IOBC/WPRS

Working Group "Integrated Control in Protected Crops"
"Mediterranean Climate"

OILB/SROP

Groupe de Travail "Lutte intégrée en Cultures Protegées"
"Climat Méditerranéen"

Proceedings of the Meeting
Comptes-Rendus de la Réunion

at/à

Tenerife, Islas Canarias, España
3-6 November 1997

Edited by R. Albajes & A. Carnero

IOBC/WPRS Bulletin
Bulletin OILB/SROP Vol. 20(4) 1997

The IOBC/WPRS Bulletin is published by the International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palaearctic Regional Section (IOBC/WPRS)

Le Bulletin OILB/SROP est publié par l'Organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles, section Régionale Ouest Paléarctique (OILB/SROP)

Address General Secretariat:
INRA Station de Zoologie
Domaine Saint-Paul
Site Agroparc
84914 AVIGNON Cedex 9
France

ISBN 92-9067-089-4

Préface

Ce volume rassemble la plupart des contributions orales présentées lors de la réunion du groupe de travail de l'OILB/SROP "Lutte Integrée en Cultures Protegées, Climat Méditerranéen" qui a tenu place à Tenerife (Îles Canaries, Espagne) du 3 au 6 Novembre 1997. D'autres contributions- y compris les posters- qui ont également enrichi les discussions au long de la réunion n'ont pas pu malheureusement être publiées.

Le groupe se mantient actif et divers. C'est à s'en feliciter. Notre diversité géographique, disciplinaire, de formation et de langue est á conserver, mais aussi à accroître. Quand on constate l'accroissement incessant d'hectares protegées dans la Méditerranée, en particulier dans la Méditerranée méridionale, quand on affirme que les maladies fongiques et viriques sont un frein important pour une plus large application de la lutte biologique et integrée dans nos serres, quand on s'impatiente de la lenteur dans l'adoption par nos serristes de techniques de lutte integrée, il faut nous exiger plus de coopération avec la Méditerranée Sud, plus de travail en collaboration entre les entomologistes et les pathologistes et plus d'effort pour que nos recherches connectent avec les applicateurs. Un long parcours est encore à faire.

Je tiens a remercier notre collègue Aurelio Carnero qui a pris en charge l'organisation de la rencontre et qui, avec le concours des autorités locales et espagnoles en général, nous a permis de jouir de l'hospitalité des Canaries. Permettez-moi de rendre hommage à deux collègues dont l'effort a été decisif pour que notre groupe ait pu naître et se maintenir au long des ces treize années et qui malheureusement nous ont quitté depuis notre dernière réunion en 1994: les professeurs Cavalloro et Degheele. Le professeur Minks a assuré cette fois la publication de ce numéro de notre Bulletin. Merci, enfin, à tous les auteurs de ce volume et aux participants à la rencontre.

Preface

You have in your hands a new volume that includes most of the oral contributions to the meeting of the working group of IOBC/WPRS, "IPM in Protected Crops, Mediterranean Climate", which was held in Tenerife, Canary Islands, Spain on November 3-6 1997. Unfortunately, other contributions that enriched the discussions during the meeting, including the posters, could not be published.

The group remains alive and plural, for which congratulations are due. We must conserve the diversity of our group in geography, discipline, training and language, but we still need more. When we note the continual increase in the number of protected hectares in the Mediterranean, in particular in the southern Mediterranean, when we find that fungal and viral diseases are an important obstacle to a wider application of biological and integrated control in our greenhouses, or when we become impatient at the slowness of our greenhouse growers to adopt IPM techniques, we should demand more cooperation with the southern Mediterranean, more joint work between entomologists and pathologists and greater efforts to connect with the implementers. We still have a long way to go.

I should like to thank our colleague Aurelio Carnero for his dedication in organising the meeting which, with the aid of the local and Spanish authorities, allowed us to enjoy the hospitality of the Canary Islands. I should further like to pay homage to two colleagues who have left us since the last meeting in 1994, Professors Cavalloro and Degheele, whose efforts were decisive for the birth of our group and its maintenance in its thirteen years of existence. Professor Minks was responsible for the publication of this volume. Finally, thanks are due to all the authors of this volume and all those who participated in the meeting.

Ramon Albajes, animateur du groupe de travail/convenor of the working group

INDEX/CONTENTS

III. Nematodes

IV. Aleurodes/*Whiteflies*

V. Thrips

VIII. Entomopathogènes et techniques moléculaires/*Entomopathogens and molecular techniques*

*****

I. IPM systems in Mediterranean regions
Systèmes integrés de lutte en culture protegée méditerranéenne

BILAN DES INTRODUCTIONS D'AUXILIAIRES DANS LES SERRES TROPICALES DU PARC PHOENIX A NICE

F. Bertaux*, J.P. Marro**

* Laboratoire National de la Protection des Végétaux, 1382 route de Biot 06560 VALBONNE
** INRA Laboratoire de Biologie des Invertébrés, Centre de Transfert, 1382 route de Biot 06560 VALBONNE-France

Résumé

Les serres du Parc Phoenix gérées par la Ville de Nice s'étendent sur une surface de 6.000 mètres carrés. C'est une collection de plantes tropicales et subtropicales, avec des secteurs plus humides (orchidées, fougères) ou plus secs (plantes grasses).

Depuis plus de 3 ans, a été mis en place un programme de lutte intégrée. On observe un enrichissement progressif de la faune auxiliaire à la suite des nombreux lâchers effectués mais aussi par des entrées naturelles depuis l'extérieur.

Les auxiliaires suivants, disponibles dans le commerce, ont été lâchés :

-sur pucerons : les parasitoïdes *Aphidius colemani* et *Aphelinus abdominalis*, les prédateurs *Aphidoletes aphidimyza* et *Harmonia axyridis*.

-sur aleurodes : *Encarsia formosa*.

-sur tétranyques : *Phytoseiulus persimilis*.

-sur thrips : *Orius laevigatus* et *Amblyseius cucumeris* aussi utilisé contre les tarsonèmes.

-sur cochenilles farineuses : le parasitoïde *Leptomastyx dactylopii* et la coccinelle *Cryptolaemus montrouzieri* efficace aussi sur ovisacs de la cochenille australienne et de pulvinaires.

D'autre part, d'autres auxiliaires provenant d'élevages de l'INRA ont été introduits :

- les coccinelles *Rhyzobius lophantae*, *Chilocorus nigritus* et *Chilocorus bipustulatus* prédatrices de cochenilles diaspines.
- contre les cochenilles farineuses : les parasitoïdes *Leptomastidea abnormis* et *Anagyrus bohemani*, en renforcement de population ; des larves de *Cryptolaemus montrouzieri*.
- le parasitoïde *Aphytis holoxanthus* contre *Chrysomphalus aonidum*.

De plus, de nombreux auxiliaires naturellement présents ont été recensés :

-sur pucerons : *Praon* sp, *Lysiphlebus testaceipes*, syrphes et chrysopes.

-sur aleurodes : *Encarsia* sp., *Macrolophus caliginosus*, *Dicyphus errans*.

-sur acariens : différents phytoséides.

-sur la cochenille *Diaspis boisduvalii* un parasitoïde : *Plagiomerus sp.*

-sur les cochenilles *Saissetia oleae* et *Saissetia coffeae* : *Scutellista cyanea* prédateur d'œufs, ainsi qu'un *Encyrtus sp.*

La plupart des auxiliaires se maintiennent d'une année sur l'autre du fait de la continuité du climat, de la permanence et de la diversité des plantes présentes. Seules quelques coccinelles comme *Harmonia axyridis* et *Cryptolaemus montrouzieri* doivent être réintroduites chaque année.

Pendant ces 3 ans, on a constaté que les pucerons, aleurodes et acariens devenaient moins importants tandis que les cochenilles posaient un problème sur certaines plantes.

Cependant, le résultat global est très positif ; de rares traitements localisés sont pratiqués avec des produits sélectifs. L'objectif actuel est d'introduire de nouveaux auxiliaires contre certaines cochenilles posant encore problème.

Présentation

Le Parc Phoenix à Nice est un parc floral de 7 hectares qui a été créé en 1990 et est géré par le Service des Espaces Verts de la ville. Il comprend aussi une des plus grandes serres d'Europe d'une surface de 6.000 mètres carrés de 26 mètres de hauteur en zone centrale.
Elle héberge une grande diversité de plantes d'origine tropicale et est divisée en différents secteurs :
- zone centrale où se trouvent les plus grands sujets : Palmiers Royaux, Arbre du Voyageur, bananiers ;
- zones à orchidées plus sombre et plus humide où se côtoient de nombreuses variétés d'orchidées et de broméliacées cultivées ainsi que des espèces rares ;
- zone australe à climat plus sec et plus frais en hiver contenant des plantes grasses et des espèces de climat méditerranéen ;
- zone à fougères à ambiance très humide et ombragée où croissent des fougères arborescentes ;
- zone Louisiane où de nombreuses plantes d'appartement comme crotons, poinsettias, Ficus, Schefflera constituent des massifs ;
- zone à papillons où de multiples espèces tropicales et des oiseaux évoluent librement.

Un système de régulation du climat est en place qui permet de contrôler la température et l'hygrométrie dans chaque zone: chauffage, brumisation, ouvertures automatiques.

Depuis 4 ans maintenant, nous développons un programme de lutte intégrée. Des visites fréquentes et régulières permettent de suivre l'évolution en temps réel de tous les problèmes phytosanitaires ; chaque mois, un compte-rendu détaillé dresse le bilan de la situation ; des solutions sont proposées qui privilégient la lutte biologique ou les méthodes culturales (taille, arrosage du feuillage, apport de matière organique).
2 types de lutte biologique sont menées :
- des lâchers inondatifs avec des auxiliaires d'origine commerciale : surtout contre les aleurodes, pucerons, cochenilles farineuses et acariens;
- des lâchers de nouvelles espèces non encore présentes dans la serre.

Dans le premier cas, nous utilisons les mêmes auxiliaires que ceux utilisés dans une lutte biologique classique, comme elle existe en cultures maraîchères sous serre.
Dans le deuxième cas, nous espérons une action à long terme par enrichissement de la faune auxiliaire. Ceci est rendu possible par la pérennité de la plupart des plantes présentes et la persistance toute l'année d'un climat favorable.

Nous avons établi une liste restreinte de matières actives utilisables en cas de pullulations de ravageurs ; mais les traitements ne sont pratiqués qu'en dernier recours et toujours en localisé.

Matière active	ravageur visé
buprofézine	aleurodes
huiles blanches	cochenilles
pyrimicarbe	pucerons
fenbutatin oxyde	acariens
abamectin	thrips

Les pucerons

La plupart des espèces de serre ont été rencontrées, en particulier *Myzus persicae* (Sulzer) et *Aphis gossypii* (Glover) très fréquent sur les Malvacées comme les hibiscus. *Acyrtosiphon pisum* (Harris) est souvent présent sur les légumineuses tandis que *Aphis nerii* (B. de F.) se développe seulement sur les plantes de la famille des Asclépiadacées ainsi que celle des Apocynacées comme le laurier-rose.

