genitalia for specific discrimination.

At the same time, sprouts and leaves were collected from Citrus and Olive plants and from weeds, in the investigated biotopes and in other areas where Citrus-trees are cultivated.

The collected larvae were bred separately in suitable containers till the emergence of adults or of their parasitoids in order to identify the species.

3.RESULTS AND DISCUSSION

Data in Tab.1, Fig.1 and Fig.2 regard the catches carried out by means of the mentioned traps. They emphasize the different life-cycles of the two species. In fact, C.pronubana shows an almost continuous development in our climatic conditions, with several flight peaks in April, June-July and October; thus the homodynamic trend of the species is confirmed, since it performs a variable number of generations per year, which is related to the environmental conditions, and ranges from two (in Central Northern Europe) to six (in Northern Africa)(4).

The flights of A.rosanus adults took place in a restricted period that, in the considered citrus-groves, began in the middle of May and finished at the end of June. This fact confirms the monovoltine behaviour of the species also in Southern environments.

The pheromones used in the present study did not show a marked selectivity on the males of the two species. In fact, at the emergence of A.rosanus adults, both types of traps captured almost exclusively specimens of this species. On the contrary, the catches of C.pronubana males were carried out by the traps primed with the specific pheromone of A.rosanus in almost the whole period of field exposition.

The samples of preimaginal stages collected in citrus-groves and on other host plants evidenced that only A.rosanus larvae live on Citrus spring vegetation, whereas C.pronubana has been found only on weeds of citrus-groves and on Olive plants (3)(5)(9).

Bio-ethological observations on the two species confirmed what is already known from previous studies.

A.rosanus adults are present in fields from the end of May to the end of June; after mating the female ovoposits about 300 eggs on the bark of the trunk and main branches, arranged in characteristic egg-masses each one made up by 50 to 150 units.

Tab. 1-Captures carried out in Sicilian citrus-groves in 1987

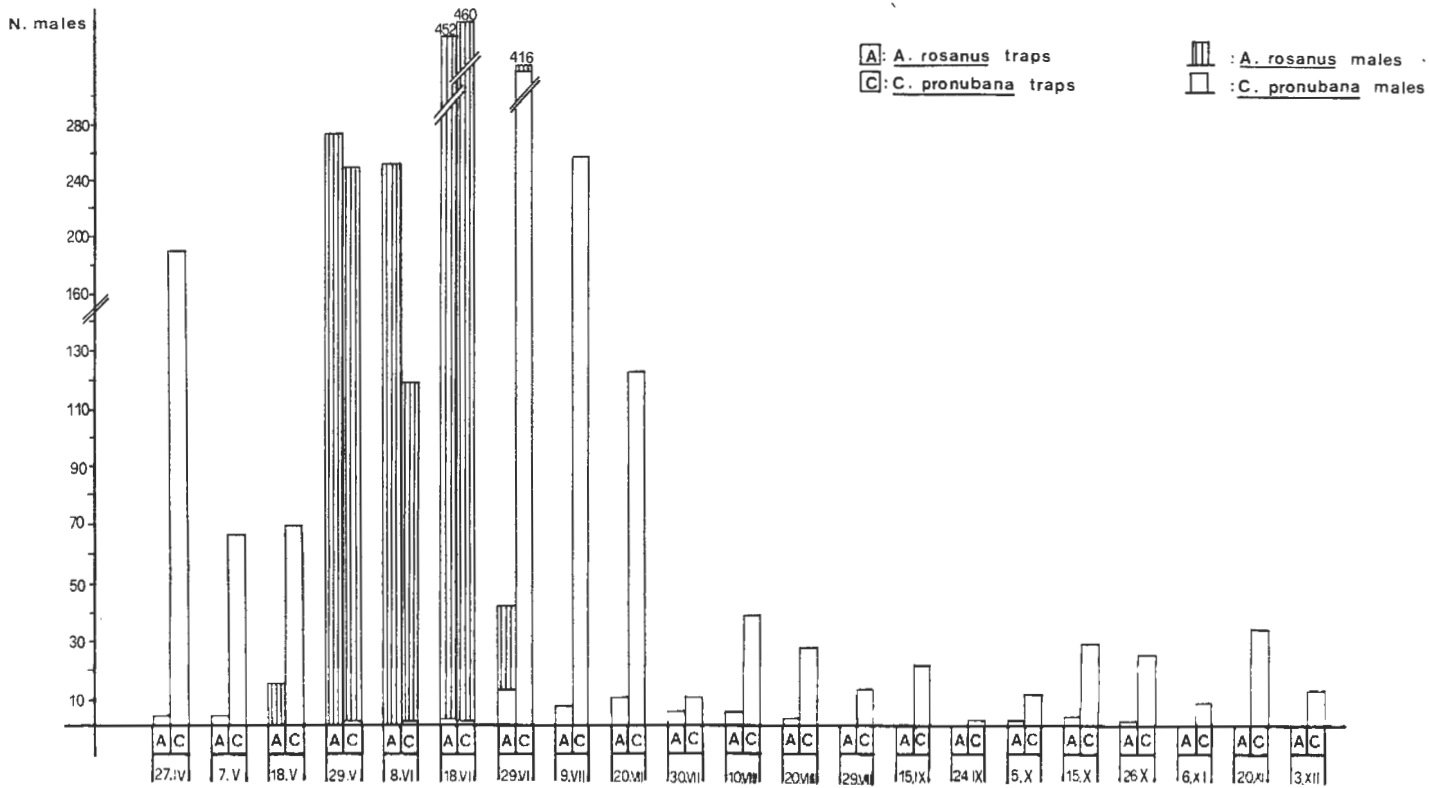
	C.pronubana TRAPS						A.rosanus TRAPS					
n.traps	1		2		3		1		2		3	
species	C*	A*	C	A	C	A	C	A	C	A	C	A
27/4	102	-	46	-	44	-	3	-	-	-	1	-
7/5	38	-	18	-	11	-	3	-	1	-	-	-
18/5	40	-	18	-	12	-	1	6	-	5	-	3
29/5	-	70	-	73	1	90	-	81	-	115	-	79
8/6	1	58	-	28	1	30	-	94	-	76	-	83
18/6	196	-	147	-	115	1	1	141	1	155	1	153
29/6	214	-	80	-	111	1	13	-	-	14	-	15
9/7	138	-	52	-	78	-	1	-	2	-	4	-
20/7	50	-	31	-	42	-	3	-	3	-	4	-
30/7	5	-	1	-	5	-	1	-	2	-	2	-
10/8	22	-	7	-	9	-	1	-	3	-	-	-
20/8	13	-	10	-	4	-	1	-	-	-	-	-
29/8	9	-	-	-	4	-	-	-	-	-	-	-
15/9	3	-	6	-	12	-	-	-	-	-	-	-
24/9	-	-	-	-	1	-	-	-	-	-	-	-
5/10	4	-	6	-	2	-	1	-	-	-	-	-
15/10	16	-	7	-	6	-	2	-	-	-	1	-
26/10	8	-	12	-	15	-	1	-	-	-	-	-
6/11	2	-	5	-	1	-	-	-	-	-	-	-
20/11	20	-	9	-	5	-	-	-	-	-	-	-
3/12	8	-	4	-	1	-	-	-	-	-	-	-

C*: C.pronubana males - A*:A.rosanus males

Tab. 2- Composition of the parasitic biocenose of A.rosanus and C.pronubana in Sicilian citrus-groves

A.rosanus	C.pronubana
PARASITES	
(Hymenoptera, Ichneumonoidea)	
Macrocentrus rossemi H.A.	Apanteles sp.
Apanteles sotades Nixon	Campoplex borealis(Zett.)
Hypomicrogaster suffolciensis(M.)	Campoplex sp.
Campoplex restrictor Aubert	Venturia canescens(Grav.)
Campoplex sp.	Pristomerus vulnerator(P.)
Pimpla instigator F.	
(Hymenoptera, Chalcidoidea)	
Brachymeria intermedia(Nees)	Trichogramma evanescens(W.)
Elachertus sp.	
(Diptera,Tachinidae)	
	Actia pilipennis(Fall.)
HYPERPARASITES	
(Hymenoptera,Chalcidoidea)	(Hymenoptera, Ichneumonoidea)
Eurytoma sp.	Stictopisthus sp.
Habrocytus sp.	

Fig.1 - Captures of Archips rosanus (L.) and Cacoecimorpha pronubana (Hb.) males carried out in Sicilian citrus-groves during 1987.



the latter are joined with a silken thread and feed upon the inside. Trophic activity may interest also the flowers. Reaching the maturity A.rosanus larvae pupate inside these shelters and emerge after about two weeks.

C.pronubana has a variable number of generations per year, correlated to the environmental conditions. In the investigated biotopes and in other citrus-groves in Eastern Sicily , its infestations neither occurred on spring vegetation nor on the summer-autumnal one.

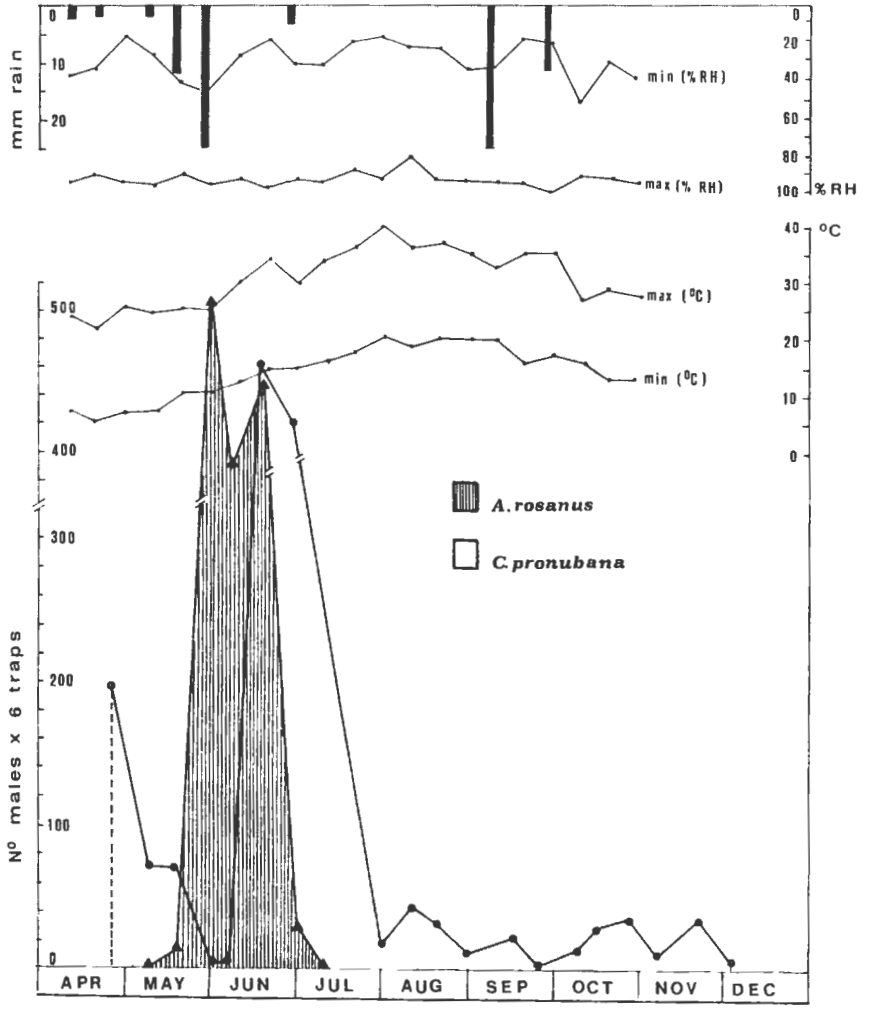


Fig.2 - Climatic state and flight dynamics of Archips rosanus (L.) and Cacoecimorpha pronubana (Hb.) males in Sicilian citrus-groves during 1987.

The newly hatched larvae move on the leaves of the new sprouts; the latter are joined with a silken thread and feed upon the inside. Trophic activity may interest also the flowers. Reaching the maturity A.rosanus larvae pupate inside these shelters and emerge after about two weeks.

C.pronubana has a variable number of generations per year, correlated to the environmental conditions. In the investigated biotopes and in other citrus-groves in Eastern Sicily , its infestations neither occurred on spring vegetation nor on the summer-autumnal one.

The investigations carried out on the parasitic biocenose of the two Tortricoid species showed the presence on A.rosanus of eleven Hymenopterous species, two of which are hyperparasites, whereas with regard to C.pronubana, eight Hymenopterous species have been reported, (one of which is a hyperparasite) and one Diptera Tachinidae (see Tab.2).

An oophagous Hymenopterous species Trichogramma evanescens Westwood,has been found only on C.pronubana , whereas Trichogramma cacoeciae Marchal, reported in France and in Switzerland as an important natural antagonist of A.rosanus(1), did not occur in Sicily.

It is significant to point out that, though only unspecific parasites were collected up to now (already reported as parasites on several Lepidoptera species), the composition of parasitic biocenose living on the two species is quite different.

4.CONCLUSIVE CONSIDERATIONS

C.pronubana and A.rosanus are both present in Sicilian citrus-groves, yet only the latter lives on Citrus, having one only generation per year.

C.pronubana performs 4 generations per year, it did not occur on Citrus,but on various weeds living in citrus-groves and on Olive plants.

The unspecificity of presently available commercial synthetic pheromones did not allow to individualize the relative period of flight of the two Tortricoid species, without the morphological observation on male genitalia.

The chemical control of A.rosanus on citrus-groves is not necessary, owing to the parasitic biocenose living on this species and the negligible damages it causes to plants.

ACKNOWLEDGEMENTS

We thank Prof. G.Viggiani and Dr. K. Horstmann for the identification of Chalcidoidea and Ichneumonidae species respectively.

K.W.: Citrus, Lepidoptera, Tortricidae

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CITRUS MITES IN THE MEDITERRANEAN AREA

V.VACANTE, A.NUCIFORA and G.TROPEA GARZIA

Institute of Entomology
Via Valdisavoia n°5
University of Catania (I)

Summary

The authors present an investigation on the mite fauna on citrus orchards in the Mediterranean area. Notes are given on the feeding habits of each family and distribution of every single species.

A. INTRODUCTION

During a first investigation, put into effect with the purpose of selecting and listing the mite-fauna of Italian citrus orchards, 64 species belonging to 17 families have been found (38) (35). Further researches made on citrus bark mites in Sicily (39) led to the discovery of 14 more species, belonging to 12 different families, six of which aren't listed among the species found on same citrus foliage.

We are then concerned with 23 families with 78 species. If we add to this figure 6 species of phytoseiids recovered by other authors (22)(23)(40) which have not previously been found during our researches and another two species recently recorded in Liguria and Sicily (37), we reach the number of 86 species recovered in Italy up today as a whole.

Before doing new researches in Italy and widen them to the Mediterranean basin, we considered useful to take a census, as complete as possible, of the species recovered up to now in the countries of the basin itself.

Bibliographical research allowed us to list 164 species belonging to 26 different families.

Here follows a list, ordered in families, of the species recovered and of those registered in literature.

B. LIST OF THE SPECIES

1. Family TETRANYCHIDAE Donnadieu	Country and references
Aplonobia histricina (Berlese)	I(38)
Bryobia praetiosa Koch	I(38),TR(11)
Epitetranychus althaeae (von Hanstein)	TR(11)
Eutetranychus orientalis (Klein)	CY(14),ET(19)(26),GR(*),RL(10) IL(15)
Eutetranychus pyri Attiah	ET(19)
Eutetranychus africanus (Tucher)	ET(19)

(*) Personal communication of dr Bakoyannis of Plant Protection Institute, Valos (Greece).