On rencontre des pucerons toute l'année dans la serre avec des maxima en mars, avril et à l'automne lorsque les plantes sont en croissance.

La lutte contre les pucerons est basée sur des lâchers périodiques de prédateurs et de parasitoïdes.

Nous utilisons :

- les parasitoïdes *Aphelinus abdominalis* et *Aphidius colemani* (Viereck) [*Hym. : Braconidae*];
- la cécidomyie *Aphidoletes aphidimyza* (Rondani) qui se maintient bien ;
- la coccinelle *Harmonia axyridis* (Pallas) qui a un bon effet de choc mais une action de courte durée.

Nous constatons aussi la présence de nombreux auxiliaires indigènes : *Lysiphlebus testaceipes* (Cresson) parasite de nombreuses espèces de pucerons (Costa *et al.*,1988), des praons, des prédateurs comme des coccinelles Scymnidae et des syrphes, surtout dans la zone australe plus ouverte sur l'extérieur.

Depuis que la lutte intégrée s'est mise en place, les proliférations sont beaucoup plus rares ; de nombreux auxiliaires naturels apparaissent très rapidement sur les foyers.

Les aleurodes

Deux espèces ont été trouvées : *Trialeurodes vaporariorum* (Westwood) et *Bemisia tabaci*(Genn.), cette dernière surtout sur Poinsettia et Hibiscus.

On en rencontre aussi toute l'année avec des maxima au printemps et à l'automne.

Nous conseillons des lâchers réguliers de *Encarsia formosa* (Gahan); nous l'avons retrouvé aussi parasitant *Bemisia tabaci* sur Poinsettia.

Deux parasitoïdes naturels se retrouvent régulièrement : *Encarsia lutea* (Masi) et *Encarsia tricolor* (Forster).

Deux punaises Mirides se sont installées naturellement dans la serre : *Dicyphus sp* en été et *Macrolophus caliginosus* (Wagner) ; cette dernière espèce est présente toute l'année sur certaines plantes refuges comme *Angelonia gardneri*, une Scrophulariacée.

Comme pour les pucerons, nous avons noté une réduction très importante des attaques d'aleurodes depuis la mise en place de la lutte intégrée. Quelques pullulations peuvent encore s'observer sur les pousses tendres de certaines plantes, souvent après une taille : Hibiscus, Sparmannia, Greyia,..

Les thrips

Le thrips californien *Frankliniella occidentalis* (Pergande) est régulièrement présent, surtout dans les fleurs, mais a occasionné peu de dégâts ; cependant, quelques plants d'impatiens ont dû être éliminés à cause du tomato spotted wilt virus (TSWV) transmis par ce thrips.

En été, on détecte régulièrement des punaises prédatrices *Orius sp* entrant naturellement dans les serres.

Une autre espèce de thrips d'origine tropicale *Hercinothrips femoralis* (Reuter) est beaucoup plus nuisible ; le feuillage attaqué prend un aspect terne grisâtre. Des plantes de

diverses familles botaniques sont touchées comme *Crinum sp*, *Eugenia sp*, *Aristolochia sp*, *Jatropha multifida*, *Hibiscus elatus*, manguier, etc...

Heureusement, chaque année, les thrips disparaissent pratiquement complètement en hiver. On suppose que les fortes humidités sont favorables au développement de champignons entomopathogènes. Par contre, ils prolifèrent en été.

La lutte n'est pas parfaitement efficace : les apports réguliers de *Amblyseius cucumeris* (Oudmans) ne font que ralentir l'évolution. Les punaises *Orius sp* ont été testées mais elles restent dans les fleurs et agissent très peu contre *Hercinothrips femoralis*.

Les acariens

Les attaques de *Tetranychus urticae* (Koch) se localisent dans les secteurs les plus secs : zone australe, parties hautes des plantes ou près des portes.

Le prédateur *Phytoseiulus persimilis* (Athias-Henriot) s'installe difficilement du fait de conditions très chaudes et sèches. Par contre, d'autres espèces de phytoséides indigènes (non identifiées) sont abondantes.

La brumisation limite les dégâts dans beaucoup de zones. On complète par des arrosages au jet du feuillage : zone australe, bordures.

Un autre acarien, le tarsonème *Polyphagotarsonemus latus* (Banks), se développe en été dans les zones chaudes et mal ventilées sur quelques plantes comme *Schefflera sp*, *x Fatshedera sp, Impatiens, Begonia* et *Lantana.*

La lutte biologique avec *Amblyseius cucumeris* (Oudemans) n'est pas très efficace. Par contre les phytoséides naturels ont sûrement un rôle non négligeable (Mc Murtry *et al*, 1984).

Les cochenilles

Elles deviennent préoccupantes dans quelques secteurs et sur certaines plantes plus atteintes.

→ cochenilles farineuses :

2 espèces ont été identifiées : *Planococcus citri* (Risso), très polyphage et *Pseudococcus longispinus* (Targioni), rencontrée sur *Beaucarnea recurvata*, *Zamia furfuracea* et différentes fougères. La deuxième espèce semble préférer les zones les plus chaudes et les plus humides. Elles prolifèrent dans les secteurs peu ventilés et qui ne sont pas atteints par la brumisation.

Chaque année, on constate un maximum des attaques en fin d'hiver, sans doute parce que les auxiliaires sont peu actifs à cette période.

La coccinelle *Cryptolaemus montrouzieri* (Mulsant) est lâchée régulièrement sous forme d'adultes. Elle est très efficace contre ces 2 espèces ; nous l'avons retrouvée aussi s'attaquant à une pulvinaire *Protopulvinaria pyriformis* (Cockerell) ainsi que sur les ovisacs de la cochenille australienne *Icerya purchasi* (Maskell). Son optimum thermique est élevé : 30°C (Ramesh Babu *et al.* , 1987) ce qui pourrait expliquer sa faible activité en hiver. Il est même nécessaire de la réintroduire chaque année. Une autre espèce de coccinelle *Nephus reunioni* (Chazeau) originaire de l'île de La Réunion vient d'être lâchée et pourrait compléter l'action de *C. montrouzieri* ; son optimum thermique est plus bas : 24-25°C, et elle tolère des hygrométries faibles (Izhevsky *et al.*, 1988).

Leptomastix dactylopii (Howard) [*Hym. : Encyrtidae*] a été introduit plusieurs fois contre *P. citri* mais reste peu fréquent. Par contre, *Leptomastidea abnormis* (Girault) qui parasite les jeunes larves de *P. citri* est très abondant. D'autres parasitoïdes sont régulièrement présents : *Anagyrus pseudococci* (Girault) contre *P. citri* (Copland *et al.*, 1985) et *Anagyrus fusciventis* (Girault) contre *P. longispinus*.

→ cochenilles diaspines :

Deux espèces polyphages sont très présentes : *Diaspis boisduvalii* (Signoret) et *Chrysomphalus aonidum* (L.) («Florida Red Scale »).

D. boisduvalii se rencontre sur de nombreux palmiers comme le cocotier *Cocos nucifera*, *Neodypsis decaryi*, des bananiers, strelitzias, héliconias, orchidées et broméliacées. Elle est d'origine tropicale et se retrouve dans les secteurs de la serre les plus chauds en hiver. *C. aonidum* est aussi à affinité tropicale et se rencontre dans les mêmes zones sur palmiers, manguier, bananiers, anthurium, uniquement sur feuilles.

Ces deux espèces, depuis 4 ans, ont augmenté progressivement leurs effectifs et ont étendu la gamme des plantes hôtes : sur les plus sensibles et dans les zones les moins soumises à la brumisation, elles ont contribué, surtout *D. boisduvalii*, au dépérissement d'espèces comme des héliconias, bananiers et palmiers.

Nous avons été contraints de préconiser des traitements locaux aux huiles blanches ; mais nous avons surtout tenté des introductions d' auxiliaires coccinelles ou parasitoïdes .
Les deux coccinelles *Chilocorus bipustulatus* L. et *Chilocorus nigritus* (Fabricius) provenant d'élevages INRA, introduites en 1995, n'ont jamais été retrouvées. Par contre la coccinelle *Rhyzobius (Lindorus) lophantae* (Blaisdell), élevée aussi à l'INRA, lâchée à la même époque en faible quantité, 50 adultes environ, s'est répandue dans toute la serre. Elle prédate surtout *D.boisduvalii* à bouclier moins épais que *C. aonidum* qui n'est attaquée que lorsque les deux diaspines sont ensemble. Elle a permis de contenir les pullulations de *D. boisduvalii*, mais elle ne s'attaque qu'à des colonies déjà bien développées lorsque les dégâts sont visibles sur la plante. Un parasitoïde *Plagiomerus sp* limite aussi les populations de *D. boisduvalii*.
Début 1996, *Aphytis holoxanthus* (De Bach) a été introduit de Floride en Quarantaine à l'INRA à Valbonne. IL semble être la parasite le plus efficace car il a permis, après son introduction en Floride, de réduire très rapidement les populations de *C. aonidum* en dessous du seuil de nuisibilité (Browning, 1990).
Quelques centaines d'adultes ont été lâchés en trois fois au printemps 1996. Très vite l'auxiliaire s'est installé et répandu dans la serre ; suivant les secteurs, le taux de parasitisme qui était quasiment nul, atteint actuellement 20 à 80% au bout d'un an . Sur des plantes très touchées comme *Dracaena marginata*, seules les vieilles feuilles restent infestées. La progression de C. *aonidum* est actuellement stoppée.

→ cochenilles Coccidae :

La cochenille *Saissetia coffeae* (Walker) est abondante dans les secteurs à températures élevées et forte hygrométrie sur les fougères *Nephrolepis*, *Asplenium*, *Polypodium*, *Polystichum* ainsi que *Ixora coccinea* et les inflorescences de *Pachystachis lutea* ou *Aphelandra sp*. Deux auxiliaires sont naturellement présents : *Scutellista cyanea* (Motschulsky) [*Hym. : Pteromalidae*] prédateur d'œufs et *Encyrtus lecaniorum* (Mayr) moins fréquent. Mais des traitements aux huiles blanches restent nécessaires pour contenir les attaques. Nous avons lâché très récemment la coccinelle *Rhyzobius forestieri* (Mulsant) que nous avons pu élever sur cette cochenille; elle est utilisable aussi contre *Coccus hesperidum* L. et *Saissetia oleae* (Olivier) (Iperti *et al.*, 1989) présentes aussi dans la serre.

Saissetia oleae se retrouve sur quelques plantes ligneuses et est abondante sur un *Ficus religiosa*. Jusqu'à présent, *Scutellista cyanea* était le seul auxiliaire notable. Bien que lâché très récemment en mai 1997, nous avons retrouvé *Metaphycus bartletti* (Annecke & Mynhardt) [*Hym. : Encyrtidae*] qui parasite le troisième stade larvaire (Panis, 1981).