Panonychus citri (McGregor)	F(24),GR(*),IL(29),I(38),YU(20), RL(34),LT(36),P(8),E(13),TR(11)
Panonychus ulmi (Koch)	GR(28)
Petrobia tunisiae Manson	I(38)
Tetranychina harti (Ewing)	E(12)
Tetranychus urticae Koch	GR(28),I(38),RL(10),LT(36),P(6)
Tetranychus cinnabarinus (Boisduval)	ET(23),GR(28),RL(10),MA(7),E(27)
Tetranychus telarius (L.)	ET(25),IL(15),P(6)
Tetranychus turkestani Ugarov & Nikolski	YU(20)

2. Family TENUIPALPIDAE Berlese

Anopalpus pulcher(Canestrini & Fanzago)	ET(26)
Brevipalpus californicus (Banks)	DZ(2),CY(14),ET(26),GR(*),IL(15), I(38),LT(36),P(6),E(12)
Brevipalpus obovatus Donnadieu	CY(14),ET(26),GR(*),IL(15),I(38), YU(20),RL(10),LT(18),E(12)
Brevipalpus lewisi McGregor	DZ(2),ET(26),GR(28),YU(20),RL(10), LT(36),P(6)
Brevipalpus phoenicis (Geijskes)	ET(26),GR(28),IL(15),I(38),LT(36), MA(7),P(6),E(12)(27)
Brevipalpus cuneatus (Canestrini & Fanzago)	I (38)
Cenopalpus pulcher (Canestrini & Fanzago)	ET(26),GR(28),P(6)
Tenuipalpus granati Sayed	GR(*)

3. Family ERIOPHYIDAE Nalepa

Aceria sheldoni (Ewing)	DZ(2),CY(14),F(24),GR(28),IL(15), I(38),RL(9),LT(36),MA(7),P(6), E(27),TR(11),TN(24)
Aculops pelekassi (Keifer)	GR(28),I(38),YU(20)
Phyllocoptruta oleivora (Ashmead)	CY(14),ET(26),GR(28),IL(16),RL(9), TR(11)

4. Family TYDEIDAE Kramer

Homeotydeus mali (Oudemans)	ET(26),IL(15),E(12)
Metalorryia magdalenae (Gerson)	IL(15),I(38)
Orthotydeus foliorum (Schrank)	I(38),LT(36)
Orthotydeus caudatus (Dugés)	I(38),E(12)

Orthotydeus californicus Banks	ET(26),IL(15),I(38),E(12),P(5)
Orthotydeus kochi (Oudemans)	ET(26),I(38),E(12)
Pronematus ubiquitus (McGregor)	ET(26),IL(15),I(38),E(12)
Triophtydeus triophthalmus (Oudemans)	I(38)
Tydeus teresae (Carmona)	I(38)
Tydeus australensis Baker	I(38)
Tydeus ferulus (Baker)	I(38),LT(36),P(5),E(12)
Tydeus reticulatus Oudemans	I(38),E(12)
Tydeus formosa (Cooreman)	DZ(2),I(38),LT(36),MA(7),P(5)(16) E(27)

D. Family STIGMAEIDAE Oudemans

Agistemus exsertus Gonzalez	ET(26),IL(15)
Agistemus cyprius Gonzalez	E(12)
Agistemus industani Gonzalez	IL(15)
Agistemus collyerae Gonzalez	I(38)
Eryngiopus pini (Canestrini)	ET(26)
Eryngiopus siculus Vacante & Gerson	I(37)
Eryngiopus bifidus Wood	I(37)
Ledermuelleriopsis plumosus Will.	I(38)
Mediolata similans Gonzalez	I(39),IL(15)
Stigmaeus lutens (Summers)	ET(26)
Zetzellia mali (Ewing)	YU(20),I(38)
Zetzellia graeciana Gonzalez	I(38)
Zetzellia languida Gonzalez	P(8)
Zetzellia hispanica Gonzalez	E(12)

E. Family CHEYLETIDAE Leach

Cheletogenes ornatus (Canestrini & Fanzago)	ET(26),IL(15),I(38),LT(36),E(12)
Cheletomimus minutus Soliman	I(38)
Cheletomimus berlesei (Oudemans)	IL(15),I(38),E(12)
Cheletomimus duosetosus (Muma)	E(12)
Cheletomorpha lepidopterorum (Shaw)	E(12)
Eutogenes citri Gerson	IL(15),I(38)
Hemicheyletia bakeri (Ehara)	ET(26),IL(15)
Hemicheyletia wellisi (Baker)	IL(15)

7. Family HEMISARCOPTIDAE Oudemans	
Hemisarcoptes coccophagus Meyer	IL(15),E(12)
Hemisarcoptes malus (Shimer)	I(38),LT(36)
8. Family ANYSTIDAE Oudemans	
Anystis baccarum (L.)	IL(15),I(39)
9. Family BDELLIDAE Dugés	
Bdella captiosa Atyeo	I(39)
Bdellodes longirostris (Hermann)	E(12)
Bdellodes lapidaria (P.Kramer)	IL(15)
Cyta latirostris (Hermann)	IL(15)
10. Family EUPALOPSELLIDAE Willmann	
Eupalopsis maseriensis (Canestrini & Fanzago)	IL(15),I(39)
Eupalopsis aegyptiaca (Zaher & Sol.)	ET(26)
Saniosulus nudus Summers	ET(26),IL(15),E(12)
11. Family RAPHIGNATHIDAE Kramer	
Raphignathus gracilis (Rack)	IL(15),I(39)
12. Family PTERYGOSOMIDAE Oudemans	
Hirstiella insignis (Berlese)	I(39)
13. Family CUNAXIDAE Thor	
Cunaxa capreolus (Berlese)	I(38)
Cunaxa setirostris (Hermann)	ET(26),I(38),E(12)
Cunaxoides oliveri (Schruft)	I(39)
Cunaxoides americanus Baker	E(12)
14. Family CAMEROBIIDAE Southcott	
Neophyllobius citri (Zaher & Sol.)	ET(26)
Neophyllobius aegyptum (Zaher & Sol.)	ET(26)

Neophyllobius lamimani (McGregor)	ET(12)
Neophyllobius burrellis (McGregor)	ET(12)

15. Family PHYTOSEIIDAE Berlese

Amblyseiella setosa (Muma)	ET(12)
Amblyseius libanesi Dosse	RL(16)
Amblyseius messor Wainstein	IL(31),I(22)
Amblyseius swirskii Athias-Henriot	ET(26),IL(31),I(38)
Amblyseius aberrans (Oudemans)	GR(28),I(38),YU(20)
Amblyseius finlandicus (Oudemans)	GR(28),IL(21),I(23)
Amblyseius meridionalis (Berlese)	GR(28)
Amblyseius citri (Meyer & Ryke)	GR(28)
Amblyseius stipulatus Athias-Henriot	DZ(3),GR(17),I(38),YU(20),E(12)(17), TR(17)
Amblyseius barkeri (Hughes)	IL(31),I(38)
Amblyseius rubini Swirski & Amitai	IL(31),TR(32)
Amblyseius setosus (Muma)	IL(21)
Amblyseius concordis Chant	YU(20)
Amblyseius vivax Chant & Baker	YU(20)
Amblyseius umbraticus Chant	YU(20)
Amblyseius zwölfrei Dosse	YU(20)
Amblyseius tiliarum Oudemans	YU(20)
Amblyseius californicus (McGregor)	I(38),E(17)
Amblyseius potentillae (Garman)	I(38),E(12)
Amblyseius bordjelaini Athias-Henriot	E(12)
Amblyseius italicus(Chant)	I(23)
Amblyseius largoensis (Muma)	I(40)
Anthoseius recki (Wainstein)	IL(1)
Anthoseius hierochunticus (Amitai & Swirski)	IL(1)
Anthoseius phialatus (Athias-Henriot)	I(23),E(12)
Euseius scutalis (Athias-Henriot)	E(12)
Iphiseius degenerans (Berlese)	GR(17),IL(30),I(38),TR(17)
Neoseiulus aleurites Ragusa & Athias-Henriot	E(12)
Neoseiulus yugoslavicus Mijskovic & Tomasevic	YU(20)

Phytoseius plumifer (Canestrini & Fanzago)	GR(28)
Phytoseius finitimus Ribaga	IL(21),I(38),YU(20),E(12),TR(32)
Phytoseius panormita Ragusa & Swirski	I(23)
Phytoseius horridus Ribaga	YU(20)
Phytoseiulus macropilis Banks	YU(20)
Phytoseiulus persimilis Athias-Henriot	I(38),E(12)
Tetramedius citri Zaher & Sheata	ET(26)
Typhlodromus pyri Scheuten	ET(25),IL(30),YU(20)
Typhlodromus rhenanus (Oudemans)	CY(14),IL(41),YU(20)
Typhlodromus talbii Athias-Henriot	GR(17),IL(41),I(38),E(17)
Typhlodromus tiliae Oudemans	CY(14)
Typhlodromus athiasae Porath & Swirski	GR(17),IL(21),TR(17)
Typhlodromus bakeri Garman	YU(20)
Typhlodromus cryptus Athias-Henriot	GR(33),IL(1),I(38)
Typhlodromus rhenanoides Athias-Henriot	I(38),E(12)
Typhlodromus athenas Swirski & Ragusa	I(38)
Typhlodromus exhilaratus Ragusa	I(38)

16. Family ASCIDAE Voigts & Oudemans

Melichares dentriticus (Berlese)	IL(15)
Proctolaelaps bickleyi (Bram)	ET(26)
Proctolaelaps pygmaeus (Muller)	ET(26),I(38)

17. Family ACARIDAE Ewing & Nesbitt

Tyrophagus putrescentiae (Schrank)	ET(26),IL(15),I(38),LT(36)
Tyrophagus tropicus Robertson	I(38)
Tyrophagus palmarum Oudemans	I(39)
Thyreophagus entomophagus (Laboulbene)	I(38)
Thyreophagus corticalis (Michael)	I(38)

18. Family GLYCYPHAGIDAE Berlese

Glycyphagus domesticus (De Geer)	I(38)

19. Family SAPROGLYPHIDAE Oudemans

Calvolia hebeclinii (Sicher)	I(38)

20. Family TARSONEMIDAE Kramer

Daidalotarsonemus vandevrieri Suski	I(39)
Fungitarsonemus monasterii (Lombardini)	I(38)
Polyphagotarsonemus latus (Banks)	GR(28),I(38),MA(7)
Tarsonemus setifer Ewing	MA(16),IL(15),E(12)
Tarsonemus confusus Ewing	I(39)
Tarsonemus sulcatus Beer	E(12)
Tarsonemus cryptocephalus (Ewing)	P(5),E(12)
Tarsonemus nr randsi Ewing	IL(15)
Tarsonemus aurantii Oudemans	IL(15),I(38)
Tarsonemus muhlei Wetzel	IL(15)
Tarsonemus nodosus Schaarschmidt	IL(15)
Tarsonemus occidentalis Ewing	P(5)
Tarsonemus smithi Ewing	ET(26),IL(15),I(38),LT(36),P(4)
Tarsonemus unguis Ewing	I(38)
Tarsonemus waitei Banks	I(38),LT(36)
Tarsonemus bakeri Ewing	I(38)
Tarsonemus setifer Ewing(sensu Karl, 1965)	I(39)(35)

21. Family MICREREMIDAE Grandjean

Micreremus brevipes (Michael)	E(12)
Micreremus gracilior Willman	I(38)

22. Family CERATOZETIDAE Jacot

Humerobates rostrolamellatus Grandjean	CY(14),GR(28),I(38)
Trichoribates angustatus Mihelcic	I(38)

23. Family ORIBATULIDAE Thor

Dometorina plantivaga (Berlese)	E(27)

Oribatula tibialis (Nicolet)	E(12)
Phauloppia lucorum (Koch)	I(39)
Siculobata sicula (Berlese)	ET(26),IL(15),I(38),LT(36)

24. Family PELOPIDAE Ewing

Eupelops acromios (Hermann)	I(39)

25. Family CYMBAEREMEIDAE Willmann

Scapheremaeus corniger (Berlese)	E(14)

26. Family TUCKERELLIDAE Baker & Pritchard

Tuckerella nilotica Zaher & Ramsy	ET(42)

C. CONCLUSION

We didn't take into account the correct systematic position of every single species in this work because we didn't have all the slides we needed. May be that some species of the list will be synonyms in a further systematic and comparative analysis.

From the above-reported list you can see that the 46 species of phytoseiids represent about 1/4 of the total number of the species found in the Mediterranean basin. Another family rich in species (14 as a whole) is the Stigmaeidae one, which for the great part are useful mites for their active ability of preying.

Stigmaeidae (14 species), the 8 species of Cheyletidae, the 19 species of Hemisarcoptidae, Anystidae and Bdellidae, Eupalopsellidae, Raphignathidae, Cunaxidae and Camerobiidae have multivalent predatory capacity against phytophagous mites and young scales. The 3 species of the family Ascidae are predacious as much as Phytoseiidae. Thence we have 44 predatory species that added to the 46 species of phytoseiids (46 in all) get 90 acarophagous or acaro-entomophagous species recovered on Citrus in the Mediterranean basin.

Tydeidae reach also a wide number which isn't ascertained but however they must be included among the beneficial mites for the cleaning done by some of theme.

Besides the above-mentioned Tydeidae, there are beneficial species differently useful among Acaridae, Glycyphagidae, Saproglyphidae whit coprophagous, saprophagous or micetophagous diet and among Oriobatulidae, Pelopidae, Cymbaeremeidae, Micreremidae and Ceratozetidae with preponderant microphytophagous feeding habit. As a whole we have 17 species that, added to the 13 species of Tydeidae, get a wide number of useful arthropods, both for their activity of "scavangers" on plants and as a substratum of feeding for the most cospicuous group mentioned before.

Among Tarsonemidae 16 species (of the 17 in list) have a micetophagous diet prevalently. Only one of them has phytophagous feeding habits.

In the end there are four families (Tetranychidae, Tenuipalpidae, Eriophyidae and Tuckerellidae) which comprehend phytophagous species, ascertained or supposed.

We are then concerned with 26 species, only some of which were esteemed particularly harmful in the citrus areas of the Mediterranean basin; harmfulness caused by them occurs where, by ignoring the biological power exerced by the predatory species and breaking the natural balance, people indulge indiscriminately in wide-spectrum chemical treatments.

Abbreviations used in the text:

I (ITALY); TR (TURKEY); CY (CYPRUS); ET (EGYPT); GR (GREECE); RL (LEBANON); IL (ISRAEL); F (FRANCE); YU (YUGOSLAVIA); LT (LYBIA); P (PORTUGAL); E (SPAIN); MA (MAROCCO); DZ (ALGERIA); TN (TUNISIA).

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HOST-FEEDING OF *APHELINUS CHAONIA* WALKER (HYM. APHELINIDAE) PARASITOID OF *TOXOPTERA AURANTII* (B.d.F.) (HOM. APHIDIDAE) (*)

G. Liotta
Istituto di Entomologia agraria, Università di Palermo, Italia

Summary

Aphelinus chaonia Walker is commonly found on *Toxoptera aurantii* (B.d.F.) in Sicily. This parasitoid is mainly active during autumn and spring time when there are outbreaks of the aphid.
A. chaonia, as many Aphelinids, exhibits host-feeding.
In laboratory trials, using *Aphis fabae* Scop. as host the host-feeding/parasitization ratio was 1:1, when we gave 5 aphids/day; it was 1:1.4 when 10 aphids/day were supplied.
The average of aphids killed by a female of *A.chaonia* giving 5 and 10 aphids/day was 12.5 and 30.5 as for as host-feeding is concerned and 25.2 and 43.5 as for as parasitization is concerned respectively.

1. Introduction

Aphelinus chaonia Walker is an Aphelinid often found in Sicily on *Aphis fabae* Scop. and on *Toxoptera aurantii* (B. d. F.), mainly in the winter-spring period and in autumn.

Previous biological data about this species (11) concerned also host-feeding, i.e. preying upon host species practised by many parassitoid Hymenoptera, are given.

Host-feeding in Hymenoptera has been known for a long time. Marchal (12) first noticed that *Tetrastichus xantomelaenae* (Rond.) (Hym. Eulophidae), parasite on the eggs of *Galerucella luteola* (Müller) (Col. Chrysomelidae), may consume body fluid of its host. The same author (13) observed a similar behaviour in *Archenomus bicolor* How. (Hym. Aphelinidae) on *Lepidosaphes ulmi* (Hom. Diaspididae). Host-feeding was later found out in several Chalcidoidea parasitoids and also in *Aphytis* of the *proclia* (Walk.) group on *Aonidiella aurantii* (Mask.) (Hom. Diaspididae) (14); in *Aphelinus lapisligni* How. on *Aphis baker* Gowen, in *Aphelinus asychis* (Walker) on many hosts (9, 17, 10, 6), in *Aphelinus thomsoni* Graham on *Drepanosiphum plata-*

(*) *Work supported by Ministero della Pubblica Istruzione's founds (60%).*

noides Sehrk. (Hom. Aphididae), in *Aphytis chilensis* How. on *Aspidiotus nerii* Bouchè (Hom. Diaspididae) (1).

Host-feeding is apparently connected with the parasitoids need to assimilate proteinic substances useful for the production of eggs (7, 5, 2, 16),and it may play a very relevant role in the control of noxious species.

This work aims to determine quantitatively the importance of host-feeding practised by the same species.

2. Materials and Methods

Trials were carried out in laboratory at a temperature of 25 ± 1 °C, with 75 ± 5 % R.H.

Aphid mummies, due to parasitization by *Aphelinus chaonia* Walker, were taken from a colony of *A.fabae* breed on *Galium aparinae* L. and individually placed in gelatin capsules.

As soon as *A.chaonia* adults emerged, one female was taken and got to mate with a male emerged a few days earlier. One day later the fertilised female was placed inside a cage containing a bud of *G.aparinae* infested with five or ten 3-8 days old larvae. 24 hours later the female was removed and placed inside another cage containing another bud infested with the same number of aphids as the previous one. 8 females were studied : 4 were supplied with 5 aphids/day and 4 with 10 aphids/day.

The following data were taken into consideration:

- the number of killed aphids per day
- the percentage of aphids killed by host-feeding
- the number of killed aphids per female
- the host-feeding/parasitization ratio.

3. Results and Discussion

Results are reported in Figs. 1, 2 and 3.

3.1. Five aphids per day (fig.1):

- the comprehensive longevity of the four females was 36 days;
- the number of aphids killed by host-feeding was 50, equivalent to 1.4 aphids per day, i.e. 12.5 aphids per female;
- the number of aphids parasitized was 51, equivalent to 1.4 aphids per day, i.e. 12.7 aphids per female;
- the total number of killed aphids, both by host-feeding and by parasitization was 101, equivalent to 2.8 aphids per day and to 25.2 aphids per female;
- the host-feeding/parasitization ratio was 1:1.

3.2. Ten aphids per day (fig.2):

- the comprehensive longevity of the four female was 48 days;

- the number of aphids killed by host-feeding was 122, equivalent to 2.5 aphids per day, i.e. 30.5 aphids per female;

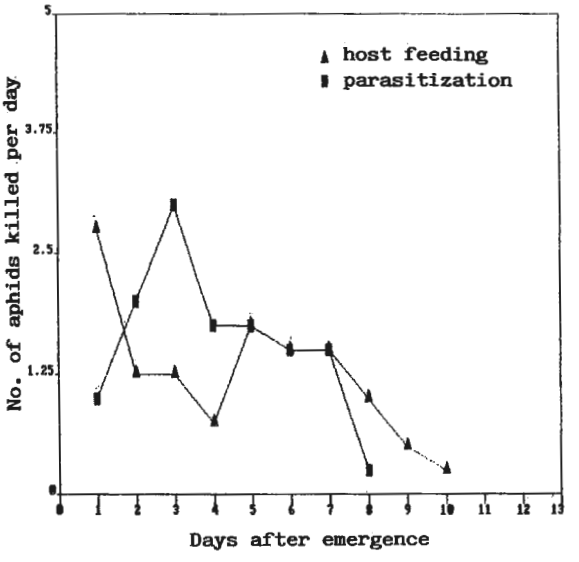

Fig. 1. Aphids killed by inividual female of *A. chaonia* (density: 5 aphids per day)

- the number of aphids parasitized was 174, equivalent to 3.6 aphids per day, i.e. 43.5 aphids per female;
- the total number of killed aphids, both by host feeding and

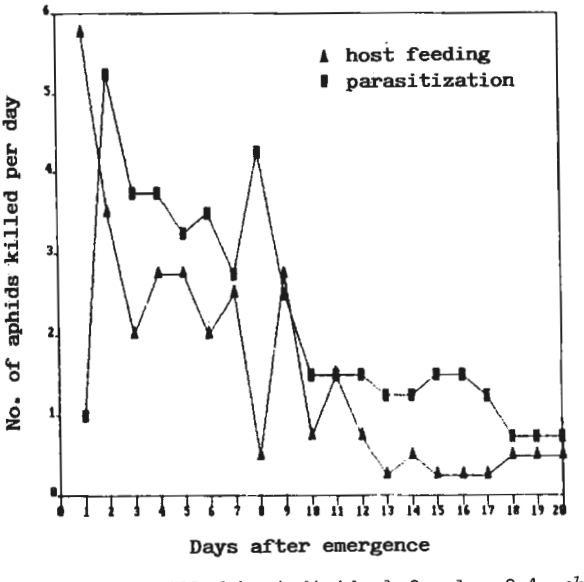

Fig. 2. Aphids killed by individual female of *A. chaonia* (density: 10 aphids per day)

by parasitization was 296, equivalent to 6.2 aphids per day and to 74 aphids per female.

- the host-feeding/parasitization ratio was 1:1.4.

In both conditions the highest mortality value due to host-feeding occurred on the first day of the life of the Aphelinid, while the highest mortality value due to parasitization occurred between the second and the third day.

A. chaonia, as well as *A. asychis* (15), can parasitize its host even on the first day of its life. In fact seven females out of eight, besides practising host-feeding, laid fertile eggs on the host.

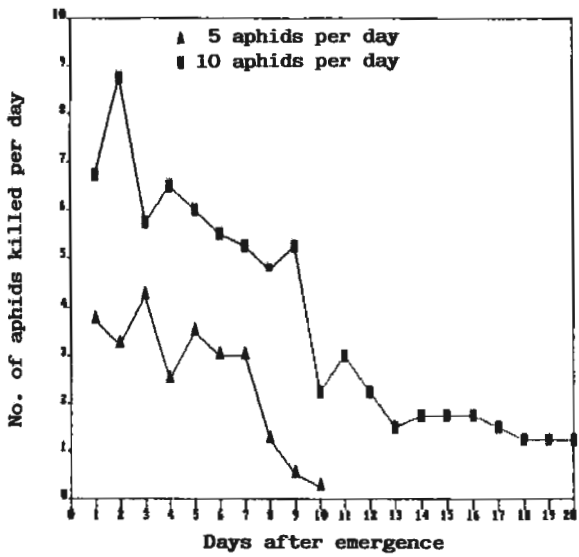

Fig. 3. Aphids killed by individual female of *A. chaonia* by host-feedind + parasitization

This fact suggests that host-feeding is not necessary for the maturation of gonads.

As De Bach (4) and Flanders(7) observed, when the density of the host increased the percentage of parasitization too increased (fig. 3): the host-feeding/parassitization ratio was 1:1 when 5 aphids per day were supplied and 1:1.4 when 10 aphids per day are supplied.

When 10 aphids per day are supplied host-feeding in *A. chaonia* was lower than in *Metaphychus helvolus* (Compere) (1:0.2-0.25) (4) and in *Aphytis chilensis* How. (1:1) (1), but higher than in *A. asychis* (1:7.9) (3)and in *A. thomsoni* (1:1.7) (8).

The comprehensive aphid-killing rate of *A. chaonia* was 6.2 aphids per day, which is higher than that of *A. thomsoni* (3 aphids per day) (8) and lover than that of *A. asychis* (13.4 aphids per day) (3).

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VERTICILLIUM LECANII AS A POSSIBLE CONTROL AGENT OF CITRUS BLACK SCALE IN SICILY.

A.RUSSO
Istituto di Difesa delle Piante, P.zza S.Francesco 2, GALLINA (RC), ITALY
G.MAGNANO DI SAN LIO and S.O.CACCIOLA
Istituto di Patologia vegetale, via Valdisavoia 5, 95123 CATANIA, ITALY
C.ASERO
Istituto di Entomologia agraria, via Valdisavoia 5, 95123 CATANIA, ITALY
entomopathogenic fungus, biological control, Italy

Abstract.
The entomogenous fungus Verticillium lecanii (Zimm.) Viegas was evaluated as biological control agent of citrus black scale, Saissetia oleae (Oliver), in Sicily. Conidial suspensions of the fungus were sprayed on orange trees in citrus groves infested by the scale. In some cases up to more than 80% of scales were parasitized by the fungus. Heavier infestations were observed during winter.

1. Introduction

Verticillium lecanii (Zimm.) Viegas is an entomopathogenic Deuteromycetes with a wide spectrum of hosts. It was applied successfully as microbial insecticide against insect pests of several crops (1,2,4,5,9). Naturally occurring epizootics of this fungus were reported on citrus scales populations in Sicily (Italy)(6,7,8). in this study we evaluated V.lecanii as biological control agent of citrus black scale Saissetia oleae (Oliver). Observations of three years (from May 1985 to December 1987) are summarized.

2. Materials and Methods

Inoculum

A mass isolate of V.lecanii obtained from naturally infected black scales in Sicily was used in the experiments. To produce inoculum the isolate was grown on carrot-broth within 250 ml Erlenmeyer flasks (25 ml of broth/flask). Flasks were incubated for 15-18 days at 25 C in constant light (4.500 lux), or total darkness.

Laboratory tests

The effect of temperature on germination of conidia of V.lecanii was tested in 1% glucose solution within van Tieghem cells. Cells were incubated in the dark at different temperatures. Conidia were considered germinated when germ tube length equalled or exceeded the length of conidium. Germ tube length of at least 100 conidia from three replicate van Tieghem cells was measured at each temperature and time interval. The tests were repeated three times.

Field tests

Suspensions of conidia (final concentrations $5 \cdot 10^5$ to 10^6

conidia/ml of water) were thoroughly sprayed on canopy of 5-years old' Navelina' orange trees, naturally infested by citrus black scale in a citrus grove located at Carlentini (Siracusa). Groups of 2-6 trees were treated at different dates from May 1985 to November 1987. Infections were evaluated by both visual and microscopic examination of vegetative and reproductive structures of the test fungus emerging from the body of parasitized scales. The incidence of mycosis on black scale population was expressed as "apparent parasitation rate" (10).

3. Results

Laboratory tests

Results of laboratory tests are diagrammatically represented in fig.1. Germination of conidia (the infective propagules) was inhibited at 35 C. Temperatures of 5 an 10 C only reduced germination rate, thus indicating that infections of V.lecanii could occur even at low temperatures, provided that other environmental conditions are favourable.

Field tests

In field tests the time interval between inoculation and emergence of mycelium from the body of infected scales (latency period) ranged from two to more than four months according to the date of inoculation. Length of latency period depended upon climatic factors (Tab.I).

In most cases epidemic outbreak of infections of V.lecanii was hampered by both climatic conditions and low population levels of black scale. On the contrary significative percentages of parasitations were obtained in trees inoculated on 25th May 1985 (Tab.II), when density of scale population on twigs was one adult female/cm of length. Mycosed scales were first noticed in October. This first occurrence was associated with 53.5 and 37.5 mm of rainfall during the last and the first decades of September and October, respectively (Fig.2). Percentage of infected scales increased throughout winter until the end of April (Tab.II). Infections of V.lecanii appeared closely associated with rains and were not inhibited by low temperatures, thus confirming the results of laboratory tests. The most susceptible to the infections of V.lecanii among black scale developmental stages appeared the 3rd instar larvae (Tab.II). Moreover percentage of parasititation was higher on twigs than on leaves (Fig.4). Anyway epidemic outbreaks of mycosis lowered black scale population below threshold levels (Fig.3).

Natural spread of the inoculum of V.lecanii occurred from trees sprayed with the fungal suspension to the neighbouring ones. However parasitation levels in naturally infected scale populations maintained low. V.lecanii remained alive for a long time on treated trees. It was recovered from mummified scales up to more than two years from inoculation.

4. Discussion

V.lecanii proved to be an effective biological control agent of citrus black scale. However like other entomogenous fungi it needs high moisture levels and dense scale population to cause epidemic infections. On the contrary it is inhibited by temperatures above 35 c and dry weather. Thus in Mediterranean climate during summer

V.lecanii.
The role of entomogenous fungi as biological control agents of citrus scales is questioned (2,3). Bodenheimer (2) stated that only such entomogenous fungi can be of practical benefit which cause epidemics under summer conditions when scale populations build up. Fawcett and Lee (3) observed that in California scale insects were not controlled to any appreciable extent by fungus parasites. Even in Sicily natural enemies of V.lecanii occur rarely. The results presented here however demonstrate that under favourable environmental conditions artificial inoculation of V.lecanii can lower scale populations below threshold levels. It appears interesting that enemies of V.lecanii occur in a period of the year when other entomophagous parasites do not exert effective control of black scale populations (10). Thus this entomopathogenic fungus seems worthy to be taken in consideration for integrated control programs.

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Tab.I Latency period of V.lecanii infections as affected by climatic factors

Date of inoculation	Latency period (days)	Enviromental factors that affected lenght of latency period
25th May '85	125	rain and high temperatures
20th December '85	118	low temperatures
4th June '86	139	rain and high temperature
13th August '87	90	rain

Tab.II Apparent parasitation rates in a black scale population artificially inoculated with V.lecanii, at different time intervals after inoculation. Inoculation was performed on 25th May 1985.