De temps en temps, la cochenille *Coccus hesperidum* L. prolifère surtout en zone australe ; elle est très polyphage ; elle produit beaucoup de miellat sur lequel se développe la fumagine. Mais cette cochenille est naturellement très fortement parasitée, en particulier par *Coccophagus scutellaris* (Dalman) [*Hym. : Encyrtidae*] (Llorens Climent, 1990) et *Encyrtus lecaniorum* (Mayr) . Aucune intervention n'est nécessaire.

→ autres cochenilles :

D'autres espèces sont présentes mais n'occasionnent pas de dégâts et sont très localisées comme *Hemiberlesia sp*, *Aspidiotus nerii* (Bouché) et *Icerya purchasi* (Mask). Le parasitisme naturel est très important pour les 2 premières tandis que des lâchers de *Rodolia cardinalis* (Mulsant) ont permis de contrôler *Icerya purchasi*.

Conclusion

L'expérience de 4 ans de fonctionnement en lutte intégrée dans les serres du Parc Phoenix est un succès. Les traitements sont très peu nombreux, toujours localisés et utilisent une gamme de matières actives très peu toxiques. Si bien que la serre est ouverte tous les jours au public, alors qu'auparavant elle devait fermer une journée par semaine pour cause de traitements phytosanitaires.

La réussite de cette expérience a été facilitée par la diversité des plantes présentes pouvant servir de refuges aux auxiliaires ainsi que leur longue durée de séjour. Il faut reconnaître aussi que les seuils de nuisibilité sont beaucoup plus élevés que dans une serre de production.

L'aspect pédagogique est aussi très important : des pancartes sont disposées pour expliquer l'intérêt de la lutte biologique ; le compte-rendu mensuel de visite est affiché.

L'objectif actuel consiste à continuer les introductions de nouvelles espèces d'auxiliaires pour améliorer l'équilibre : par exemple contre certaines cochenilles et le thrips *Hercinothrips femoralis*.

Il faut cependant rester très vigilant sur les introductions de plantes qui pourraient héberger de nouveaux ravageurs : inspection, quarantaine.

La compétence et la motivation du personnel sont une des clefs du succès : il a appris à reconnaître les principaux auxiliaires et ravageurs et détecter rapidement tout nouveau problème.

Remerciements

.à MM Brun, Kreiter, Malausa et Pinet du Centre INRA d'Antibes pour la fourniture de différents auxiliaires.

.à MM Bouet, Menuet et Widmann des Espaces Verts de la Ville de Nice pour leur implication dans le programme de lutte intégrée.

Références

BROWNING, H.W.1990. Classical biological control of citrus scale insects. In « Pest Management in the Subtropics. Biological Control- a Florida Perspective » D. Rosen , F.D. Bennett, J.L. Capinera ed. Intercept Limited, Andover, 49-78.

COPLAND, M.J.W., TINGLE, C.C.D., SAYNOR, M., PANIS, A. 1985. Biology of glasshouse mealybugs and their predators and parasitoids. In Biological Pest Control : The Glasshouse Experience (N.W. Hussey, N. Scopes, eds) 82-86. Cornell Univ. Press, Ithaca, NY.

COSTA, A., STARY, P. 1988. *Lysiphlebus testaceipes*, an introduced aphid parasitoid in

Portugal [*Hym.: Aphidiidae*]. Entomophaga **33** (4), 403-412.
IPERTI, G., GIUGE, L., ROGER J.P. 1989. Installation de *Rhyzobius forestieri* [*Col. : Coccinellidae*] sur l'île de Porquerolles. Entomophaga **34** (3), 365-372.
IZHEVSKY, S.S., ORLINSKY, A.D. 1988. Life history of the imported *Scymnus (Nephus) reunioni* [*Col. : Coccinellidae*] predator of mealybugs. Entomophaga **33** (1), 101-114.
LLORENS CLIMENT, J.M. 1990. Homoptera I. Cochenillas de los citricos y su control biologico. Pisa Ediciones. Alicante.
Mc MURTRY, J.A., BADII, M.H., JOHNSON, H.G. 1984. The broad mite *Polyphagotarsonemus latus*, as a potential prey for phytoseiid mites in California. Entomophaga **29** (1), 83-86.
PANIS, A. 1981. Note sur quelques insectes auxiliaires régulateurs des populations de *Pseudococcidae* et de *Coccidae* [*Hom. : Coccoidea*] des agrumes en Provence orientale. Fruits **36**, 49-52.
RAMESH BABU, T., AZAM, K.M.1987. Biology of *Cryptolaemus montrouzieri* Mulsant [*Col. : Coccinellidae*] in relation with temperature. Entomophaga **32** (4), 381-386.

DEVELOPMENT OF INTEGRATED CROP MANAGEMENT SYSTEMS FOR SWEET PEPPERS IN SOUTHERN SPAIN

Garcia, F.,[1] GreatRex, R. M.,[2] and Gómez, J.[1]

[1] Novartis B.C.M. España S.A., Polig. Industrial La Redonda, Sta Ma del Aguila, 04170 El Ejido, (Almeria, España)

[2] Novartis B.C.M. Limited, Aldham Business Centre, New Road, Aldham, Colchester, Essex, CO6 3PN, England.

Abstract

Novartis B.C.M. has been working to develop Integrated Crop Management (I.C.M.) programmes in Spain, in collaboration with Novartis Agro, since 1994. Implementation of successful programmes in some protected salad vegetables is now beginning after three years of trials. Earliest successes have been in cucumber crops. Work has continued in sweet pepper crops and is now yielding success in some areas. Sweet pepper crops are grown in two main geographical regions, with different growing seasons. The severity of pest problems is different in the two regions, and control measures also have to be different. For example, transmission of Tomato Spotted Wilt Virus (TSWV) is particularly important in Murcia and Alicante. Control of the vector of TSWV, *Frankliniella occidentalis*, is therefore of crucial importance in this area. In Almeria, where incidence of this virus is lower, the control of *F. occidentalis* is necessary mainly because of the direct damage it causes the crop. The aim has been to reduce chemical inputs by the incorporation of biological control agents into control programmes. The first step has been the rationalisation of chemical control programmes. This and grower training are prerequisites for success. Sweet pepper pests other than thrips can be controlled with chemicals compatible with thrips predators, but those chemicals currently available for thrips control are harmful to most beneficials. As a result the control of thrips using beneficial arthropods has been a key part of the development of I.C.M. programmes. The development of commercially acceptable programmes also requires close attention to the costs of the various elements included, and to the normal costs of chemical control. Acceptable costs are influenced by external factors such as consumer pressure as well as by the internal economics of the industry.

In terms of production of horticultural crops, Spain is second only to Italy within the European Union, producing 20% of the total tonnage (Table 1). Salad vegetables are a major component of this production, and are grown both in the open field and under protection. Holland was once pre-eminent in the production of protected salads for consumption in Northern Europe, but has now yielded this position to Spain, at least in terms of area under cultivation. This is illustrated below, where areas used for production of specific crops in the most important Spanish regions are compared to those in Holland over the same period (Table 2).

Country	Tonnes x 1000	Percentage of Total
Italy	13035	25
Spain	10328	20
France	7229	14
Great Britain	4160	8
Greece	4013	8
Germany	3927	8
Holland	3702	7
Portugal	1800	3.5
Belgium	1600	3
Austria	455	1
Denmark	314	1
Sweden	246	0.5
Ireland	228	0.5
Finland	214	0.5
European Union Total	**51,411**	**100**

Anuario De La Horticultura Española. (1996)

Table 1. Production of Horticultural Crops in Countries of the Europe Union

	Holland [2]	Almeria [1]	Murcia[1]
Tomatoes	1239ha	5800ha	3800ha
Cucumbers	826ha	2300ha	1490ha
Peppers	1016ha	7000ha	420ha
Aubergines	82.5ha	800ha	140ha
Others	1308ha	14200ha	6000ha
Total	**4475ha**	**30100ha**	**11850ha**

[1] Anuario De La Horticultura Española. (1996)

[2] Centraal Bureau voor de Statistiek, Divisie Landbouw. (1996)

Table 2. Comparative Areas of Protected Crops: 1995

	Surface area (hectares)	Production (Tonnes)	Yield (kg/hectares)
Almeria[1]	7090	394,065	55,580
Murcia[1]	1492	119,062	79,800
Alicante[1]	507	25,037	49,383
Holland[2]	1016	-	-

[1] Anuario De La Horticultura Española. (1996)

[2] Centraal Bureau voor de Statistiek, Divisie Landbouw. (1996)

Table 3. Quantities of Protected Peppers grown in Holland and Different Regions of Spain

Sweet peppers are one of the most important protected crops grown in Southern Spain, with approximately 7000 hectares grown in Almeria and 1,500 hectares in Murcia in 1995. This compares to a total of 1016 hectares grown in Holland over the same period. With the area now grown, the crop occupies a similar total area of protected cultivation to tomatoes, which

have 5,800 hectares in Almeria and 3,800 in Murcia. Large areas of peppers are also grown in the open field, with the total area, including protected cropping, being close to 23,000 hectares.

Crop	Surface area hectares	Yield (Tonnes)	Exports (Tonnes)
Peppers	23,000	779,000	324,783
Tomatoes	55,000	2,706,000	758,740

Anuario De La Horticultura Española. (1996)

Table 4. Total Pepper and Tomato Production in Spain (1995)

A large proportion of the total crop is exported to Northern Europe, where it is sold through the major supermarket chains. As such, pepper production provides a significant source of export earning for Spain and for its regions, and will continue to do so.

	Tonnes	Value (Thousands of pesetas)	Value ($US) (1$ =140ptas)
Germany	79,306	11,209,903	80,070,736
France	18,704	2,643,810	18,884,357
Holland	35,713	5,048,033	36,057,379
Italy	12,464	5,048,033	36,057,379
Great Britain	17,404	2,460,055	17,571,821
Total	**163,591**	**23,123,588**	**165,168,486**

Source: Direccón General de Aduanas- FEPEX

Table 5. Exports of Peppers from Almeria to European Union Countries in 1995

This total is 84.5% of total pepper production in Almeria. Similar figures are not available for the other growing regions.

Crops are generally grown in polythene houses without heating, and yields may be lower than those in glasshouses in northern Europe, which have more sophisticated environmental controls. Yields in Murcia are higher than those in Almeria because the spring and summer growing period is more favourable to the crop, but prices obtained may compensate for the lower yield.

Novartis BCM have been working in Spain since 1995, as part of a joint project with Novartis Agro Espana S.A. The initial phases of the project, carried out by Novartis Agro (Acebes *et al* 1997, Newton *et al*, 1996a and b), concentrated on a rationalisation of pesticide use, leading to an overall reduction in the quantity of pesticide used. This prepared the way for the introduction of beneficial insects and mites into the crop, which would previously have been impossible because of the widespread use of persistent, broad spectrum insecticides.

The overall aim of the work we have been doing has been to develop strategies for the control of the most important pests and diseases of the pepper crop. The incorporation of beneficial arthropods into rationalised chemical programmes is seen as the most practical way to do this, and will enable the growers to meet the demands of their most important customers, the

supermarkets. It is this pressure from the supermarkets which is driving the entire process. They have expressed concern over the heavy use of chemical insecticides and fungicides, and the perceived risk to the consumer from residues left in the produce. The supermarkets are increasingly important as retailers of fresh produce in Europe, and are able to exert considerable economic pressure on growers in consequence. Table 6 illustrates this growing dominance.

Country	1985	1990	1995
France	76	82	81
Germany	60	66	72
Great Britain	55	66	74
Spain	36	55	66

Source: A.C. Nielsen Company S.A.

Table 6. Proportions of Fresh Produce (%) Sold by Supermarkets in Various European Countries

At present there is a tendency to rely heavily on chemical control of pests and diseases, and many applications are made either preventatively or in response to pest presence. There is no recognition of the true risk to the crop, as in the number of pests which may be present before economic damage occurs. In some cases it seems that there is no understanding of what is a pest, and the presence of anything alive on the crop may trigger pesticide application. This may be counterproductive, as even naturally occurring beneficial arthropods may be perceived as the enemy.

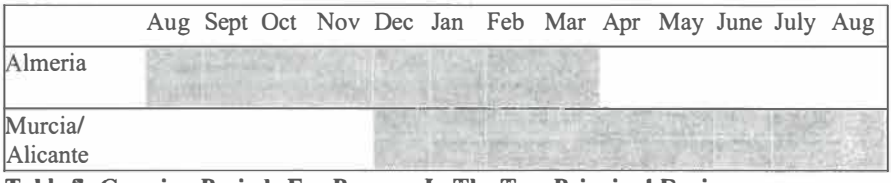


Table 7: Growing Periods For Peppers In The Two Principal Regions.

The sweet pepper crops grown in the two regions present totally different situations. The growing season in Almeria starts in the late summer or early autumn (August or September) and continues through the winter to finish in late February or early March. In Murcia and Alicante the crop is planted in December and cropping continues until the following July or August.

The different growing seasons and regions naturally present quite different pest and disease situations. In Murcia and Alicante, transmission of Tomato Spotted Wilt Virus (TSWV) by the Western Flower Thrips (*Frankliniella occidentalis*) is extremely serious, (Sanchez *et al* 1997) and can lead to the destruction of a large proportion of the crop. In fact the virus is the single most important cause of economic loss for pepper growers in this area: no treatment is available and infected plants cease to yield marketable fruit and must be destroyed. The problem is made more severe where the other crops around the greenhouses, such as artichokes and lettuce, provide alternate hosts for the thrips and may also harbour the virus. Weed hosts may act as virus reservoirs without themselves exhibiting symptoms. Thus

control of thrips is essential not only because of direct damage, but also because of the virus risk.

In Almeria, *Frankliniella* is also the main problem for pepper growers, but the incidence of virus is very low, , with less than half of one percent of plants being affected during the last three years. This is mainly because of the cultural methods adopted in the region. Recognition of the threat posed by TSWV has lead to the removal of weeds which might act as alternate hosts, and to the alternation of pepper crops with crops of melon, cucumber and courgettes which do not support the virus. In addition, pepper crops are not surrounded by field crops which act as reservoirs for the virus (Cuadrado, 1996).

	Almeria	**Murcia/Alicante**
Frankliniella occidentalis	Medium	High
Tomato Spotted Wilt Virus	Almost zero	High
Bemisia tabaci	Medium	High
Myzus persicae	Medium	Medium
Caterpillar	High[1]	High[2]
Tetranychus urticae	Low	High
Polyphagotarsonemus latus	High	Low

[1] *Spodoptera exigua, Heliothis armigera*

[2] *Spodoptera exigua, Ostrinia nubilalis*

Table 8. Principal Pest Problems in the Two Regions

The different growing seasons also influence the beneficials which can be used. One key example of this is in the use of *Orius*. Release of *Orius* into winter grown crops is not successful because of diapause induction in short daylength. Spring crops on the other hand may be suitable for *Orius* if it is released when daylength is sufficient to prevent diapause. There are thus more available options for thrips control in some crops, as well as differences in pest pressure.

Where pollen is an important additional food supply for beneficial mites and insects, variations in the total number of flowers on the plants may affect establishment. Where the beneficial or pest is closely associated with the flowers, as is the case with thrips and its predators, a decrease in flower numbers can also lead to an apparent increase in population density because of concentration of the same number of insects into fewer flowers. Thus growing practices and the cultivar grown can affect actual and perceived levels of control.

Previous seasons trials had identified those beneficials which would work in the two regions. This information, and a knowledge of the available insecticides, was used to construct initial crop programmes for further testing. Rationalised chemical programmes themselves permit native beneficials to enter the crops, and these can form an important part of the programme.

Novartis BCM has concentrated its efforts in this crop principally on control of *Frankliniella*. There are several reasons for this. *Frankliniella* is the most important pest of the crop in both growing regions, for reasons already referred to. The majority of chemical treatments are made against this pest, and it therefore offers the greatest scope for a reduction in the total number of chemical interventions in the crop.

Lepidoptera can largely be controlled by applications of *Bacillus thuringiensis* products, whilst other pests can be controlled with chemical treatments which are compatible with some or all of the beneficials used for thrips control. The reverse is not the case, as most chemicals which are effective against thrips are toxic to beneficial mites and insects and consequently severely disrupt any existing control programmes. Without the use of beneficial arthropods for thrips control, it then becomes impossible to use anything other than chemical control on the crop. The use of beneficial arthropods for thrips control, on the other hand, presents the grower with a series of options. He can use beneficials for control of thrips but rely on selected chemical controls for his other pests, or he can increase the number of beneficials he uses. These may be either naturally occurring or introduced, but the important feature is that the grower now has a choice.

Full programmes, incorporating all those beneficials which have proven effective in sweet peppers in Spain, have been costed in terms of the materials used, and are considerably more expensive than the chemical control that the grower currently uses. More restricted programmes for thrips control alone are far closer in cost, and provide a crucial first step for growers to approach the concept of integrated pest management. Because the procedures used are closer to their existing ones, they can feel greater confidence, and thus find this an easier step to take. They then have the choice of increasing their commitment, or of remaining at the same level. This is extremely important in regions where Integrated Pest Management and Integrated Crop Management are not well established. Growers in Northern Europe regard IPM as a standard practice, and have recognised the benefits which it can bring to them. This has however taken more than twenty years, and it is unreasonable to expect Spanish growers to adopt the same techniques without question in the face of the more severe pest problems.
The current perception of many growers in Southern Spain is that chemical control is cheap and effective. Available chemicals may have a broad spectrum of activity, and even if one no longer works something else will be available to replace it. Some growers may have tried biological control agents before and found them unreliable and expensive. In order to enable growers to make the change from chemical use to ICM, we have developed training programmes which emphasise the impact of chemicals, the differences between broad spectrum and more specific or 'softer' chemicals, and the general principals of ICM and pest recognition. The same principal has been applied successfully to cucumber crops in Almeria (GreatRex and Garcia Jimenez 1997), and is a critical component of the commercial programmes we are developing. We regard it as so important that we consider it an obligatory part of the programmes. That is to say that growers must take part in training before they are able to take part in one of our programmes.

The benefit of this approach is that the growers are totally committed to and involved in the programme. They are able to manage their crops more successfully: they can take samples, make informed decisions and discuss these decisions and the progress of their crop with the technicians employed by their co-operatives. This has been a very significant change for many of the Spanish growers.

In order to facilitate the change from a trial situation to a practical commercial application we have tested a simplified monitoring system with good results. The system is non random and was chosen specifically with the needs of the growers in mind. Few will have either the inclination or time to carry out random monitoring of the type used in scientific trials, so it is essential to develop a system with is both practical and reasonably sensitive. The system

chosen has now been tested in a range of crops for a range of pests, and has met our criteria well.

The true costs of the programmes are difficult to assess. Costs of materials can of course be specified, but any prediction will tend to apply to a worst case scenario, where pest and disease problems are at a maximum level. Direct labour costs for application of pesticides and beneficials can also be estimated with reasonable accuracy, as can the costs of the necessary training. It is much harder to assess the cost and value of time spent examining the crop or taking samples. There are also a series of costs to the grower from the excessive application of pesticides, not in terms of time and material but rather in terms of potential damage to their own health and that of their families, and to the environment in which they live and work.

It is these latter costs which are now gaining increasing publicity in local and national newspapers. This, together with the perceived risk to the health of the final consumer, has persuaded the supermarkets to demand a reduction in overall pesticide use. The financial power of the supermarkets is the final stimulus which is persuading growers and grower co-operatives to change the way they grow their crops. We, as commercial practitioners of I.P.M and I.C.M, can benefit from this by developing and providing the alternative systems which the growers need to fulfil their own aspirations and the demands of the supermarkets.

Our work on peppers has so far yielded promising results. Initial programmes have been under trial during 1996 - 1997. These trials have been less sucessful than those in cucumbers, but the problems revealed have been analysed and changes made to the programme structure. These refined programmes are being tested in the current season, and we are confident that we will have viable commercial programmes for both growing regions within two years.

References.

ACEBES, A., GUERRA, J.A., AND LOPEZ DE HIERRO, N. 1997. Development of and integrated pest management (I.P.M.) programme in plastic house vegetables in Spain. Proceedings of the IOBC Symposium ' Integrated Protection and Production in Horticulture' Agadir, Morocco. *In Press*

ACEBES., A. 1997. Novartis Agro España. Personal communication.

ANUARIO DE LA HORTICULTURA ESPAÑOLA. 1996. Ediciones Cantón S. L., Almeria, España

CENTRAAL BUREAU VOOR DE STATISTIEK, Divisie Landbouw, Voorburg, Holland. 1996.

CUADRADO - GOMEZ, I.M. Ph.D Thesis, 1995. Estudio sobre las enfermedades causadas por Tomato Spotted Wilt Virus en cultivos de invernadero de Almeria. Universidad de Granada, Facultad de Ciencias Biologias.

GREATREX, R.M. & GARCIA JIMENEZ, F. 1997. Practical crop management systems in southern Spain. Proceedings of the IOBC Symposium ' Integrated Protection and Production in Horticulture' Agadir, Morocco. *In Press*

NEWTON, P., BAUR, F., FLUCKIGER, C., TICÓ, J., AND FIDGETT, M. 1996a. IPM entry points: CIBA's basic steps to combine chemicals, biologicals and modelling for optimum crop protection: A case study from the vegetables of Spain. Proceedings of the XX International Congress of Entomology, Florence

NEWTON, P., NEALE. M.C., ARSLAN-BIR, M, BRANDL, M., FIDGETT, M., AND GREATREX, R.M. 1996b. Proceedings of the Symposium 'Biological Control Introductions; Opportunities for Improved Crop Production'. Brighton ,U.K.