Developmental stages of the scale	"apparent parasitation rate" %		
	9th Dec.'85	24th Jan.'86	8th May '86
2nd instar larvae	3	4	35
3rd instar larvae	17	51	82
adult females	13	3	45
Total	12	28	69

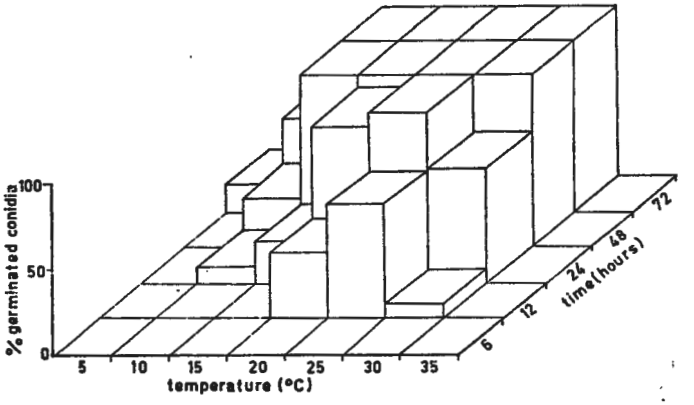

Fig.1 Effects of temperature on germination of conidia of V.lecanii

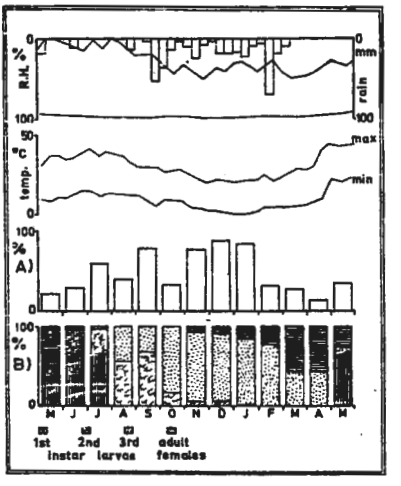

Fig.2 Monthly mortality caused by both abiotic and biotic factors, excluded infections of V.lecanii, A), and seasonal trend of citrus black scale population B), as related to climatic conditions, in the grove located at Carletini.

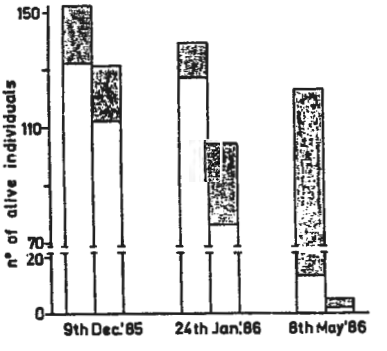

Fig.3 Dynamics of citrus black scale population as affected by infections of V.lecanii. Numbers of individual were determined on 16 randomly collected leaves and 40 cm of twigs. On the left the not inoculated population. White=3rd instar larvae; grey=adulte females. 2nd instar larvae were not considered because their incidence was nor relevant.

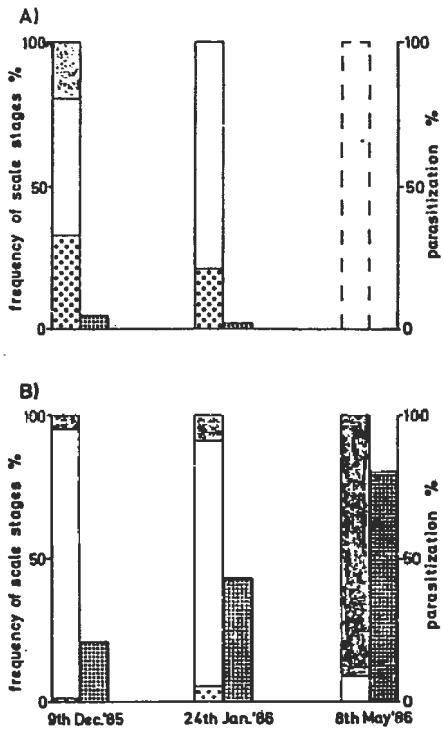

Fig.4 Apparent parasitation rate and dynamics of black scale population artificially inoculated with **V.lecanii**. on twigs (A) and leaves (B), respectively. Dots=2nd instar larvae; white=3rd instar larvae; grey=adult female. In May 1986 average scale population density on twigs increased to six individuals/40 cm.

EFFECTS OF OXYDEMETON-METHYL ON CRYPTOLAEMUS MONTROUZIERI MULS

M. CASTAÑER, A. GARRIDO, T. DEL BUSTO
Institute for Agricultural Research of Valencia (I.V.I.A.)
Moncada, Spain.

Summary

The incidence of oxydemeton-methyl on mortality of various stages of the evolution cycle and on oviposition has been determined on Cryptolaemus montrouzieri Muls, predator of Planococcus citri (Risso).

From the studies conducted, it can be inferred that direct treatment with oxydemeton-methyl causes mortality rates higher than 85% on eggs and 4-day-old larvae, whereas on adults this is about 50%, and on 15-day-old larvae is 8%. Indirect treatment, using two different concentrations of insecticide, has caused mortality exceeding 95% in 4-day-old larvae, and 100% in adults, using the two dosages. In 15-day-old larvae mortality was 44% when using dosis of 0.1% and 28% when the dosis was 0.025%.

Key words: Coccinellidae, Cryptolaemus montrouzieri Muls, Oxydemeton-methyl.

1. Introduction

The economical and ecological importance involved in the utilization of beneficial insects, either parasiting, predating or pathogenic organisms of pest control and the fact that completely omitting use of chemical insecticides appears not to be possible, prompts investigators to study the effects that such compounds may produce on the beneficial fauna, in such a way that both control forms be compatible.

Our objective has actually been the study of the effects of the organophosphorate insecticide oxydemeton-methyl on Cryptolaemus montrouzieri Muls. (Coleoptera, Coccinellidae) an important predator of Planococcus citri (Risso) (Homoptera Coccoidea, Pseudococcidae), one of the most damaging citrus pests (METCALF, FLINT, 1952).

The selection of a determined insecticide among the large variety of products available in the market, was initially restricted to those showing systemic action, i.e., insecticides that are absorbed by the plant, basically roots and leaves, with subsequent translocation throughout vascular systems, at sufficient amounts to allow effectiveness on action points for some time (PRIMO YUFERA, CARRASCO, 1976). Since a systemic insecticide undergoes a translocation in the sap of the plant, while sucking this sap, a phytophagous takes in the compound and, since C. montrouzieri is a predator feeding throughout its life cycle (lifespan) on several specimens of P. citri, an insecticide concentration effect my take place; such a situation does not occur in a parasiting

organism, since this feeds on one single host.

Among a wide range of insecticides we selected oxydemeton-methyl because of its broad spectrum basically as aphicide, and not restricted only to citrus.

The fact that oxydemeton-methyl is now a widely used insecticide has prompted the development of a number of studies about its potential effects on various beneficial insects (SELL, 1985, on Aphidoletes aphidimyza (Rond); MISHRA, SATPATHY, 1985, on Coccinella repanda Th.; BULL, COLEMAN, 1985 on Trichogramma spp.) as well as about compounds closely related, such as oxydemeton-methyl (TEWARI, MOORTHY, 1985, and CHAUDARY et al., 1983, on Menolichus sexmaculatus F.; RATHORE, PATHAK, 1983, on Menolichus sexmaculatus F. Coccinella septempunctata L. and Brumus suturalis F.).

On the other hand, in order to render control efficient -whether chemical, biological or both altogether- an understanding of the beneficial insect biology is essential as to determine how and when to accomplish this control. The knowledge of the biological cycle of C. montrouzieri (GRANDI, 1951; GOMEZ CLEMENTE, 1928) is allowing us to determine the effects of the insecticide on various stages of the insect.

2. Material and Methods

C. montrouzieri rearing was done in the conventional fashion (GOMEZ CLEMENTE, 1928) in a controlled environment chamber with a relative humidity of 60±10% and a temperature of 24±1ºC.

Origin of insect strains

The strain of C. montrouzieri comes from adults originating in mass rearing carried out in the Plant Protection Station at Silla.

Without any records of sex rates, ten transparent plastic cillinders (29 x 10 cm.), both ends covered with muslin, with 15 adults in each one were placed. Food was provided daily, this consisting of potato stems (Solanum tuberosum L.) infested with P. citri; with a binocular magnifying glass (WILD-MSA) the stems from the previous day were observed to collect eggs of C. montrouzieri. The eggs were taken with a thin brush (Nº 0.0) and then placed on petri dishes 10 cm. diam. on filter paper moistened with water, thus providing some humidity to the environment where the C. montrouzieri embryos were to be developed.

In this fashion, the species of C. montrouzieri was maintained; simultaneously obtaining specimens from the various evolutionary stages of the insect needed in the experiments.

The phytophagous P. citri was originating in a species that had been rearing for some time past in the controled environment chamber previously described, in a compartment adjacent to the used for rearing C. montrouzieri.

Utilization of oxydemeton-methyl

A sprayer of the sigma spray type was used, keeping constant spray time and distance, so that the application were homogeneous. Considering the toxicity of the insecticide,the treatments were always done under a campana de extracción de vapores.

To prevent formulation excess on the material under study, it was placed on a precipitate vase covered with a mesh (0.3 x 0.3 mm) to allow drainage of excess insecticide. Two types of trials, direct and indirect were conducted.

2.1. Direct trials

Insecticide was applied at recommended dosages, 0.1% (by the Official Record of Plant Protection Products of the Ministry of Agriculture and Fisheries) on eggs, 4-day-old larvae (1st instar), 15-day-old larvae (4th instar) and adults of C. montrouzieri. Under these conditions we were able to determine the direct effect of oxydemeton-methyl on mortality and oviposition of the predator insect.

Three series of five trials each were conducted for immature stages. One of the series dealt with egg treatment and the other two, with treatment of 4 and 15-day-old larvae respectively. Each trial had 5 replications.

For the egg treatment, these were placed on filter paper circles 5 cm diameter, using 20 in each replication. Once treated, the paper circles were placed into petri dishes of the same diameter. Daily observations were done counting eggs hatched.

Larvae were treated in the same way, placing them, subsequently, into petri dishes (7 cm), using five of them in each replication. Every two days they were provided with fresh food and observed for mortality.

The experiment with adults was composed of one trial with ten replications. The insecticide was applied on newly emerged specimens to avoid possible matings. To make handling easier, adults were anaesthetized exposing them to chloroform vapours for one minute, in a closed container. Sex segregation was done subsequently with the aid of a binocular magnifying glass (WILD-MSA), treating them separately, and finally, three couples were placed into plastic vases (7.5 x 9 cm) covered with muslin.

The results of all tests were compared with the same number of controls, treated with water.

To carry out the study on oviposition, eggs from adults treated with insecticide and control adults were collected daily during two months (approximate insect lifespan). Simultaneously egg hatching was observed to determine their viability.

2.2. Indirect trials

The indirect effect of the insecticide on C. montrouzieri was studied through the food source. Within this section, there were two trial variants as follows:

A. The insecticide was applied on potato stems infested with P. citri at the same dosis as used in the direct treatment (0.1%). Since the absorption of the chemical by the stems was minimum, as no leaves or roots were present, the oxydemeton-methyl presumably acted as a contact insecticied on P. citri.

These trials were conducted on 4 and 15-day-old larvae and on adults following the same method and doing the same number of tests as on the first section.

B. The insecticide was applied on sour orange plants

(Citrus aurantium (L) which were subsequently infested with the phytophagous.

These plants, originating from seed germinated under a controlled environment chamber, adjacent to the previous one in which was kept a temperature of 26 ± 1ºC and a relative sterile soil composed of peat and washed sand at equal proportions. When the plants reached about 15 cm in height and developed 6-7 leaves (this was approximately three months after sowing), were taken out of the bin, minding the roots, subsequently rinsing them to detach soil and placing them into planstic flasks (100 cc and 5 cm diam.) with a plastic screw plug with central hole 6 mm diam. to introduce the plant and maintain it erect. The plant nutrition was achieved by adding a nutrient solution which maintained the plants in the flasks in proper conditions for over 10 months, a period in which there is no significant development of the plant. The nutrient solution was as that used by MOUTOUS and FOS (1973) which efficiency was confirmed in young orange tree plants (GARRIDO et al., 1976).

In this fashion the absorption of oxydemeton-methyl was favoured, accumulating in the plant for some time. After subsequent infestation, the phytophagous sucks the sap of the plant, taking in the compound. Finally the predator completes the trophic chain, incorporating the insecticide through P. citri.

To secure a food source for C. montrouzieri during the duration of the experiment, it was necessary to estimate a sub- lethal dosis of oxydemeton-methyl for P. citri with a 50% efficiency, therefore allowing phytophagous survival of about 50%. To this purpose, orange plants were treated with 4 different dosages, and subsequently were infested with P. citri and a counting of specimens, dead or alive, was done until the population was maintained constant. The results were compared with untreated control plants.

The efficiency evaluation was done following the formula of ABOOT (1925) as follows, and with application to the mean values:

$$\text{Efficacy} = \frac{\text{Alive controls} - \text{Alive treated}}{\text{Alive controls}} \times 100$$

Dosages Oxydemeton-Methyl (%)	Efficacy (%)
0.1	100
0.05	80
0.033	62
0.025	46

Survival of P. citri of about 50% was obtained using a 0.025% dosis, which was used throughout the assay.

The plants were treated and, after 24 hours, were infested with the phytophagous. When the infestation was abundant enough (4-5 days afterwards) the specimens under study were placed on each one of the plants and 5 of them were used in each replication. To avoid escapes, the plants were introduced in plastic cillinders (29 xd 10 cm) with both ends

covered with muslin. the insect stages treated as well as number of assays and replications per assay were the same as in section A.

In the two forms of indirect treatment the results were compared with untreated, fed controls.

3. Results and Discussion

3.1. Direct treatment

The variance analysis applied to the results (Table 1) showed the existence of significant differences ($P \leq 0.05$) on mortality of all of the evolutionary cycle stages of C. montrouzieri treated with oxydemeton-methyl us the controls, treated with water.

Such high mortality (non-hatched eggs) on 4-day-old larvae and eggs, might be explained considering that these stages of development are the most vulnerable and therefore the insecticide is likely to penetrate easily. However, on 15-day-old larvae convered with and abundant waxy secretion that may become a barrier for compound penetration, the mortality rate decreases if compared with younger stages.

Observations carried out SELL (1985) about the influence of oxydemeton-methyl (0.1%) on 2, 3 and 4-day-old larvae of A. aphidimyza (Rond) show that susceptibility of these larvae is inversely proportional to age.

STORK-WEYHERMULLER (1984) examined the effects of oxydemeton-methyl and pirimicar at different concentrations on aphid natural enemies attacking cereals, and found that when treated with dosages as recommended by the manufacturers, the predator larvae rate of mortality increased by 50 and 75% with respect to larvae treated with lower dosages.

Adults obtained from treated surviving larvae, did not show any abnormality and the rate of mortality did not show significant differences ($P \leq 0.05$) if compared with that for adults originating in control larvae. This seems to indicate that during nymphosis there is an elimination of insecticide residues.

Mortality higher than 50% in control eggs might be due to humidity (moisture) fluctuations occuring inside the petri dishes from water evaporation, since although the chamber conditions are constant, the eggs are in direct contact with the microclimate afforded by such dishes. An additional consideration is a potential canibalism amongst larvae which would increase natural mortality.

In adults, their chitinous back may partially prevent penetration of the insecticide; however, it does not result in such efficient a barrier as the waxy cover of the 15-day-old larvae since through the extremities articulations, where the cuticle narrows, penetration could occur. In connection to control adults, there is a possible deleterious effect of chloroform, used to facilitate manipulation, which might increase natural mortality. In subsequent trials we fell back on low temperatures (6-7ºC) to make insects drowsy; the results, however, were similar.