SANCHEZ, J.A., GARCIA, F., LACASA, A., GUTIERREZ, L., ONCINA, M., CONTRERAS, J., GOMEZ, Y.J. 1997. Response of the Anthocorids *Orius laevigatus* and *O. albidipennis* and the Phytoseid *Amblyseius cucumeris* for the control of *Frankliniella ocidentalis* in commercial crops of sweet peppers in Murcia (Spain). Proceedings of the IOBC Symposium ' Integrated Control in Protected Crops in Mediterranean Climate' Tenerife, 1997. *In Press*

CHARACTERISTICS OF IPM IN VEGETABLE CROPS IN HUNGARY

Ilovai, Zoltan

Biological Control Laboratory of Plant Health and Soil Conservation Station of County Csongrád, 6800 Hódmezovásárhely, Rárósi út110 Pf. 99.

Key words: general plant protection aspects, IPM models

Abstract

In protection of vegetable crops, besides control of polyphagous and host-specific pests causing severe outbreaks, establishment of a system of preventive procedures is very important. Good examples of it are the production of healthy propagating materials and the control of virus vectors. A characteristic of vegetable growing is the increasing appearance of new pests (natives and introduced ones) Besides their direct control, phytosanitary regulation and sanitary system shall gain more importance. In the control of pests and diseases, treatments based on the biology and forecasting of the pests as well as the application of more selective biopesticides and the mass-reared beneficial arthropods shall become conspicuous instead of using the conventional chemical substances. The management programmes for decreasing losses caused by the pests shall be replaced by the better use of biological methods influencing population of the pests, biotechnological techniques improving tolerance or resistance of the crop and cultural methods. Modern agriculture requires sustainable management programmes. Sustainable agriculture means a high-level integration of the production, of which the key elements are crop protection and nutrient supply.

Introduction

Vegetable growing has great tradition in Hungary, it occupies key position in the Hungarian horticulture. It ranges from family gardens till large-scale industry-like production. Besides fresh vegetable growing in city-surroundings, there formed vegetable growing regions based on traditions mainly as field growing. Some key production data of main vegetables are displayed in Table 1. The early vegetable growing in greenhouses and plastic foil tunnels takes about 6000 ha.

Intensification of the growing started in '70-s - it means introduction of modern varieties, application of agrochemicals, mechanisation, processing, storage, packaging. Large-scale industry-like production was developed in the '80-s. Production systems appeared also in vegetable production. Formation of the development can be illustrated by the formation of usage of agrochemicals. The rate of development was stopped by the energy- and economical crisis in the 90-s. The political changes and the striving to European Union made necessary elaboration of new economical programmes also in our country. Environment saving and wide integration became a basis for both of production and processing.

Integrated Pest Management makes efforts against excessive pesticide usage or striving towards object-aimed selective methods instead of wide-range materials. This paper is to demonstrate the characteristics of the integration.

Methods

Pest management of vegetables - beside chemical weed control - is based on battling of pests endangering the safety of production with epidemics. These pests must be separated into

polyphagous and specific ones. Besides traditional - mainly chemical - methods, new control methods and materials are demonstrated too, what are promising for the future.

Together with presently available biopesticides, introduction of natural arthropod- and microbial organisms and resistant crop varieties have their place as well. Besides recounting of results, criteria for inte.grated crop protection are drawn up too.

Results

Pests and disease problems

General polyphagous pests in vegetable growing:

Soil-borne arthropods: Elateridae, Melolonthidae, Noctuidae
Root-gall nematodes: *Meloidogyne* spp.
Stem- and bulb nematodes: *Ditylenchus* spp.
Wilt diseases: *Fusarium* spp., *Verticillium* spp.
White- and grey mould: *Sclerotinia* spp., *Botrytis cinerea*
Virus vector aphids: *Myzus persicae*, *Aphis gossypii*, *A. nasturtii*
Plant viruses: TMV, CMV, AMV, PVX, PVY, TSWV, etc.
Foliage pest Noctuids: *Mamestra* spp., *Phytometra* spp
Whiteflies, thrips, spider mites

Host plant specific pests and problems:

Tomato: Besides *Phytophthora* leaf- and fruit rot Alternaria and Botrytis infections are common. *Corynebacterium michiganense* occurs rarely. Control measures must be taken against aphids and Noctuid moths.

Pepper: Bacterial leaf spot causes epidemy in early stage of development. Fruits are damaged by Noctuid moths and corn borer. Aphid gradations are changing in every year but preventive control is necessary because of virus vector activities.

Leveillula taurica appeared in 1972. Alternaria fruit and seed rot is a special problem.

Onion: Besides mildew, bacterial bulb rot can be dangerous in some years. Grey mould commonly occurs on irrigated plots. In dry years tobacco thrips causes problems. Together with soil-borne pests overpopulation of root mites is frequent - they spread Fusarium dry rot.

Garlic: Ditylenchus nematodes, thrips and tulip leafmites mean usually danger (Kerenyi-Nemestothy *et al.*, 1996).

Cabbages: Cabbage fly causes problem in early -and autumn season equally. Control of aphids is a constant problem. Caterpillars of cabbage noctuids make necessary control measures against all generations.

French beans: Beside bacterial diseases and pod diseases spidermites and aphids are damaging regularly.

Green peas: Mildew and powdery mildew occur regularly. Early damage caused by moths and seed damaging beetles is substantial.

Cucumber: Because of *Pseudoperonospora* epidemies production became unstabile. In dry weather powdery mildew and mites are causing problems. Good quality seeds are necessary to avoid virus problems. Control of insect vectors is a must.

Greenhouse crops: The crops are grown in soil - this is why soil-borne diseases, nematodes and viruses have general importance. Whiteflies, aphids, spider mites, leafmites, thrips, require constant control measures. Presence of vectors and lack of resistance contribute to virus infection. As a consequence of technical problems, epidernies can be caused by Phytophthora, Botrtis and powdery mildew (Ilova *et al.*, 1996).

After this short introduction the most characteristic pest gradations and epidemies must be mentioned - these occurred especially at the beginning of large-scale production. Such were: onion mildew and bacterial rot in 1970 and 1975, and the field pepper virus crisis in 1974. Increase of epidemy danger was caused not only by the concentration of production but by sudden insect invasions too. Such was the invasion of *Loxostege sticticalis* in 1976, *Pseudoperonospora* infection of cucumber at the end of 80-s, of locusts in draught period in 90-s in many regions of the lowland. Frequent occurrence of aphids in potatoes resulted in several virus epidemies. Some other problems like nematode infestation in garlic, Elateridae larvae damage in several crops, Noctuid caterpillars in tomato and pepper w-ere promoted by technological difficulties. On the other hand, modernisation of field pepper Browing - sowing from seeds instead of seedlings - contributed to epidemies of bacterial leaf spot, insect damage at the emergence, *Tanymecus dilaticollis* in paprika, *Sitona* spp. in peas, *Phyllotreta* spp. in cabbage, spider mites in cordon type of cucumbers, etc. In the beginning stage of greenhouse growing damping-off and viruses in seedling growing, aphids and overpopulation of root gall nematodes were characteristic

Usage of selective pesticides and introduction of biological control caused appearance of secondary pests what earlier did not caused any problem, like *Polyphagotarsonemus latus* and *Aculops lycopersici* and powdery mildew in tomatoes. Introduction of new, non-native pests should be taken into consideration also in vegetable production. Pepper powdery mildew appeared in 1972, *Botrytis squamosa* leaf spot occurs in onions. cucumber mildew is spreading from south, *Globodera* spp. have already infested some potato fields.

Several new pests appeared in greenhouse growing, mainly spread by- seeds and stockplants of ornamental and vegetable crops: *Trialeurodes vaporariorum* spotted in 60-s, has become a common pests by now, *Liriomyza trifolii* had been eradicated already twice. New pests of beginning of 90-s were *Frankliniella occidentalis* and *Bemisia tabaci*, and from 1995 *Helicoverpa armigera*. Some of these pests are potential vectors and because control of them is difficult, new and new viruses appear (see Table 7).

Development of control measures

Outdoor vegetables

Vegetable growing has always meant an intensive concentration and specialisation. In spite of classical crop rotation. overpopulation of pests is a regular problem because of monocropping. This became more of a problem after the really large-scale production started. The safety of production was critical on two fields: mechanisation in case of herbicide treatments and protection of the yield from pests. In 70-s, after determination of key pests, formation of pest management technologies became necessary. This was made possible after the network of Plant Protection Stations were created and strengthened by laboratories and each county was divided into districts with plant protection inspectors. Main characteristics were introduction and registration of pesticides in different crops, determination of rates, technical measurements concerning spraying technics, waiting time measurements, residue control, etc. Other direction of practical research concerned elaboration and introduction of forecast and survey methods.

The Integrated Plant Protection Forecast System was founded in 1970. This network could produce regional and countrywide forecasts. There were intensive research studies in order to determine damage thresholds and adapt to different pests and production levels. This way directed and pest-oriented, supervised control was applied. The mass market production, striving towards maximal security, kept to zero tolerance Principle - this way the programmed

pest management concentrated on prevention. Prevention of epidemies and gradations became basis of the forecast.

First stage of development was directed to soil disinfecting and seed dressing. Practically all vegetables required different principles for the development. Crops protected at "plot level" meant pesticide integration under strict inspection. The high degree industrialised concentrated production required protection measures for the mass yield products after harvest. Instead of direct battering of storage pests and diseases prevention at production sites was aimed. Breakthrough to real integration began in our country with widening of breeding for resistance and selection for varieties proper for different regions.

The other field was providing good quality sowing seeds and propagation material what created basis for growing on favourable sites with minimal pesticide control. Real development of integration of production was required by the demand for high quality products. One purpose of regular inspection was raising of product integration on such level so that to be able to limit the input quantity. New age of integrated pest management means also rational nutrient supply, crop fertilisation, integrated weed management including soil cultivation and agro-environment management too (Kajati *et al.*, 1989, 1994, Csikai, 1996).

We have arrived to that limit where pesticide usage should already be limited. This lead to withdrawal of several wide-effect products and active ingredients. Counterbalancing of the narrowing of pesticide usage is possible only by biological methods. Further possibilities for quantity limitations are stripe applications and perfection of seed treatments. Good spray cover, prevention of nonpurposeful dispersion, airspace treatments by cold- and thermo foggings and electrostatic technics are parts of novel efforts for rational application technologies (Ilovai, 1987; Ilovai *et al.*, 1992, ceglarska-Hodi *et al.*, 1996).

Modern pest management can not exist without biopesticides. Registration of *Bacillus thuringiensis* products also for vegetables is a good advantage for us. Introduction of entomopathogenic micro-organisms can be promising against soil-borne pests (Simon *et al.*, 1992). There are several microbial antagonists against soil-borne pathogens. Beside traditional pesticides there appeared some botanical and microbiological preparates on natural basis (see Table 5) (Dormanns-Simon *et al.*, 1996).