Absence of significant differences ($P \leq 0.05$) observed while doing the variance analysis with data on oviposition and on egg laying mortality (Table II), suggest that the existence

of a competition amongst surviving adults against insecticide treatment, baing lower than that amongst control adults, might increase fecundation and therefore oviposition and, on the other hand, the insecticide should not be largely accumulated within the insect, so that developing eggs could not become damaged.

3.2. Indirect treatment

In both forms of indirect treatment the variance analysis disclosed the existence of significant differences ($P < 0.05$) among specimens provided with food treated with insecticide and control specimens that had untreated food supply (Table III).

The same analysis applied to the results obtained for 4-day-old larvae and adults of the two forms of indirect treatment, showed the absence of significant differences ($P \leq 0.05$). Mortality in 4-day-old larvae was virtually 100%, and in adults was 100%, probably owing to the fact that these were the two evolutionary cycles of C. montrouzieri most greedy in eating and, therefore, taking in lethal amounts of the product, even when using lower insecticide dosages.

In 15-day-old larvae the mortality was lower, basically du to two reasons: the short period (4-5 days) between initial supply of treated food, until the start of nymphal stage and, on the other hand, the low feeding activity taking place during this period.

When comparing the results obtained in 15-day-old larvae having fed on treated food, following the two forms of indirect treatment, there were marked differences ($P \leq 0.05$) the rate of mortality being lower in the second form where the insecticide dosage was lower, probably due to the little amount of food ingested, typical of this stage, which did not allow formation of lethal insecticide concentrations in most specimens.

It was not possible to carry out a study on oviposition because of 100% mortality resulting in all adults, in both forms of indirect treatment.

Comparative studies on insecticide toxicity carried out by MISHRA and SATPATHY (1985) on beneficial arthropods, with 8 of the compounds tested, oxydemeton-methyl was found to be the least toxic to C. repanda Th, predator of Brevicoryne brasicae L., as well as it was the most harmful to the aphid.

Finally, to ascertain that the mortality rate in C. montrouzieri in the second form of indirect treatment was caused by the presence of insecticide on the plant food where the phytophagous was feeding on, a high pressure chromatography analysis was accomplished in the Laboratorio Agrario at Burjasot (Valencia) on different parts of treated plants, on P. citri and on various stages of C. montrouzieri larvae. The results were as follows:

Sour orange plants (100 g)	Leaves and stems	0.05 ppm*
	Roots	0.04 ppm
Planococcus citri (5 g)		3.5 ppm
Cryptolaemus montrouzieri (5 g)		6.5 ppm

*Resolution limit 0.01 ppm

The insecticide concentration, whether in leaves, stems

or roots, was negliglible owing to sap sucking, on the one hand, ant to chemical processes of plant rotting, such as hydrolysis, oxidation, trans-alquilación, etc., on the other hand; whereas C. montrouzieri showed the highest oxydemeton-methyl concentration due to its acumulation through P. citri.

4. Conclusions

Direct application pf oxydemeton-methyl (0.1%) on eggs and 4-day-old larvae of C. montrouzieri causes high mortality rate since these are the most vulnerable stages of the evolutionary cycle of the insect and the compound can penetrate easily. The 15-day-old larvae are representative of the stage with lower mortality rate. This lower susceptibility lity to the insecticide is caused by the abundant waxy secretion protecting them, and acting as a barrier preventing entry of the product. In adults, their chitinous back restrains partially penetration of the chemical; however, this protection is not so efficient as the waxy cover developed by 15-day-old larvae, thus mortality is higher.

Oviposition and mortality on egg laying of specimens treated with insecticide, does not show significant differences ($P \leq 0.05$) with the controls, treated with water. Possibly, the higher mortality in insects treated with the compound reduces competitiveness, which may increase fecundation and therefore oviposition. On the other hand, the insecticide might not be substantially acummulated within the insect, since egg formation and subsequent evolution does not appear to be affected.

The two forms of indirect treatment, in which the food source for C. montrouzieri is treated with varying dosages of oxydemeton-methyl, give similar results in 4-day-old larvae in which mortality is almost 100% and in adults in which mortality is 100%, since these are the most voracious stages of the evolutionary cycle, taking in larger amounts of the product. In 15-day-old larvae, mortalaity is much lower as they develop little feeding activity. This reduction in mortality is even more marked when the oxydemeton-methyl dosage is lower.

Evolution of surviving larvae, both in the direct treatment and in the indirect treatment, does not show significant differences ($P \leq 0.05$) with the evolution of control larvae, probably because the insecticide is eliminated during nymphosis.

Acknowledgements

The authors wish to express their appreciation to Mr. Miguel Gamón, Bachelor of Chemistry, for his analyses performed at the Laboratorio Agrario of Burjasot, Valencia.

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TABLE I. Mortality caused by application of oxydemeton-methyl on varios stages of the evolutionary cycle of *C. montrouzieri*.

	Development stage											
	egg			4-day-old-larva			15-day-old-larva			Adult		
	$\bar{X}$	E.S.	n	$\bar{X}$	E.S.	n	$\bar{X}$	E.S.	n	$\bar{X}$	E.S.	n
Water	54.00	± 9.90*	20	16.80	± 7.42	5	0.80	± 1.68	5	24.37	± 15.92	10
Oxydemeton-methyl	97.99	± 2,62*	20	87.20	± 4.13	5	8.00	± 5.65	5	49.02	± 9.75	10

*Not hatched
n: number of specimens per replication.

TABLE II. Influence of oxydemeton-methyl on oviposition and on viability of egg laying.

	Water			Oxydemeton-methyl		
	$\bar{X}$	E.S.	n	$\bar{X}$	E.S.	n
eggs/female	547.49	± 68.15	5	552.94	± 61.14	5
% mortality	42.00	± 6.52	20	43.45	± 5.65	20

*Not hatched
n: number of specimens per replication.

TABLE III. Mortality percentage caused by ingestion of food treated with Oxydemeton-methyl in varios stages of the evolutionary cycle of C. montrouzieri.

	Development stage					
	4-day-old larva		15-day-old larva		Adult	
Water	13.60 ± 6.00	5	2.40 ± 3.60	5	22.31 ± 14.60	10
Oxydemeton-methyl (0.1%)	99.29 ± 1.78	5	44.00 ± 10.00	5	100.00 ± 0.00	10
Water	22.40 ± 2.84	5	9.40 ± 2.24	5	24.74 ± 12.90	10
Oxydemeton-methyl(0.025%)	98.40 ± 3.72	5	28.00 ± 5.65	5	100.00 ± 0.00	10

n: number of specimens per replication.

STUDIES OF MAL SECCO ISOLATES FROM SEVERAL AREAS OF GREECE

C.C. Thanassoulopoulos and E. Gogou
Aristotelian University, Faculty of Agriculture, Plant Pathology Laboratory,
540 06 Thessaloniki, Greece

Summary

The morphological characteristics and virulence of natural population of the Mall secco fungus (Phoma tracheiphila) from several lemon areas of Greece were studied.
The study of morphology of various isolates showed that were not existed serious differences among them to distinguish different strains. All isolates produced red pigments. The only considerable distinction was that all isolates did not produce pycnidia on PDA, since their original isolation.
Virulence of isolates was studied with artificial inoculations on sour orange seedlings five-months-old. The final enaluation of results, 169 days after inoculation, was madewith disease index 0-5, the length in cm of vascular discoloration and isolations. Isolates forming pycnidia showed significant higher variables than those non forming. The results showed that the isolates could be distinct in 5 groups of virulence.

Introduction

Since 1930 it had been considered by Petri (5,6) that there were two races of the fungus Phoma tracheiphila while later Baldacci (1) reported that a natural third race existed. Other authors, later on (2, 3, 7), considered that the fungus is a monotypic species while it is also reffered that these are strains with various virulence (4, 5, 7, 8).

The results of this effort i.e the investigation of the natural population of the fungus, in several lemon areas of Greece, are presented in this paper.

Materials and Methods

Isolates of the fungus were isolated from lemon tree samples grown in several areas of Greece (Table 1). Only one isolate was obtained from an orange tree sample. Ten of the isolates were isolated during 1985, whereas number 8 in 1983 and numbers 9 and 11 in 1978. Isolations were made from discoloured vascular bandles of shoots on PDA (Oxoid Ltd.,Code CM 139). Fungus growth as well as preservation in 4°C were made on the same medium. Colonies were grown in Petri dishes 9cm in diameter or in test tubes, in 18°C for 15 days. At the end of this time the colony diameter was measured and the colonies were examined macroscopically and microscopically.

For testing virulence of several isolates, 12-16 sour orange seedlings, five months old, were used. Each seedling was grown in a plastic pot in the greenhouse. Temperature during the experiment fluctuated from 18-20°C.

Inoculum was prepared by adding 10ml tap water in a fungus colony grown in test tube, scraping slightly the mycelium and strongly agitating by hand the tube so a dilution of hyphae and spores was obtained. Inoculum propagules density was not considered necessary to be counted.

Seedlings were inoculated with a 2ml hypodermic syringae containing the prepared inoculum. Each seedling was injected 2-4 times on its stem, depending of its height and diameter, and also by scratching 5-6 leaves. With the content of the syringae were inoculated 4-5 seedlings.

Disease progress was observed frequently, after the first 20 days after inoculation. Disease development was evaluated with a 0-5 disease index as follows:

0: Plant apparently healthy. No chlorotic areas around leaf scratchings.
1: Chlorotic spots of various sizes and intensity of yellowing around leaf scratchings.
2: Chlorotic leaf veins, twisting, wilt and dropping of some leaves.
3: Dieback of shoots, defoliation in more than 50%.
4: Almost complete defoliation of upper leaves, advanced dieback of lateral shoots and main stem.
5: Seedling practically dead. Main stem completely dried out or a small basic part is still alive but completely chlorotic.

After last observation, isolations were made from three places of the main stem. Then the stem was decorticated and the length of discoloration of the vascular bandles was measured in cm. When the discoloration was not distinct the stem was dipt in 1% KOH solution for some minutes, then the characteristic red color was distinctively apparent. Healthy shoots treated the same way do not appeare red discoloration remaining white.

Statistics of disease index, vascular discoloration and colony diameter was made with analysis of variance, while the rate of increase of disease per unit of inoculum and time was made with Van der Plank's (9) method, and the evaluation of dead plants and positive isolations with X^2 test.

Results and Discussion

All isolates did not produce pycnidia on PDA, nor in the original isolation (Table 1), while other morphological differences were not evident at the end of 15 days of growth. Isolates had not any difference in the rate of colony growth and the colony colour; all isolates produced several tints of red pigment with various intensities. The variability of pycnidia and pycnidiospores sizes, in the isolates that formed pycnidia, were not out of species deviation. On the other hand there was observed great variability in subcultures of the same isolate, as it is reported elsewhere (2,4).

These results indicate that isolates had no morphological differences those that could differentiate distinct "races" (1, 5, 6). Otherwise in later research (3, 7) it is considered that P. trachelphila is a monotypic species and this observation seems that it is certified in this work.

On the contrary virulence of the isolates was quite variable (Table 2), as it is evident from the disease index, the number of dead plants and the number of plants from which positive isolations were obtained. Mean disease index variated from about 0.5 to 4.2, so five groups of isolates could be distinguished. The rate of increase of disease per unit of inoculum and time (Van der Plank's r, 9) provides a better consideration of the forementioned results. The same results are also observed from the number of dead plants (Table 2) while positive isolations results (Table 2) are not so conclussive, and this is probably the consequence of complete dryness of a number of plants before isolations be effected. These results are quite similar with those obtained by italian authors (7, 8).

It was also observed that 4 out of 5 isolates which formed pycnidia showed the highest values of disease index and dead plants. Generally isolates with pycnidia showed clearly higher virulence than isolates without pycnidia (Table 3). Differences observed were significant in 0.1% level

of significance. This is clearly evident in figure 1 with disease index and r coefficient, of which the rate of increase of disease was definitely higher in isolates with pycnidia. This observation indicates that pycnidiospores are more significant in disease development than mycelia or phialoconidia, producing more extensive infection.

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Table 1

Cultural characteristics of several isolates of Mal secco fungus

Area of isolation[1]	Colony diameter 15 days old mm	Pycnidiospores sizes µm	Production of red pigment
Isolates with pycnidia			
1. Messinia I	60[2]	2-4X1-2	+[3]
2. Poros	60	2-4X1	+
3. Mytilini	73	2-3X1	+
4. Kriti I	75	3-4X1-2	+
5. Messinia II	80	2-6X1-2	+
Isolates w/o pycnidia			
6. Messinia III	78		+
7. Kriti II	67		+
8. Achaia I	85		+
9. Achaia II	65		+
10. Korinthia I	74		+
11. Achaia III	85		+
12. Korinthia II	67		+
13. Chios	77		+

1. All isolates but one from lemon; No 9 from sweet orange.
2. Mean of three Petri dishes in 18°C for two weeks.
3. +, presence of red pigment.

Table 2

Virulence of several isolates of Mal secco fungus in artificial inoculations on sour orange seedlings.

Area of isolation[1]	Disease index 0-5[2]	Dead plants %	Positive isolations %[3]	Vascular discoloration cm	Rate of increase r'[4]
Isolates with pycnidia					
1. Messinia I	4.18a[5]	64.3	50.0	18.7	0.0106
2. Poros	3.22b	42.8	71.4	15.8	0.0063
3. Mytilini	3.17b	48.8	41.6	16.0	0.0059
4. Kriti I	3.03b	48.8	35.8	19.9	0.0055
5. Messinia II	2.37c	25.0	50.0	11.5	0.0040
Isolates w/o pycnidia					
6. Messinia III	2.95b	41.6	58.3	15.0	0.0053
7. Kriti II	2.90bc	50.0	14.3	15.8	0.0050
8. Achaia I	2.67c	41.6	41.6	17.3	0.0045
9. Achaia II	2.25c	16.7	16.7	19.3	0.0032
10. Korinthia I	1.47d	0	0	8.6	0.0025
11. Achaia III	1.45d	0	61.5	2.3	0.0022
12. Korinthia III	1.45d	6.3	0	11.5	0.0022
13. Chios	1.12d	0	0	15.1	0.0015
Control	0	0	0	0	0

1. All isolates but one from lemon; No 9 from sweet orange.
2. 0: apparently healthy plants, 5: dead plants.
3. Isolations were made from three sites of each plant from all the plants.
4. Rate of increase of disease per unit of inoculum and time.
5. Means followed by the same letter are not significant in 0.1% level of significans with the Duncan'a meltiple test range.

Table 3

Virulence and growth of isolates with pycnidia and without pycnidia of the fungus of Mal secco of lemon.

	Isolates	
	with pycnidia	without pycnidia
Colony diameter (mm in 15 days)	69.60a[1]	74.33a
Disease index (0-5)	3.21a	2.08b
Rate of increase of disease (r)	0.0061a	0.0032b
Dead plants[2], %	43.1a	18.8b
Vascular discoloration (cm/plant)	16.38a	13.87a
Positive isolations[2], %	48.6a	19.6b

1. Means of each parameter followed by the same letter are not significnant in 0.1% level of significance.
2. Statistics with X^2 test. Statistics for all the rest with analysis of variance.

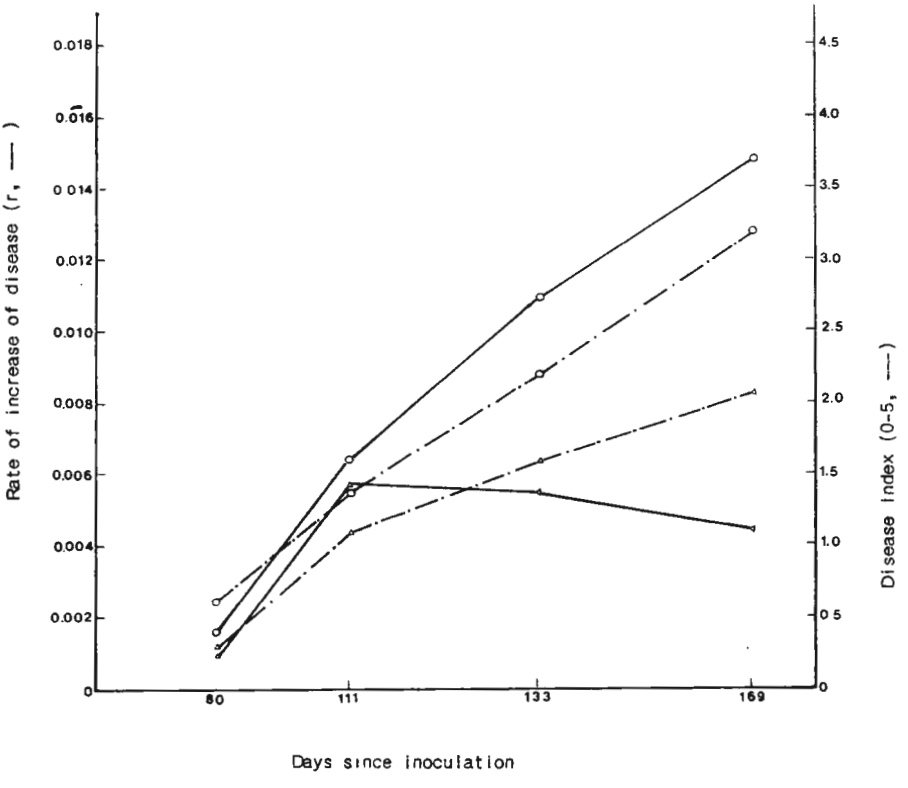

Fig. 1. Disease index and rate of increase of disease during 169 days since the inoculation day of the fungus Phoma tracheiphila an sour orange seedlings.

XYLEM FACTORS IN SUSCEPTIBILITY TO CITRUS MAL SECCO

G.Lanza*, V. De Cicco, A. Ippolito** and G. Cutuli*****

*Istituto Sperimentale per l'Agrumicoltura, Acireale.
**Dipartimento di Patologia vegetale, Bari.
***Osservatorio per le malattie delle piante, Acireale, Italy.

Summary - In an attempt to obtain indication on possible mechanisms of lemon susceptibility toward Citrus mal secco, caused by **Phoma tracheiphila**, some anatomical and physiological xylem factors of three cultivar with different susceptibility were analyzed. Investigations were also extended to nucellar clones considering their higher susceptibility in comparison with ordinary clones. The xylem factors considered were: density and grouping vessels, as well as water conductivity during the resting periods.

The results obtained from our observations did not reveal any sure relationship between the investigated anatomical and functional xylem factors and susceptibility of the lemon clones towards mal secco.

Since the first researchs on mal secco of Citrus, caused by **Phoma tracheiphila** (Petri) Kanc. **et** Ghik.(10), many investigations have examined the possible factors responsible for the different level of susceptibility of Citrus varieties to the disease. Many research works have been performed on chemical mechanisms (1, 2, 3, 9, 12, 13), whereas little attention has been paid to anatomical and funtional ones. The results obtained in studies of Dutch elm disease, which demonstrated a significant relationship between xylem factors and disease susceptibility (4, 6, 14) induced us to carry out this study. Our aim was to evaluate if there were such relationships in the mal secco disease and our results are reported in this paper.

Materials and Methods

Trials were performed on three lemon cultivars [**Citrus limon** (L.) Burm.], with different level of susceptibility towards mal secco, these being 'Femminello' comune (susceptible), 'Monachello' (resistent) and Santa Teresa (intermediate). Because of their higher susceptibility towards this disease (Perrotta and Tribulato, 1977), nucellar clones were also tested.

Anatomical characteristics of xylem vessels. Five trees (about 15 year old) grafted onto sour orange (**C. aurantium** L.) were tested for each clone. In the summer 5 one year old twigs

varying in diameter from 4.5 to 6 mm were removed from each tree at a height of about 1m. These twigs were then cross sectioned and from each section a print was obtained using the Leva's method (1977) conveniently modified. A drop of acrylic glue for plexiglass (vedricol E1 Montedison) was put on the cut surface and the print was removed by adhesive tape and then put on a microscopic slide. These prints were used to determine the density of the secondary xylem vessels, their diameter and the number of isolated and contiguous vessels. The average vessel group size was calculated by multiplying the average vessel diameter by the number of contiguous vessels.

Water conductivity (WC). During the resting period (January and July) 3 one year old twigs were removed from each of the before mentioned trees, in order to perform observations on WC. This age of twig was studied to give maximum data uniformity. Twig WC was calculated using Melching and Sinclair's formula (8) for WC determination in elms ($WC = v / t \cdot a \cdot l \cdot p$; where v= volume in ml of collected water, t= time in sec 5 ml water takes to go through the twig, a= area of the twig cross section in cm^2, l= twig lenght in cm, p= water pressure in atm). Diameters at the tips and at the centre of twig without bark were measured to determine average area of the twig section. A rubber tube pipe was used to connect the twig up to a small tank containing distilled water maintained at constant pressure (1 atm) by means of a Millepore pump. The time it took 5 ml distilled water to pass through a 10 cm twig segment was calculated.

Results and discussion

The results of observations of the anatomical characteristics of the twig secondary xylem were statistically elaborated and reported in Table I. Vessels density, expressed as average vessel number per mm , appears higher in nucellar than in old line clones. More exactly, in the 'S. Teresa' and 'Monachello' cultivars there was a statistically significant difference (P=0.05) between the nucellar and old line clones. Apart from the different specific statistical significances, analysis of data regarding the vessel diameter clearly shows that it is markedly greater in the old line of 'Monachello' as compared with other clones. Percentage of contiguous vessels in the two 'S.Teresa' clones is only lower than that observed in 'Femminello' and 'Monachello' old line clones (P=0.05). The size of the contiguous vessel groups tends to be lower in nucellar clones when compared with old line clones. In this connection it must be pointed out that there were rarely more than 2 contiguous vessels.

The results of the twig WC in the lemon clones tested were statistically elaborated and are reported in Table II. WC was highest in the 'Monachello' old line clone followed by old line 'Femminello', nucellar 'Monachello' and then 'S.Teresa' old line.

Table I - Anatomical factors of lemon twigs from nucellar (n.l.) and old line (o.l.) clones with different behaviour towards Citrus mal secco.

Clones (in descending susceptibility)	Vessels density (n/mm2)	Vessel diameters (μ) max.	Vessel diameters (μ) min.	contiguous vessels (%)	size of vessel groups
Fem. n.l.	218.7 ab	30.8 aA	23.6 aA	4.5 ab	123 a
Fem. o.l.	185.9 a	33.7 abAB	25.8 abAB	12.6 c	380 b
S.Ter.n.l.	237.6 b	30.7 aA	23.2 aA	3.0 a	79 a
Monac.n.l.	240.5 b	33.6 abAB	25.1 aAB	6.0 abc	172 ab
S.Ter.o.l.	190.9 a	31.0 aA	23.9 aA	3.2 a	89 a
Monac.o.l.	185.3 a	36.2 bB	28.3 bB	10.4 bc	350 b

Each datum is the mean of 500 measurements obtained from 25 twigs. Within each column the values marked with the same letter are not statistically different. Small and capital letters apply to P = 0.05 and P = 0.01, respectively.

Table II - Water conductivity of lemon twigs from clones with different behaviour towards Citrus mal secco determined in two differents seasons.

Clones (in descending susceptibility)	summer	winter
'Femminello' o.l.	0.0052 ab	0.0013 bAB
'Monachello' n.l.	0.0038 b	0.0007 bcBC
'S.Teresa' o.l.	0.0018 c	0.0003 cC
'Monachello' o.l.	0.0072 a	0.0020 aA

Each datum is the mean of 15 measurements obtained from as many twigs removed from 5 plants. Within each column the values marked with the same letter are not statistically different. Small and capital letters apply to P = 0.05 and P = 0.01, respectively.

In this last clone the WC is lower than in the other clones tested, sometimes showing a markedly statistical difference (P=0.01). WC values obtained from the twigs removed during the winter was always comparatively lower than those obtained from twigs collected in the summer. Data obtained from our study revealed that the susceptibility towards mal secco in the clones tested does not seem correlated to anatomical and functional xylem factors here considered. This does not agree with the literature on Dutch elm disease, where it has been demonstrated that the secondary xylem vessels are shorter and narrower in diameter and have lower WC in resistant elms as compared with susceptible ones (4,6) showed that also the size of contiguous

vessels was smaller in more resistant elms. These data were used to determine a diametric marker of xylem vessels in order to select resistant elms to **Ceratocystis ulmi** (Buism.) C.Moreau (15).

The results obtained from our observations did not reveal any sure relationship between the investigated anatomical and functional xylem factors and the level of susceptibility of lemon clones towards mal secco. They also showed that where there was an indication of such relationship it was the opposite as reported in Dutch elm disease. So, the lesser susceptibility of 'Monachello' lemon seems to be associated with both, larger vessel size and higher WC. These findings as well as the data obtained, regarding the size of contiguous vessels, contrast with those observed in elm.

Of considerable interest were, in our opinion the data regarding higher density and lower vessel grouping size in nucellar clones as compared with old line clones known to be less susceptible to the disease. In this connection it should be kept in mind that the above mentioned factors could be an expression of the greater strength of the nucellar clones related to their youth characteristic and to the absence of viruses, rather than because they were directly responsible for their greater susceptibility. In fact, mal secco is known to be more severe in strong trees in active growth and in trees without virus infection, which present a lower amount of specific phenolic compound after mal secco infection than that found in virus infected plants, as in plants infected by mal secco (13).

The greater vessel size and higher WC observed in the 'Monachello' seem to be peculiar to this cultivar which is known to be very resistant to mal secco. However, this resistance seems to be linked to the cultivar's capacity to localized the pathogen rather than to its anatomic characteristics (7,15).

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Investigation on the mal secco through a DNA probe of Phoma tracheiphila

I. Di Silvestro, L. Cupperi & M. Leonardi
A.I.D. S.p.A. Research Center , Catania, Italy.

A. Catara
Plant Pathology Institute, University of Catania,Italy

Summary

The presence of Phoma tracheiphila in citrus tissues and in soil samples was investigated by DNA hybridization techniques. Samples were enriched through culture on nutrient media for two days and submitted to nucleic acid extraction. Results show a high percentage of infection in symptomless plants; in soil samples, collected from field, the fungus was detected few times.

1 . INTRODUCTION

The causative agent of mal secco disease is Phoma tracheiphila, a fungus that affects the citrus culture in Mediterranean countries (1).

Determining the presence of fungus inside the tissue or into the soil by traditional methods is slow (2) and sometimes difficult.

For this reason a rapid qualitative and quantitative method was developed which utilizes a DNA probe obtained from cloning the pathogen DNA (8, 10).

The DNA probe is highly specific and sensible for detection P.tracheiphila in plant or in seedling (3).

In the present work we review the results of the pathogen detection by probe pPhoB25 (8) in citrus tissue and in the soil.

2. MATERIALS AND METHODS

2.1. Plant samples.

Samples of trunk, branches and twigs of different species of Citrus, collected in the field, were utilized to detect the presence of P.tracheiphila.

Trunk pieces and small pieces (4/5 cm length) of

twigs of different Citrus (from one year to 28 years old trees), cut from young branches, were longitudinally sectioned, surface sterilized with NaClO (0,4% active Cl) rinsed few times in sterile distilled water and placed in culture in Sabouraud medium supplemented with carbenicillin (50 ug/ml). After two days in the dark at 25°C the internal surfaces of branch sections and the medium below were scraped with a razor blade and the mycelium obtained was homogenized in a mortar with a pestle in the presence of a phenol-EDTA mixture and submitted to DNA extraction (4).

2.2. Soil samples.

The fungus was detected after putting experimentally inocula into the soil. Field samples of soil were processed directly or after the sterilization (2 hours at 121°C or 3 hrs at 400°C in oven). Mycelium obtained from three days old static cultures (6000 conidia in 100 ml carrot broth), or different phialoconidia concentration (1.2-12-120x10) were added to samples. Incubation was done at room temperature for different times (Fig. 4).

About 10 grams of the sample were transferred in carrot broth flasks supplemented with carbenicillin (50 ug/ml) in shaken culture (120 rpm in dark 20°C) for two days. The obtained mycelia were harvested by filtration and homogenized in a mortar in presence of phenol-EDTA mixture for nucleic acid extraction.

2.3. Hybridization.

The dot blot (6) and Southern blot (11) against probe pPhoB25 (9) were performed as usual. The membranes were washed three times at 68°C for 45 min each in 2xSSC, 0,1%SDS. The DNA was fixed on the nylon membranes by U.V. crosslinking.

DNA concentration and specific activity of probe (7) were calculated by scintigraphic methods (5)

3. RESULTS AND DISCUSSION

3.1. Plant samples.

DNA of P.tracheiphila was easily detected in seedlings experimentally inoculated grown in supplemented medium in growth chamber and/or in pots in greenhouse and in woody samples taken from the field (3,10). Healthy looking and affected samples of Femminello trees and trunks of Tarocco and Navelina

orange showing internal wood discoloration were tested successfully (Fig.1). In many cases mal secco symptoms appeared some months later in tested plants, some of the plants died after two years.

Spot or Southern hybridizations were compared also with traditional methods of diagnosis (visual inspection and plating of xylem explants).

For what concerns twig tests 75% of samples tested with pPhoB25 probe appeared infected, whereas mal secco was suspected only in 25% of them. Plating of xylem explants from trunk sections appeared poorly effective for diagnosis giving less than 10%.

3.2. Soil samples.

Spot intensity was directly proportional to the age of culture and concentration of inocula. The method allowed to ascertain the phialoconidia growth faster than mycelia.

To know optimal culture time for enrichment different inocula concentrations (1.2, 12, 120 x 10) were cultured in vitro in not sterilized soil for 5 intervals (6,22,30,48.72 hours). The highest spot intensity was detected after 30 hours (Fig.4).

In other experiments sterilized or not sterilized soil samples were inoculated with phialoconidia (12 x 10) and left for different times at room temperature for 0,2,16,21 days. Phialoconidia and mycelia survived until 16th days, after the spot signal went down (Fig.3,5).

Field samples from different areas were collected to survey the natural occurrence of P.tracheiphila. Out of 130 tested samples put in culture 24 were positive by dot blot and Southern hybridization . Scintillation counter revealed between 100 and 426 cpm.

The use of the probe (pPhoB25),appears able to detect the presence of P.tracheiphila in trees and in the soil. The results show the method suitability for epidemic researches and studies on the relationship between host-pathogen or other application.

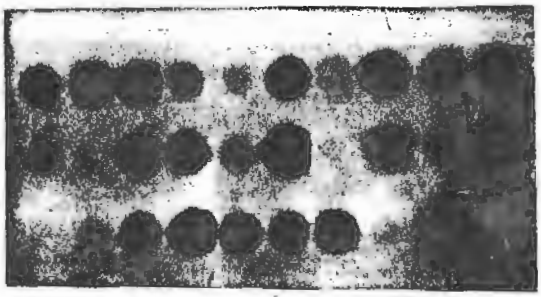

Fig. 1 Total DNA isolates from healthy looking lemon trees; spot n°1 represents DNA from infected tissue as control.