Artificial, seasonal introduction of arthropod antagonists during vegetation is still costly and technically not solved. Presently real possibility is saving and activating of host-parasite system on the plots at growing sites with introduction of alternative control methods.

In outdoor integrated pest management we have good chances for wide application of biorational and bioinformatical materials, baits . pheromones and physical methods. This way the controlling system develops into good direction (Szocs *et al.*, 1996)..

Greenhouse vegetables

Greenhouse growing in Hungary takes place on real soil, in heated and non-heated greenhouses. Plastic tunnels and blocks require regular soil disinfection - it is done mainly by chemicals. Fungicide drench and individual plant treatments are used against soil-borne fungi. Besides systemic fungicides, contact partners are applied too. In soil-block seedling growing chemicals are mixed into the soil. In every 2-3 years nematode control is necessary - mainly it is used before cucumber or tomato crops. Prevention treatments by spraying or having the agents absorbed through roots is necessary to carry out against aphids, *P. latus* and western flower thrips. Control of whiteflies and leafminers is usually intensive in the first part of growing season. There are several fungicides against foliage diseases and flower infections. Since 1987 intensive development is going on concerning introduction of pesticides; number and usage of pesticides many times more than those in outdoor growing. Its value is 4-10 times

more than the average number of treatments in the country. This means that in a long period number of control measures can go over 40. Summertime the surroundings serve as infection source - by this the number of necessary measures increases. Majority of new pests are virus vectors - this mean further endangering of protection - prevention is even more needed. Selection of local pest populations accelerated in the latest 25 years at family farms and establishments. Pesticide resistance problems were put into surface by the exaggerated pesticide usage. Insecticide resistance developed in case of acaricides, aphicides, and even *H. armigera* caterpillars. These factors made necessary to look for new ways.

Introduction and promotion of biological control is a requirement. Beside of already introduced arthropod parasitoids application of biopesticides is necessary (Hatala-Zseller, 1992) (see Table 6). Integration of biological control methods in pepper and testing of virus inhibiting materials has begun (Ilovai *et al.*, 1996). The low technical level forces the developers to change the growing methods and by this to help the environmental saving and effective plant protection.

Discussion

Analysing the main domains of plant protection in vegetables, we can conclude that Hungary could not avoid introduction and wide-scaled application of chemicals. Such an intensive branch as vegetable growing could not afford the fluctuation of yield. Thanks to state integrated forecast system from the beginning we made efforts for working out reasonable control technologies based on the damage-thresholds. Endeavouring to provide complex control, integrated plant protection aimed to solve the weed problem. That's why herbicides are still composing more than a half of pesticides applied. The industry-like growing systems following the principle of prevention runned on an offensive control leaved out of consideration the condition of agroecosystems. High number of treatments against diseases was also characteristic for horticultural production. Due to mechanisation the size of fields grown and application of less effective air-treatments increased the pesticide emission into the environment. In the effect of one-sided fertilisation crops become susceptible to diseases.

Decreasing of soil viability and diversity resulted sometimes decreasing of number of pests which in monocultural crops leaded to domination of certain pests as Fusarium, Botrytis, or bacteria. There are still unsolved problems in vegetable crops. The crop-breeding concentrated its attention on speeding up the productivity of plants and the question of breeding resistant varieties squeezed to background. Epidemies and invasions of a new pests mentioned above stimulated a selection for resistance. Due to the political and economic changes, market-oriented economy, domination of export interests production has decreased. It become necessary to turn in the direction of sustainable production. Protection of environment which become a public demand, aims a new, economic, energy and cost-save methods. A rational plant protection cannot make shift without biologically-based classical integrated control systems (Finch, 1992). Taking into consideration the damage-thresholds, it utilises different principles instead of eradication of pest.

In the future the strategy of modern control technologies should be based on the following principles:

-Prophylaxy: more attention and international collaboration is needed for prevention of pest invasion in the conditions of free market,
-Reduction of chemicals: much rigorous selection of pesticides for utilisation in vegetables, according to IOBC directives, commercialisation of supervised control, taxes for hazardous chemicals, development of application technics and formulations.

-Stimulation of biological control: registration of natural beneficial organisms in a way different from that of classical pesticides, introduction of the positive list of beneficials, national foundation for introduction of natural products into the IPM technologies, organisation of associations for implementing of IPM.
-Strengthening of agricultural control methods: field inspection: phytosanitary qualification (diseases, pests, weeds), soil-management, saving of soil-life, soil improvement, space and time isolation of fields and crops,
-Genetic control: breeding of resistant and tolerant varieties forward to unsolved problems.

References

CEGLARSKA-HODI, E., DORMANNS-SIMON, E., APONYI-GARAMVOLGYI, I. (1996): Some aspects of biological control of soil-borne pathogens in greenhouse paprika - the Hungarian experience and prospects. Proceedings of International workshop on biological and integrated pest management in greenhouse pepper. Hodmezovasarhely, (HU), 10-14 June, 1996. (in press)
CSIKAI, M., KAJATI, I. (1996): Some efforts to produce integratedpepper in greenhouse. Proceedings of International workshop on biological and integrated pest management in greenhouse pepper. Hodmezovasarhely, (HU), 10-14 June, 1996. (in press)
DORMANNS-SIMON, E., BUDAI, CS., ILOVAI, Z.(1996): Biological control in Hungary: Present situation and Possibilities. Proceedings of EPPO/CABI Workshop on Safety and Efficacy of Biological Control in Europe, Streatley on Thames (GB)? 2S28 Marchs 1996 (in press)
FINCH, S. (1992): Appraisal of integrated pest management sxstems for controlling insect pests of vegetable crops Proceedings of an international conference organized by IOBC/WPRS, Veldhoven, (NL), 8-13 Sept. 1991. 49-66 pp.
HATALA-ZSELLER, I., BUDAI. CS. (1992): Possibilities of biological control against pests in vegetables. Proceedings of XXIII. Conference on Integrated Production in Horticulture 01. Dec. 1992.. Budapest (HU). 17-22 pp.
ILOVAI, Z. (1987): Development of integrated plant protection in red pepper in Hungary. Proceedings of International Symposium on Scientific-Technical Development in Hungarian paprika (red pepper). Kalocsa-Szeged, (HU), 17-19 Sept., 1987. 239-243 pp.
ILOVAI, Z., SZABO, P., VARGA, A., (1992): Development of IPM in onion, possibilities in the future. Proceedings of Conference on integrated production of horticultural crops. Budapest, (HU), 12 Jan. 1992., 63-68 pp.
ILOVAI, Z., BUDAI, CS., DORMANNS-SIMON, E:, KISS, E.F. (1996). Implementation of IPM in Hungarian greenhouses. Bulletin IOBCMTPRS vol.19(1) 1996, 63-66 pp.
ILOVAI, Z., KISS, E.F. (1996): Survey made in greehouse pepper for evaluating the phytosanitary situation. Proceedings of International workshop on biological and integrated pest management in greenhouse pepper. Hodmezovasarhely, (Hl-). 1W14 June, 1996. (in press)
ILOVAI, Z., KISS, E. F., KAJATI, I., BUDAI, CS., CARNERO A, TORRES, R., HERNANDEZ GARCIA, M., HERNANDEZ SUAREZ, E. (1996)- Development of IPM for protected paprika with particular attention to beneficial arthropods. Proceedings of International workshop on biological and integrated pest management in greenhouse pepper. Hodmezovasarhely, (HU), 10-14 June, 1996. (in press)

KAJATI, I., KISS, F., KOLBER, M., BASKY, ZS., NASSER (1989): Effect of light summer oils on aphid transmission of viruses in bell and red pepper fields. Proceedings of XXXX International Symposium on Crop Protection, Gent (BE).

KAJATI, I., KISS, F., KOVACS, G., VARGA, I. (1994): Vektafid A and R - new efficient integrated control of virus transmitting aphids and virus diseases. Foodapest International Exhibition, VII. Biokultura Days, Budapest, (HU), 25-25 Nov. 1994 1-5 pp.

KERENYI-NEMESTOTHY, K., BUDAI, CS., REGOS, A, SZEREDI, A. (1995): Occurence of Aceria tulipae Keifer in garkic. Abstracts of Conference on Plant Protection, 21-22 Feb. 1995., Budapest (HU), 50 p.

SIMON, E., CEGLARSKA-HODI, E., APONYI-GARAMVOLGYI, I. (1992): Microbial control of soil-borne pathogens in Hungary. Joint EPPO-IOBC/EPRS Conference on Plant Protection in Greenhouses. 04-07. 06. 1991. Poznan-Naramowice (PL). Bulletin OEPP/EPPO 22: 457-461

SZOCS, G., TOTH, M., UJVARI, 1., SZARUKAN, I . SZABO, P. & ILOVAI Z. (1996): Three years' experience in monitoring the flight of the cotton bollworm *Helicoverpa armigera* Hbn. (Lepidoptera: Noctuidae), an upcoming pest in Hungarv. Proceedings of International workshop on biological and integrated pest management in greenhouse pepper. Hodmezovasarhely, (HU), 10-14 June, 1996. (in press)

Crop	Area (in Ha)	Production to.	Yeald kg/Ha
Cabagge	3 613	84.427	23.370
Onion	7.423	170.814	23.010
Tomato	11.656	306.060	26.260
Bean (green)	4.708	19.788	4.850
Peas (green)	30.269	328.920	10.880
Pepper(green)	6.114	59 433	9.720
Pepper (red)	7.151	46.688	6.530

Table 1: Field Vegetable Production in Hungary (1990). Data Base: KSH (Central Statistic Bureau)

Year	Active ingredient kg/ha/year	
	Pesticide	Fertilizer (N+P+K)
1960	1,9	23
1975	3,7	224
1980	5,07	262
1981-1985	4,78-5,24	2,79-2,31
1986-1989	4,1-3,35	256-231
1990-1994	1,99-1,08	127-51

Table 2. Use of agrochemicals in Hungary

Year	Zoocides	Fungicides	Herbicides	Other	Number of active ingredients
1970			no separate registration		
1978	38	25	6	19	-
1988	52	38	10	30	87
1996	74	73	20	20	82

Table 3. Pesticides Registered for Greenhouses in Hungary. (Movai-Kiss, 1996)

Pesticides	Zoocides	Fungicides/bactericides
Synthetic	56	55
Natural	6	14
Biorational	5	2
Biopesticide	6	2
-microorganism	3	2
-macroorganism	3	-

Table 4. Distribution of Pesticides among different types. (Ilovai-Kiss. 1996)