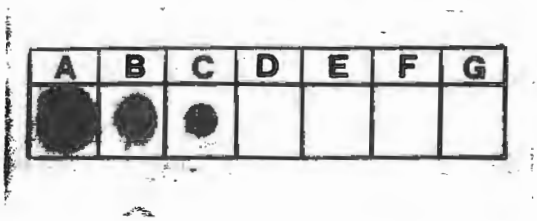

Fig. 2 Phoma tracheiphila DNA probed with radioactive pBPhc25; spot A represents 200 ng of DNA, B 40ng, C 8 ng and D 1.6 ng. The dot blot were autoradiographed at -80°C for one day.

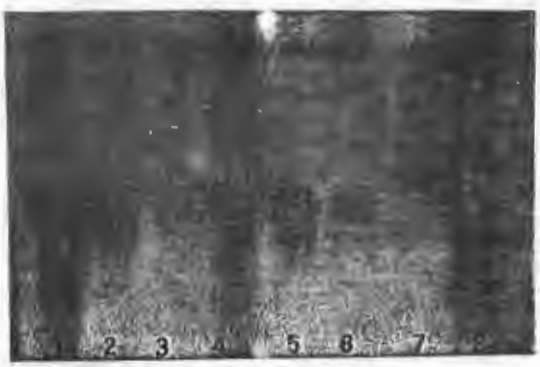

Fig. 3 Southern hybridization of phialoconidia inoculated soil samples.
1) DNA from 120 millions of phialoconidia.,2) from 12 millions, 3), 4) 12 millions after 2 and 15 days of incubation, 5) DNA from mycelia, 6), 7) DNA from 12 millions of phialoconidia after 15 and 21 days of incubation. 8) Purified P.tracheiphila DNA

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PARTIAL CHARACTERIZATION OF A PHOMA TRACHEIPHILA STRAIN WITH PROPERTIES OF HYPOVIRULENCE

A.Ippolito, V. De Cicco and D. Gallitelli

Dipartimento di Patologia vegetale, Università degli studi Bari, Italy.

Summary - A hypovirulent isolate of **Phoma tracheiphila** (Pt52), previously used in growth chamber trials for inducing resistance in sour orange seedlings against citrus mal secco, was tested in the field. The purpose of this study was to verify the stability of hypovirulence associated with Pt52 isolate as well as its ability to induce resistance in relation to both, inoculum concentration and time of inoculation.

The possible presence of a mycovirus associated with hypovirulence was also investigated.

The hypovirulent Pt52 isolate expressed hypovirulence also in the field, in spite of variation in both, inoculum concentration and the environmental condition, but there was no significant induction of resistance towards a challenging virulent isolate of the pathogen.

Under our experimental conditions, we were unable to demonstrate the presence of dsRNA in the hypovirulent isolate.

The utilization of biological means in the control of fungal and bacterial diseases in plants is becoming of everwidening interest due to technical and economical problems that often arise. One of the possibilities available in the biological control, with which promising results have been obtained particularly in vascular diseases, is the induction of resistance in the host plant. Resistance may be induced by a pre-inoculation of the host either with hypovirulent forms of the same pathogen, or with taxonomically different entities, dead cells, cellular extracts and catabolites (5, 11).

In the case of mal secco, a vascular disease of citrus caused by **Phoma tracheiphila** (Petri) Kanc. **et** Ghik., the control still involves considerable difficulties and uncertainties (9), thus it seemed appropriate to investigate the possibilities of using biological control against the disease. In growth chamber trials on 1-2 year-old sour orange (**Citrus aurantium** L.) seedlings, induction of resistence was

The valuable technical assistance of Mr. V. Maurantonio is gratefully acknowledged.

obtained by pre-inoculating a hypovirulent strain of **P. tracheiphila** 2-4 months before inoculating the virulent one (3). On solid media the following peculiarities distinguished this hypovirulent from the virulent isolate of the pathogen: (i) hyphae with a small diameter; (ii) scarce production of phialoconidia; (iii) absence of aereal mycelium; (iv) rapid lysis starting from the centre of the colonies; (v) abundant production of bright red pigment.

In order to verify the stability of the character of hypovirulence of the previously used isolate, as well as its ability to induce resistance in relation to both, inoculum concentration and inoculation time, we extended the study to field trials.

As in some phytopathogenic fungi (**Endothia parasitica, Rizoctonia solani** and **Elmintosporium victoriae**) the hypovirulence proved to be associated with a mycovirus infection in mycelium (1, 2, 10), it was decided to carry out some investigations in order to verify if this was also the case with our hypovirulent isolate.

Materials and Methods

1- Field trials. The field trials were carried out on 4-5 year-old sour orange seedlings grown in an experimental field protected by an anti-hail net. The isolates of **P. tracheiphila** were the same ones previously used (3), namely hypovirulent Pt52 and virulent Pt1. The inoculum was obtained according to Salerno and Catara's technique (8) with a slight modification. The inoculations were performed by introducing the conidia suspension into the transpiration stream by means of two cuts made with a knife at different levels on opposite sides of the stem. The hypovirulent isolate (Pt52) was inoculated about 20 cm above ground level and the virulent one (Pt1) 5 cm higher. Seedlings inoculated with sterile water were used as control. The presence of external disease symptoms were evaluated periodically utilizing an empiric scale consisting of the following classes: 1= no symptom; 2= a few apical leaves with vein chlorosis; 3= considerable number of leaves with chlorosis or fallen; 4= initial withering of the apical twigs; 5= extensive withering of the canopy; 6= dead plant. At the end of the trials, i.e. about two years after the first inoculations, the seedlings were uprooted and cut into 20 cm sections to evaluate the extend of internal discoloration and to proceed with isolation attempts.

2 - Search for dsRNA. The fungi utilized were Pt52, Pt1 and an isolate of **Penicillium chrysogenum** (ATCC 9480); the latter, infected with a mycovirus, was obtained from the American Type Culture Collection and used as control. Pt52, Pt1 and ATCC 9480 were grown for two weeks at 25 °C in still culture of potato dextrose broth. The dsRNA was extracted according to the method described by Morris and Dodds (6) and purified from the other species of nucleic acid by fractioning it with LiCl (4). The extracts were then analyzed in 1.2% agarose gel in TBE (90 mM of boric acid, 90 mM Tris, 1 mM, of EDTA, pH 8,9) and the segments of nucleic acid were stained with ethidium bromide.

DsRNAs from two phytoreoviruses, i.e. rice gall dwarf and maize rugose dwarf (kindly supplied by dr. G. Boccardo, Istituto di Fitovirologia applicata, Torino, Italy) were used as mol. wt. markers.

Results and discussion

Table I reports the results obtained with Pt52 isolate alone. To this regard, it must be specified that only seedlings inoculated with 10^6 and 10^7 conidia/ml were followed up to the end of the trials, as those inoculated with 10^4 and 10^5 conidia/ml were inoculated again after 5 months with the virulent isolate ($2 \cdot 10^6$ conidia/ml). Table I shows that hypovirulent isolate Pt52 expressed hypovirulence also in the field, as it induced only a slight chlorosis on a few plants (5 out of a total of 80). Those symptoms, observed only in the seedlings inoculated with the highest inoculum concentrations and in the first reading, showed no statistical significance and do not seem to have been influenced by the time of inoculation. The average percentage of discolored woody cylinder and the average number of plants per treatment showing discoloration tended to be higher at the highest concentration of inoculum. However, the discoloration was very slight, limited to small bunches of vessels and had poorly-defined edges. Isolation attempts were always negative.

Table I - Field behaviour of a hypovirulent isolate of **P. tracheiphila** (Pt52) inoculated in sour orange seedlings at different concentrations and over two separate times. The data indicate: A- average intensity of external symptoms, five months after inoculation; B- average percentage of discolored woody cylinder and, in brackets, the number of plants showing discoloration.

Treatments (conidia/ml)	Inoculation in November		Inoculation in April	
	A	B	A	B
10^4	1	-	1	-
10^5	1	-	1	-
10^6	1.2	1.4(6)	1.1	1.5(5)
10^7	1.1	2.3(8)	1.1	6.0(8)
water	1	0	1	0

10 seedlings were inoculated for each treatment.
There was no statistical significance in the results.

Table II reports the results of the trials carried out to evaluate the induced resistance in plants pre-inoculated with the hypovirulent isolate Pt52 in three different concentrations of inoculum and in two seasons. There was no statistical significance in the intensity of external symptoms of the disease. The average percentage of discolored woody cylinder also showed no statistical significance, even though the wood of all plants

inoculated with the virulent Pt1 isolate in April was discolored, while in the plants inoculated in September, the discoloration affected fewer plants. In both trials, the isolation attempts, positive in only 10% of the inoculated seedlings, produced colonies similar to those of the virulent isolate.

Table II - Field behaviour of a hypovirulent isolate of **P. tracheiphila** (Pt52), in relation to inoculum concentration and inoculation time, against a virulent isolate (Pt1) inoculated five months later. The data indicate: A - average intensity of external symptoms 6 months after the inoculation of Pt1; B - average percentage of discolored woody cilinder and, in brackets, number of plants showing discoloration; C - number of plants in which the fungus was re-isolated.

Treatments	Pt52 inoculated in Nov.			Pt52 inoculated in Apr.		
	A	B	C	A	B	C
Pt52 10^4 +Pt1	2.0	6.2(10)	0	3.1	25(8)	1
Pt52 10^5 +Pt1	2.5	9.5(10)	1	3.2	33(8)	2
Pt52 10^6 +Pt1	1.6	6.6(10)	1	3.6	24(8)	0
water +Pt1	1.9	10.0(10)	2	3.0	20(7)	1

10 seedlings were inoculated for each treatment.
There was no statistical significance in the results.

Under our experimental conditions, we were able to isolate dsRNA only from **P. chrysogenum**. In agarose gel electrophoresis the dsRNA preparation separated into three bands with an estimated mol. wt. of 2.2 - 2 - 1.9 x 10^6 daltons. These results were confirmed by observations of the mycelium dips with electron microscope which revealed the presence of virus-like particles (VLPs) only in **P. chrysogenum**.

The results of field trials seem to be only in partial agreement with those previously obtained in the growth chamber. In fact, the Pt52 isolate expressed hypovirulence also in the field, in spite of variations in the environmental conditions and inoculum concentrations, but there was no significant induction of resistance, which disagrees with our previous results.

Thus, the induced resistance observed in young seedlings kept in a growth chamber (20 °C), was no longer obtained under field conditions. This could be hypothetically explained by the changing seasons and, in particular, by the intense, prolonged summer heat during the trials. These severe climatic conditions very probably had a negative effect on both the induction of resistance, known to be influenced by temperature (11), and the vitality of the same hypovirulent isolate, apparently rather weak; as stated before, attempts to isolate it from the plants in which it had been inoculated alone or from plants with a double inoculation failed. Another reason why no induced resistance was observed may be sought in the fact that the two fungal isolates were localized in different vessels and,

resistance, known to be influenced by temperature (11), and the vitality of the same hypovirulent isolate, apparently rather weak; as stated before, attempts to isolate it from the plants in which it had been inoculated alone or from plants with a double inoculation failed. Another reason why no induced resistance was observed may be sought in the fact that the two fungal isolates were localized in different vessels and, consequently, remained separate in the host plant. Certainly, such a separation did not occur in the previous growth chamber trials, in the course of which the seedlings had not appreciable growth.

The hypovirulence associated with **P. tracheiphila** isolate does not seem to be due to a mycovirus infection. To this regard it should be remembered that results similar to ours were obtained by Rosciglione **et al.** (7), utilizing, as inductors, hypovirulent isolates of **P. tracheiphila**, obtained with U.V. radiation.

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CITRUS TRISTEZA IN ITALY

Davino, M. and R. Areddia
Istituto di Patologia vegetale, Università dgli Studi,
Catania, Italy.

G. Polizzi
Istituto di Difesa delle Piante, Università degli Studi,
Reggio Calabria, Italy.

Abstract. Further investigation on citrus tristeza in Italy are reported. After a five year survey it appears that the disease did non spread from the orchard where it was located for the first time. The following aphid species: Aphis fabae, A. craccivora, A. gossypii, Aulacorthum solani, Macrosiphum euphorbiae and Myzus persicae, collected on infected citrus plants, were negative for CTV when tested by ELISA assay. Attempts to transmit the virus from citrus to citrus under strict isolated conditions were also negative by a A. fabae, A. gossypii, A. citricola, M. persicae and Toxoptera aurantii but a low efficiency for two isolates imported from abroad was observed.

Introduction. Citrus tristeza virus (CTV) was first reported in Italy in 1955 in Meyer lemon and Satsuma mandarin (9). Subsequently other citrus species were found to be infected in various locations (4).

Preliminary investigations on natural spread of CTV carried out by ELISA assays of the trees near the infected ones did not show any aphid transmission (5). Since the disease is a threat for our citriculture we have been monitoring the trees for five years and attempting transmission tests in screenhouse to evaluate the efficiency of potential vectors of CTV and the transmissibility of different isolates found in Italy. Results are here reported.

Materials and Methods

Field surveys. The surveys were carried out in May-June and September-October from 1983-87 in orchards were CTV infected trees had been discovered many years ago. 3,850 samples were tested by ELISA method, using a standard procedure (1).

Virus isolates. Five CTV isolates were used in this study on transmission by aphids. They included: two CTV isolates recently discovered near Monasterace (Reggio Calabria) coded

CTV-GB and CTV-SM, a severe isolate discovered on Satsuma mandarin coded CTV-J, a mild isolate imported from Florida (6) coded CTV-T4 and another from California coded CTV-T8.

All isolates were increased in seedlings or budlings of Mexican lime and Madame Vinous sweet orange about one or two years old.

Plants. For transmission trials we used Mexican lime seedlings and budlings of Madame Vinous sweet orange grafted on sour orange or volkamer lemon. All plants were grown in a steam-sterilized potting medium, in a greenhouse protected by nets, to avoid infestation with afids or other insects, far from commercial plantings.

Testing aphids. Aphids colonies collected from infected trees in the field (Monasterace), were tested by ELISA method or were fed on Mexican lime or Madame Vinous sweet orange. The following aphid species were tested: Aphis fabae, A. craccivora, A. gossypii, Aulacorthum solani, Macrosiphum euphorbiae and Myzus persicae. Colonies collected on young shoots were put in a refrigerated plastic bag and then tranferred to the laboratory. After 24 hrs a sample of 100-120 specimens (adults and 4th instar nymphs) were transferred to a pot and homogeinized in a phosphate buffered saline solution pH 7.2 additionated with polyvinil-pirrolidone (1%) and tested by ELISA assays (2). An other group of the same colonies were transferred to Mexican lime or Madame Vinous sweet orange plants and left to colonize them for 24 hrs under a cage.

In other experiments young shoots carrying the following aphid species were collected in the field: A. fabae, A. citricola, A. gossypii, M. persicae and Toxoptera aurantii. A. fabae was collected from brad bean, while other species were collected from citrus. The shoot infected with one aphid species were placed on young leaves of donor Mexican lime or Madame Vinous sweet orange inoculated with an isolate of CTV after a 24 hrs or 48 hrs acquisitions time aphids were removed. The young leaves of donor plants carrying feeding aphids were cut in two parts and attached the young leaves of Mexican lime (acceptor host). After 48 hrs, the plants were sprayed with an aphicide. Mexican lime seedlings were inspected every 7 days and tested by ELISA assys for CTV infections after three months and subsequentely every month during one year.

Results and Discussion

Out of 3,850 local varieties tested by ELISA assays for CTV infections only three healthy looking clementine mandarin located near Golden Buckeye sweet orange and Satsuma mandarin were infected (table 1). This can be done either to a very low spread or to a regraft of previously infected trees.

Since the oldest infected trees present in the orchard is 30 years old it can be concluded that over the same period no spread (or a very poor one) of CTV has occured in Italy.

Aphid species collected from citrus plants infected by

CTV were negative for CTV when tested by ELISA assays and were not able to infect Mexical lime seedlings on which they had been transferred. Results of transmission attempts for five CTV isolates and aphid tested are shown in table 2. A. citricola was able to transmit CTV-T4 and CTV-T8 isolates whereas A. gossypii was efficient only when tested with CTV-T8. Also early transmission trials have pointed out that a very small population of A. citricola could be able to transmit CTV (3). None out of aphid species tested were able to transmit local isolates of CTV.

From the above results the transmissibility of found isolates appears to be very poor. Nevertheless CTV remains a threat for the italian citrus groves and for other mediterranean area since:

a) T. citricitidus can be accidentally introduced into the mediterranean basin;

b) Citrus aphids present may differentiate new clones more effective in the transmission of CTV;

c) From CTV isolates present in Italy mutants may arise transmissible by local aphid populations;

d) Other CTV isolates, including the seedling yellow component, which are easily transmissible can be introduced accidentally.

Since at this moment the disease is not epidemically spread in Italy only an eradications program is hopeful. Eventually in future a cross protection program can be established to protect sensitive cultivar against CTV infection as has occurred in other countries (7), also the use of biotechnology will be hopeful.

Table 1. Results of ELISA test (1983-87) for CTV of local varieties in three different citrus orchards where infected trees were detected

Citrus species	No. of trees	
	tested	infected
Lemon	60	0
Mandarin and similar	1100	3
Sweet orange	2750	0
Total	3910	3

Table 2. Results on transmission attempts of five CTV isolates by aphid species

APHIDS	Average of aphids	No. of positive transmission out of total for each CTV isolate					Total
		T4	T8	T-GB	T-SM	TJ	
A. fabae	30-118	0/15	-	-	-	0/3	0/18
A. citricola	15-140	8/32	6/20	0/10	0/12	0/25	14/99
A. gossypii	15-115	0/10	5/15	-	-	0/9	5/34
M. persicae	20-108	0/6	-	-	-	0/6	0/12
T. aurantii	75-125	0/19	-	0/5	0/8	-	0/32
Total		8/82	11/35	0/15	0/20	0/43	19/195

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CITRUS TRISTEZA VIRUS (CTV): A MAJOR THREAT TO THE MEDITERRANEAN AREA

P. Moreno
Departamento de Protección Vegetal, I.V.I.A., Moncada (Valencia)
SPAIN.

SUMMARY

The Mediterranean basin is one of the citrus growing areas that has been less affected by citrus tristeza virus (CTV). Severe CTV strains carrying the seedling yellows component and causing stunting and stem pitting in commercial varieties at present, seems to be rare in the area. The rate of CTV spread in the field is still low and the most efficient vector, Toxoptera citricidus, has not yet been found in the area. Introduction of this vector and new severe CTV strains would accelerate tristeza spread and destruction of the citrus industry. A regional program based on quarantine measures, surveys for CTV detection and strain identification, inoculum control, and quick exchange of information between countries, should be established to preserve this biogeographical area free of the ravages caused by tristeza in other citrus growing areas.

Citrus tristeza virus, a closterovirus about 2000 nm long (4) is the causal agent of one of the most destructive diseases of citrus. According to some estimations, more than 50 million citrus trees grafted on sour orange have died from this disease (6) and an uncountable number of them are infected and suffering variable damages depending on virus strains, citrus species and scion-rootstock combinations.

The Mediterran basin is one of the citrus growing areas that has been less affected by tristeza. However, recent data indicate that, in Spain alone, about 15 million trees grafted on sour orange have died from tristeza infection, and that 7-8 million more are at present infected and in different stages of decline (10). In Israel, about 20 thousand tristeza-infected trees have been rogued and the estimations are that 50-100 thousand more be infected (Bar-Joseph, personal communication). In Italy, tristeza has been detected in about 100 trees out of more than 20 thousand indexed by ELISA (11). Isolated trees infected with tristeza have been found in most Mediterranean countries. In some cases, infected trees have been removed and no obvious spread of the disease has been observed. In other instances, the actual situation regarding tristeza has not been carefully checked.

1. Diagnosis of CTV infection

Sour orange is the major rootstock in the Mediterranean

citrus growing areas. Since most CTV isolates induce decline of citrus trees grafted on this rootstock, field trees are frequently self-indicators for CTV infection. A more accurate symptom for field diagnosis of CTV is the presence of inverse pitting (or honeycombing) in the inner face of sour orange bark immediately below the budunion. In citrus areas where stubborn infections are not present or are symptomless due to low temperature, the presence of inverse pitting on sour orange rootstock is a good diagnostic symptom for tristeza. Nevertheless, trees suffering quick decline usually do not have any visible inverse pitting and CTV-infected trees may not show any conspicuous decline even if grafted on sour orange. Thus, biological or serological tests are usually necessary to confirm CTV infection.

Graft inoculation of buds or bark particles from the candidate tree onto Mexican lime seedlings is the commonest biological test to diagnose the presence of CTV (23). Most virus isolates induce vein clearing and stem pitting on this indicator citrus species, but recently, a CTV isolate has been discovered which does not induce any symptoms on lime (7). In addition, this test is expensive and long lasting (2-6 months), and this is a serious drawback when numerous tests have to be performed or quick results are required to eliminate infected trees.

Serological tests, namely ELISA (5)(9), immunoelectronmicroscopy (14) or immunofluorescence (26) have overcome the above mentioned limitations of biological tests. Monoclonal antibodies specific to CTV have been obtained (27), which react with most known virus isolates. Depending on the circumstances, one of the serological tests alone, or combined with graft-inoculation on Mexican lime should be used to confirm the presence of CTV in any suspicious field tree.

2. Virus strains: identification procedures

CTV exists as numerous strains which differ in host range, symptom intensity induced on certain hosts or aphid transmissibility. Moderate strains usually induce stunting and decline on trees grafted on sour orange and stem pitting in a few susceptible citrus species (limes, citron, Citrus macrophylla, etc.). Damage produced by these strains can be easily avoided by propagating virus-free budwood on tristeza-tolerant rootstocks.

Severe strains may induce, in addition, stunting and stem pitting on different sweet orange or grapefruit cultivars, even if grafted on tolerant rootstocks, and a seedling yellows reaction (13) on lemon, sour orange or grapefruit seedlings. Once severe strains are endemic, damage can be controlled only by cross-protection with mild strains. This method requires long periods of trials and adequate protecting strains may not always be available. Protection may also eventually breakdown.

Severe CTV strains seem to be in minority in the Mediterranean area at present, but isolates carrying the seedling yellows component have been found at least in Israel, Turkey and more recently in Spain (2), and eventually they could become widespread.

For the above reasons, identification of CTV strains

present in different Mediterranean countries is of paramount importance and the necessity of quarantine measures to avoid introduction of severe CTV strains from other countries cannot be overemphasized.

CTV strains can be characterized by symptom intensity induced in a range of hosts, (1) (3), but this is a long lasting procedure and the results are not always clearcut.

Faster and more precise results are usually obtained with biochemical procedures, including characterization of specific nucleic acids and virion coat protein. Analysis of ds-RNA in extracts from infected plants has been used to identify CTV strains (12) (18). This is a faster method of strain identification, but CTV isolates having the same ds-RNA pattern may still differ in biological properties (18).

Nucleic acid hybridization using cloned cDNAs has also been tried to distinguish CTV strains (25). P32-labeled probes were prepared that reacted with several CTV isolates but failed to react with one of them, thus providing evidence that the isolates assayed had detectable genomic differences. Positive identification of CTV strains would probably require to sequence the genome in order to localize useful areas for construction of specific probes. Recently, a cDNA library covering about 70% of the genome has been obtained from a Florida virus isolate (8), which in a near future might enable to prepare some strain-specific probes.

Analysis of peptide maps obtained by digestion of coat protein with specific proteases has enabled us to distinguish CTV isolates having the same ds-RNA pattern and similar biological properties (unpublished results). This method is not adequate for routine identification of CTV strains but it can be a powerful complement of faster methods like ds-RNA analysis.

Serology has been a useful means to compare and classify strains of some viruses, but polyclonal or monoclonal antibodies so far available to CTV, were not strain specific. Recently, mono-clonal antibodies specific to CTV have been obtained, which by ELISA react only with certain virus strains, and not with others (Garnsey, personal communication). This opens new possibilities for a quick and reliable identification of CTV strains in the near future.

An adequate combination of these methods, depending on specific circumstances, would enable identification of new severe CTV isolates introduced in the Mediterranean area or arisen by natural mutation within the area. These isolates are a serious threat to citrus production and a cooperative effort should be made to prevent their becoming widespread in the Mediterranean basin.

3. Natural spread of CTV

CTV is naturally spread in the field by different aphid species. Toxoptera citricidus (Kirk.) is considered the most efficient vector for CTV, although Aphis gossypii Glover has shown ability to transmit some virus isolates with efficiencies close to that of T. citricidus (24).

The situation of CTV spread by aphis in the Mediterranean countries has not been throughly studied. In Italy, trials to transmit by aphids the CTV isolates detected by ELISA have been

so far unsuccessful (11) and the disease does not seem to be spreading in the field. In Israel, A. gossypii is the most efficient vector (the rate of transmission varies with virus strains) and A. citricola Van der Goot (A. spiraecola) is able to transmit some isolates with very low efficiency (22). In Spain, three aphid species (A. gossypii, A. citricola and T. aurantii Boyer de Fonscolombe) have proved to vector local CTV strains (15). A.gossypii is the most efficient vector, although it usually accounts for less than 4% of the total aphid population feeding on citrus. Contrarily, A. citricola is far less efficient transmitting CTV but it often accounts for about 90% of the aphid population. Thus, both species are probably the causal vectors of most new infections under field conditions. The efficiency of CTV transmission is affected, not only by aphid species, but by virus strains (15) (16) and the donor and receptor citrus species (17) (24).

The rate of CTV spread in the Mediterranean area is low when compared with high number of trees that 50 years ago were killed by the disease in South America in less than two decades. Furthermore, it is well known that CTV was present in Israel and in Spain for more than 25 years before any obvious spread of the disease could be observed. This seems to be also the case in Italy, and probably in many other countries where tristeza has been found in isolated trees or never detected. However, the experience of Israel and Spain indicates that this is only a transitory situation and eventually virus and vectors fit each other and tristeza spread occurs with an ever increasing rate. We are monitoring the rate of CTV spread by ELISA in different Spanish citrus areas and have observed that while in areas with low to moderate incidence of tristeza (less than 10% infected trees) the rate of diffusion is low (unpublished results), in those being heavily infested (more than 50% infected trees) the rate of diffusion is extremely high (21). This situation should be a warning for those areas where tristeza is in an apparent non-spread situation.

T. citricidus has not yet been found in the Mediterranean basin, but there is no reason to think that this aphid would not breed on citrus of this area. The appearance of this vector would probably accelerate tristeza spread in the area and severe isolates might be transmitted with higher efficiency (24).

4. Control measures

The most effective control of CTV in a citrus area is the inoculum exclusion by a well operated quarantine system. Availability of quick diagnosis tests like ELISA using monoclonal or polyclonal antibodies makes this control easy as far as the quarantine system is able to enforce control of any budwood introduced in the area. A sanitary program to obtain virus-free budwood available to growers for new plantings is the necessary complement of any quarantine system.

Once the virus has been introduced in an area, removal of infected trees can help slow down tristeza spread or even to eradicate it from the area. An inoculum suppression program has been operating in the Central Valley of California for about twenty years combined with quarantine measures to prevent budwood introduction from CTV-infested areas. As a result of

this program the incidence of CTV in the area has actually decreased. The operation costs of this type of programs are very low if compared with the benefits obtained from delaying or even suppressing the exponential increase in the number of infected trees. In the Mediterranean basin, this type of survey and removal of infected trees has been done in Italy. In Israel, a CTV eradication program has been in operation for more than fifteen years (22). In Spain, general surveys to assess CTV incidence have been carried out in Sevilla (19) and in the Eastern coastal areas (10). A classification of zones according to the percentage of infected trees is being defined in order to establish specific recommendations (removal of infected trees, control of budwood source trees and trees to be topworked, replanting with virus-free plants on tolerant roootstocks, etc.) Several ELISA labs have been established by the Plant Protection Services to lend a free diagnostic service to growers. In this way, they can select CTV-free budwood when they need topworking a citrus planting or remove scattered infected trees when the CTV incidence is very low. Using these diagnostic services will prevent CTV spread by growers through infected budwood and slow down natural diffusion in many areas.

A more complicated problem is the control of new severe CTV strains introduced or arisen in areas where moderate CTV strains, not causing damage on trees propagated on tolerant rootstock, are widespread. Two difficulties have to be solved in this case: the first one is to detect that a new virus strain is present in the area, and the second one to assess if it is spreading in the area already infested by common isolates.

This is the situation in some citrus areas in Spain, where a severe CTV isolate carrying the seedling yellows component has been propagated with an early satsuma cultivar illegally introduced, probably from Japan (2). An eradication program for this severe strain is being implemented by the Plant Protection Service of Valencia (Spain) on the basis of the mandatory declaration of growers that have propagated this cultivar and who are given new virus-free plants to replace those destroyed. Rapid, reliable methods of CTV strain identification are needed now to assess possible natural spread of this severe strain to other citrus cultivars close to the original source trees.

5. Final considerations

The Mediterranean basin, with more than 500 thousand hectares of citrus is, at present, one of the world areas that has been less affected by tristeza. The incidence of CTV is still low, compared with other citrus areas, moderate virus strains are prodominant and Toxoptera citricidus, the most efficient vector for CTV, has not yet been introduced into the area.

This is a fortunate situation, but by no means will it last forever. On the contrary, it should be expected that natural spread of the virus by A. gosypii and other aphid species will occur at an increasing rate.

It would be difficult to avoid natural spread of CTV isolates already established in the area, but it could be delayed with a few control measures. ELISA testing of propagative budwood would prevent establishment of new CTV foci

by growers and periodical survey and removal of isolated infected trees would keep inoculum density low, and slow down the rate of natural spread by aphids. The effective cost of such a program would be negligible compared with the benefits that could be obtained by avoiding or delaying CTV spread in areas where sour orange is the predominant rootstock. In those locations where inoculum control would become unfeasible, progressive replanting with virus-free cultivars on CTV-tolerant rootstocks, would be an alternative solution.

These measures can be implemented by individual countries, but they should be complemented with cooperative actions directed to preserve this biogeographical area in its present situation avoiding the introduction and dispersion of new severe CTV strains and the efficient vector Toxoptera citricidus.

A regional program based on quarantine measures, surveys for new aphid species, CTV detection and strain identification, inoculum control and quick exchange of information between countries, should be established to prevent the ravages caused by tristeza in other world areas.

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