BIOAGENT	PRODUCT	PRODUCER DISTRIBUTOR
Encarsia formosa	ENCARSIA-LAP	Biocontrol Bt (HU)
	EN-STRIP	Koppert Biosystem (NL) H&T Bioprotect Bt (HU)
	BIOBEST ENCARSIA	Biobest Trading BVBA (BE) Árpád Coop. (HU)
Bacillus thuringiensis var. kurstaki	BACTUCID	Caffaro (IT) Kwizda (AT)
	DIPEL ES	Abbot (BE) Summit Agro Hungaria Ltd.
	DIPEL	Abbot (BE) Summit Agro Hungaria Ltd.
	THURICIDE HP	Sandoz (CH)
Bacillus thuringiensis var. tenebrionis	NOVODOR FC	Novo Nordisk A/S (DM) Summit Agro Hungaria Ltd.
Streptomyces griseoviridis	MYCOSTOP	Kemira OY (SF) Kemira Agro Ltd. (HU)
Trichoderma harzianum T-39 strain	TRICHODEX WP	Makhteshim (IL)

Table 5 Biopesticides and Bioagents registrated in Hungary

Continent/Country	Area under biocontrol	n. of producers	greenhouse are ha
West & South Europe	8000	25	70.000
Russia & related states	5000	10	10.000
Central Europe	500	8	10.000
North America	300	10	5.500
Asia	<100	?	50.000
Australia & N. Zealand	<50	3	1.000
South America	<50	?	6.000
Mediterranean & Africa	-	-	18.000
Hungary	<100	1	4.000

Table 6. Biological control of pests in greenhouses. World situation and Hungary 1992/96

Transmission	Virus name	Family/Group	Acronym	Crop (importance)*
soil/seed/mechanical	Tobacco mosaic	TOBAMO	TMV	pepper(+++) tomato(+++)
	Tomato mosaic	TOBAMO	ToMv	pepper(+++) tomato(+++)
	Pepper mild mottle	TOBAMO	PMMV	pepper(0)
by soil fungi	Pepper yellow vein	?	PYVV	pepper (0)
by aphids	Cucumber mosaic	CUCUMO	CMV	pepper(+++) tomato(+++) cucumber(+++)
	Potato virus Y	POTY	PVY	pepper (++) tomato (++)
	Alfalfa mosaic Tomato aspermy	AMV	AMV	pepper (++)
	Broad bean wilt	CUCUMO	TAV	pepper (+)
		COMO	BBWV	pepper (+)
by thrips	Tomato spotted Wilt	TOSPO	TSWV	pepper (0) tomato (0)
by whiteflies	Tomato yellows	GEMINI	?	tomato (?)
	Cucumber yellows	GEMINI and ?	?	cucumber (?)

Table 7. Most important Virus Diseases of Vegetables in Hungary (Ilovai *et al.*, 1996). Legend *: (+++): widely spread since long time, presently also very important (++): widely spread since long time, presently less important (+): appeared long ago, lately is diminishing (0) appeared in latest years, gets spreading (?): symptoms of the disease appeared in latest years, identification of the virus is going on.

Diseases	Main host plants	Control agents
Sclerotinia spp.	paprika lettuce cucumber	*Coniothyrium minitans* (Micon)
Botrytis cinerea	paprika tomato lettuce cucumber	*Trichoderma* spp.[2] *Trichoderma harzianum* (Trichodex WP[3])
Fusarium spp.	paprika tomato carnation gerbera	*Streptomyces griseoviridis* (Mycostop[4])
Damping-off diseases (Rhizoctonia, Alternaria, Pythium, etc.)	greenhouse crops	*Streptomyces griseoviridis* (Mycostop)
Powdery mildew (*Erysiphe* spp., *Sphaeroteca fuliginea*, *Leveillula taurica*)	cucumber tomato paprika	*Ampelomyces quisqualis*[5] Vektafid A[6]

Table 8 Possibilities of Biological and Alternative Control against Diseases of Greenhouse Crops in Hungary (Ilovai *et al.*, 1996)

[1] Biopreparate of Plant Protection Rsearch Institute of Hungarian Academy of Ssciences (Dr. László Vajna), in experimental stage, not yet registered

[2] There are standard Trihoderma preparates developed by the Hungarian plant protection network, but they are not registered and not manufactured because of the lack of sponsors

[3] Preparate of Makteshim Chemical Works Ltd. (Israel) registered in Hungary

[4] Preparate of Kemira Oy (Finland) registered in Hungary

[5] Experimental preparate of Plant Protection Research Institute of Hungarian Academy of Sciences (Dr. L. Vajna, Dr. Levente Kiss)

[6] Alternative preparate of Chemark Ltd. (Hungary) based on light summer oils

MEDITERRANEAN BIODIVERSITY AS SOURCE OF NEW ENTOMOPHAGOUS SPECIES FOR BIOLOGICAL CONTROL IN PROTECTED CROPS

G. Nicoli[1], G. Burgio[1/2]

[1] Istituto di Entomologia 'G.Grandi', Università, via Filippo Re 6, 40126 Bologna, Italy; gnicoli@agrsci.unibo.it
[2] Biolab-Centrale Ortofrutticola, via Masiera Prima 1191, 47020 Cesena, Italy

Abstract

The recent contribution of the Mediterranean biodiversity (insects and predatory mites) to the biological control in protected crops is discussed, underlining the most significant steps for the selection of candidates. The examples reported are: 1. the new association between the palaearctic parasitoid *Diglyphus isaea* (Walk.) and the American leafminers *Liriomyza trifolii* (Burg.) and *L. huidobrensis* (Blanch.); 2. the Mediterranean strains of the predatory mite *Phytoseiulus persimilis* Athias-Henriot reared to control the red spider mite *Tetranychus urticae* Koch; 3. the Mirid bug *Macrolophus caliginosus* Wagn. preying upon the whiteflies *Trialeurodes vaporariorum* (Westw.) and *Bemisia* spp.; 4. the Anthocorid bug *Orius laevigatus* (Fieber) preying nearctic and palaearctic thrips pests; 5. the parasitoid *Eretmocerus mundus* (Mercet) controlling *Bemisia tabaci* (Genn.) and *B. argentifolii* Bell. and Perr.; 6. the nearctic *Lysiphlebus testaceipes* (Cress.), established in Southern Europe, parasitizing several species of aphids, including *Aphis gossypii* Glover; 7. the natural control of aphids provided by some polyvoltine species of Coleoptera Coccinellidae which fly from the first-generation development sites (i.e. wheat canopies) to colonise mainly the open field crops infested by *A. gossypii* at the beginning of summer. In the first six cases, some European producers started to mass-rear the selected natural enemies, improving the opportunities of biological control for the whole continent. In the case of the aphidophagous Coccinellidae, the possibility of forecasting the movements of the first-generation adults in the Mediterranean agroecosystems allowed for a wider application of the Integrated Pest Management.

Introduction

In the past 20 years, new arthropod pests seriously attacking vegetable and ornamental crops were imported in Europe from other continents (mainly America) and, at the same time, some native pests became resistant to selective insecticides previously used in the Integrated Pest Management (IPM). For both reasons, plant protection by conventional chemical control became often very difficult for several protected and open field crops; therefore biological control by the 'seasonal inoculative' release of mass-reared entomophagous species appeared to be an effective alternative. In addition, thanks to the increasing application of IPM in the Mediterranean basin, the reduction of wide-spectrum insecticides allowed the colonisation of vegetable and ornamental crops by several species of predators and parasitoids, which are potentially very effective for the natural control of some harmful pests. Some of the entomophagous species (or strains) showing a good effectiveness in the natural control of both exotic and native pests were collected and evaluated (in the laboratory and in the field) in order to mass-produce and release them in protected crops of the whole continent. From the climatic point of view, the North-European glasshouses can be considered small temperate or sub-tropical agroecosystems, practically isolated from the surrounding continental climate for a long period of the year, and some natural enemies which are endemic in the Mediterranean basin proved to be able to develop and control pests also in the northern-European glasshouses.

The recent contribute of the Mediterranean biodiversity (insects and predatory mites) to the biological control in protected crops in Europe is discussed, underlining the most significant steps of the selection of candidates for mass-production and release by the 'seasonal inoculative' method on vegetable and ornamental crops.

1. *Diglyphus isaea* (Walk.) (Hymenoptera Eulophidae)

Two polyphagous American leafminers (Diptera Agromyzidae) were accidentally imported from America in the past 20 years. In northern Europe, *Liriomyza trifolii* (Burg.) was first recorded in 1976 (Minkenberg and Van Lenteren, 1986) and then it spread to all the continent; *L. huidobrensis* (Blanch.) was initially recorded in some cases without establishment, as in the United Kingdom in 1980 and 1981 (Trouvé *et al.*, 1991), then finally it established and became a widespread pest around 1989 (van der Linden, 1990). The two species colonised quickly the Mediterranean area (probably travelling with pot-plants) and, in Italy, they were respectively recorded in 1978 by Arzone (1979) and in 1991 (Süss, 1991). These pests show a strong resistance to many insecticides, and the use of wide-spectrum active ingredients sometimes determines even worse outbreaks. From the importation of the exotic *L. trifolii*, several authors reported that the native ecto-parasitoid *D. isaea* was able to enlarge its host-range to the new pest, forming a 'new association' with an high efficacy in the natural/biological control. Studying the potential of the natural enemy in the laboratory, Nicoli and Pitrelli (1994) recorded a large amount of host larvae killed by parasitization (up to more than 300 eggs laid/female) or simply paralysed without oviposition (up to more than 800 larvae/female). Many new associations between other native parasitoids and *L. trifolii* were recorded in Europe, as in Central Italy by Del Bene (1989), as well as a new association was recorded between *D. isaea* and the later-introduced leafminer *L. huidobrensis*.

In the Mediterranean basin, particularly when IPM is applied, *D. isaea* can colonise naturally both protected and open-field crops, flying from cultivated and wild plants growing in the agroecosystem; Minkenberg and Van Lenteren (1986) report that naturally occurring parasitoids, mainly *D. isaea*, can invade greenhouses in May/June also in northern Europe. Nevertheless, Lyon (1985) was the first to show the possibility of rearing *D. isaea* in the laboratory for releasing the parasitoids by the so-called 'beneficial insect in first' method. After that initial experience, the Mediterranean producers (in France, Italy and Israel) collected *D. isaea* in nature and started to mass-rear the parasitoid for the protected crops of the whole continent (as some northern-Europe producers). In southern Europe, the mass-reared parasitoids are generally released at rather low release rates (sometimes 0.1 parasitoids/m^2) to prevent *L. trifolii* outbreaks or to reintroduce the natural enemy after non-selective treatments; *D. isaea* is released at higher rates mainly for the biological control of *L. huidobrensis* on lettuce, celery and gerbera. In northern Europe, the wild parasitoids usually colonise the protected crops from outdoor late in the growing cycle and the seasonal inoculative release of mass-reared *D. isaea* (frequently associated with other parasitoids) is very common and effective also for the biocontrol of the palaearctic leafminer *L. bryoniae* (Kalt.).

2. Mediterranean strains of *Phytoseiulus persimilis* Athias-Henriot (Acarina Phytoseiidae)

P. persimilis was first described by Athias-Henriot (1957) using specimens collected in nature in Algeria in 1955-1957 on various plants. Dosse (1958) found the species (reported as *P. riegeli*) on a consignment of orchids received in Germany from Chile and distributed the progeny in several European Countries (Hussey, 1985). In Italy, Lombardini (1959) was the first to report *P. persimilis* which he found in Sicily on citrus leaves, describing the predator as

a new species (*Amblyseius tardi*) until Kennett and Caltagirone (1968) showed the synonymy with *P. persimilis*. Other Italian reports of wild populations followed in Sicily (Ragusa, 1965; 1974; 1977; 1986; Liotta *et al.*, 1977; Vacante and Nucifora, 1985; 1986; 1987a; 1987b), in central Italy (McMurtry, 1977) and near the northern Adriatic seaboard (Celli *et al.*, 1987). *P. persimilis* has also been found in other Mediterranean areas such as southern France (Rambier, 1972), Greece (Swirsky and Ragusa, 1976; 1977), Spain (Ferragut *et al.*, 1983; Garcia *Marì et al.*, 1986; 1987) and as an 'imported beneficial' in Lebanon (Dosse, 1967) and Israel (Swirsky and Amitai, 1968).

In northern Europe, *P. persimilis* is mass-reared for the biological control of the red spider mite, *Tetranychus urticae* Koch (Acarina Tetranychidae), in protected crops from late 1960s. The initial experiences of commercial production of natural enemies started with the predatory mite, and the list of the mass-reared species was enlarged only afterwards (Hussey, 1985). Also the Mediterranean producers starting the activity in the 1980s included *P. persimilis* in their list of the mass-reared species. The predatory mite is very effective for biological control, and Van Lenteren *et al.* (1992) estimated that, in 1990, *P. persimilis* was released in ca. 4,000 ha over the ca. 13,000 ha of the world-wide application of IPM in protected crops.

Nevertheless, in the past years, some problems emerged in the mass-production and therefore in the predator's effectiveness for plant protection. A microorganism might be involved in some changes in the reared predatory mites (as a loss of response to synomones), and it seems most probable that transmission occurs via contact between mites or via debris or faeces (Schütte *et al.*, 1995; 1996). Moreover, the continuous rearing of the same strain could increase the risk of a genetic selection of predators adapted to the rearing conditions, but not anymore to the variable growing conditions, particularly to the wide range of temperature and relative humidity in the Mediterranean. For these reasons, some producers recently begun to substitute periodically the mass-reared strain, in which case southern Europe can be a source of predators to start a new production cycle. The performances of the collected-in-nature strains compared with those reared in the laboratory or in a biofactory for several generations were investigated by Galazzi and Nicoli (1996a; 1996b).

3. *Macrolophus caliginosus* Wagn. (Rhynchota Miridae)

For a long time, the greenhouse whitefly *Trialeurodes vaporariorum* (Westw.) (Rhynchota Aleyrodidae) has been considered the worst pest for many protected crops (Hargreaves, 1915; Lloyd, 1922). *T. vaporariorum* is nearly cosmopolitan, but the origin of the species is probably the tropical or sub-tropical America (Vet *et al.*, 1980). In Europe, the initial experiences of biological control were carried out in England by Speyer (1927), using a parasitoid involuntary imported from America: *Encarsia formosa* Gahan (Hymenoptera Aphelinidae). After the second world war, the mass-production of this parasitoid started in northern Europe at the beginning of the 1970s, and its application increased up to ca. 7,000 ha in 1990, as estimated by Van Lenteren *et al.* (1992).

When in the 1980s the release of *E. formosa* was tested also in the Mediterranean Countries, and the use of wide-spectrum insecticides was strongly reduced, several researchers observed that some species of Miridae colonised the greenhouses from outdoor. These polyphagous predators are entomophytophagous and some of them produce occasional damages, particularly when the preys' density becomes too low as compared to that of the predators (Arzone *et al.*, 1990) and, in some cases, they can also predate the pre-imaginal instars parasitized by *E. formosa* (Delrio et al., 1991).

M. caliginosus is endemic in the Mediterranean and *Dicyphus errans* (Wolff) is known in all Europe (Wagner and Weber, 1964). No damages were observed on plants to attribute to *M. caliginosus* (Malausa *et al.*, 1987; Malausa, 1989), while *D. errans* can produce small damages to tomato when preys are rare. *Dicyphus tamaninii* Wagner shows the same habit of *D. errans* and plays an effective role for natural control particularly in Spain (Gabarra *et al.*, 1988; Alomar *et al.*, 1991; Albajes *et al.*, 1996).

Nesidiocoris tenuis (Reuter) was initially considered only a pest for vegetable and ornamental crops in Egypt, with a non-relevant predation activity (El-Dessouki *et al.*, 1976), but more recently its effectiveness in the natural control of whiteflies was recorded in several areas, such as Sicily (Vacante and Tropea Garzia, 1994a). N. *tenuis* shows a very large geographic distribution, including Mediterranean basin (Vacante and Tropea Garzia, 1994b)

In the Mediterranean basin, natural control by these predators is enhanced by the biodiversity in the agroecosystems. Several cultivated and wild plants host these predators that can refuge, feed on vegetable or other arthropods (ex. aphids) and reproduce. Alomar *et al.* (1994) recorded 24 different plant species that supported several Dyciphinae mirids during winter. A perennial plant very common in the Mediterranean, *Inula viscosa* (L.) (Compositae), plays an important role as host of *M. caliginosus* (Costanzi and Pini, 1991); this plant should be considered as a 'natural source' of predators on a farm basis. *M. caliginosus* (the species considered generally harmless to plants) is now mass-produced for 'seasonal inoculative releases' in both Mediterranean and northern Europe to control whiteflies in general and other arthropod pests, thanks to its polyphagy. The successes obtained demonstrate that the richness of the southern biodiversity can provide advantages also for the continental areas, where the local entomophauna is poorer or the cold climate limits strongly the natural colonisation of protected crops by natural enemies coming from outdoor.

4. *Eretmocerus mundus* Mercet (Hymenoptera Aphelinidae)

In the Mediterranean, until a few years ago, two species of whiteflies were generally considered pests for protected crops: the greenhouse whitefly *T. vaporariorum* and the cotton whitefly *Bemisia tabaci* (Gennadius, 1889), that was first described in Greece on tobacco. *B. tabaci* is reported as a species commonly found in Italy on various plants by Silvestri (1939), that underlines the very efficient natural parasitization of a small wasp: *E. mundus* (=*masii* Silv.). For many years, *B. tabaci* was considered a non frequently-harmful whitefly for a large part of the Mediterranean (with the exception of cotton and a few vegetable crops in some areas) and specific crop protection procedures were rarely adopted for vegetables in southern Europe.

In the USA, two whitefly populations have been referred to *B. tabaci* and distinguished as 'strains': strain A, or 'cotton strain', and strain B, or 'poinsettia strain' (Bethke *et al.*, 1991). The strain B was recognised first in USA in 1986 (Price *et al.*, 1987) and it is supposed to have spread to many greenhouse areas in the world via the intercontinental trade of plants. It was accidentally imported in Europe in late 1980s becoming a serious pest for poinsettia, but also for tomato, due to the transmission of the TYLC virus (Credi *et al.*, 1989). Recently, Bellows *et al.* (1994) demonstrated that the so called 'strain B' has to be considered as a new species, namely *B. argentifolii* Bell. & Perr. genetically distinct, reproductively isolated, different in several biological and morphological characteristics from the species *B. tabaci* (previously identified as *B. tabaci* strain A).

Some researchers evaluated the effectiveness of the parasitoid *E. formosa* to control *Bemisia* spp. on poinsettia. Although *E. formosa* shows an evident preference for *T. vaporariorum* (Boisclair *et al.*, 1990), it can control *B. tabaci* too, parasitizing and killing by

host-feeding a large number of juvenile instars (Enkegaard, 1993). A sort of 'inundative release' strategy was defined for *E. formosa* on poinsettia, but a very large amount of parasitoids was necessary (Benuzzi *et al.*, 1990). Another opportunity for biological control was evaluated by testing the American parasitoid *Eretmocerus californicus* How. (Hoddle, *et al.*, 1996). In the Mediterranean, screens are used to prevent the colonisation of vectors in vegetable crops, at least during the initial part of the crop cycle.

Greenhouse experiments in the Mediterranean area showed the frequent natural occurrence of the parasitoid *E. mundus*, showing a high incidence of parasitization. *E. mundus* is now mass-produced in Italy, mainly for biological control of whiteflies on poinsettia. A commercial programme is under development for this ornamental crop (Manzaroli *et al.*, 1997), while the application on other plants infested by *Bemisia* spp. is currently under evaluation.

5. *Orius laevigatus* (Fieber) (Rhynchota Anthocoridae)

The first appearance in Europe of the western flower thrips, *Frankliniella occidentalis* (Perg.) (Thysanoptera Thripidae), dates back to 1983 in The Netherlands (Van de Vrie, 1987). This American species has spread quickly to protected crops throughout the continent, reaching the Mediterranean Countries in a few years, and it was detected for the first time in Italy and France in 1987 and in the other southern European Countries between 1988 and 1991 (Tommasini and Maini, 1995). The exotic pest is very polyphagous and produces severe damages to protected crops and in open field (in the warmest areas of the Mediterranean) due to the virus transmission too. The resistance to insecticides makes chemical control generally ineffective (Brødsgaard, 1994).

Several species were tested for the biological control of *F. occidentalis*, such as the predatory mites *Amblyseius cucumeris* (Oud.) (widely used in the northern European glasshouses), *A. barkeri* (Hughes) (used until some years ago in northern Europe), *A. degenerans* Berlese, the Eulophidae *Ceranisus menes* (Walk.) parasitizing the larvae of thrips (Galazzi and Bazzocchi, 1993; Loomans and van Lenteren, 1995) and a predator imported from America and mass-reared in the past by some European producers: *Orius insidiosus* (Say). The predators of *F. occidentalis* and other thrips pests potentially effective for biological/natural control were reviewed by Riudavets (1995).

After the introduction of *F. occidentalis*, many native predators started to prey the exotic pest and a specific attention was paid to some species of the genus *Orius* that often were able to provide a good natural control (new associations), mainly in the Mediterranean area. The species most frequently found to predate *F. occidentalis* were compared in the laboratory and in greenhouse with the aim of selecting the most favourable one for mass-production and release in protected crops. The activity of three palaearctic species -*Orius majusculus* (Reuter), *O. laevigatus* and *O. niger* Wolff- has been compared with the nearctic *O. insidiosus* (Tommasini and Nicoli, 1993; 1994). *O. niger* appeared the least suitable species for mass-rearing, for the higher mortality and lower fecundity compared to the other three species. Although *O. majusculus* and *O. insidiosus* showed good performances in survival, predation and fecundity, short-daylengths induce a reproductive diapause in a large portion of females (Van den Meiracker, 1994) and, therefore, these two species appeared unsuitable for seasonal inoculative releases in autumn and winter, when *F. occidentalis* can remain active in the warmest areas of the Mediterranean or in the heated glasshouses. In addition, the establishment of the exotic species *O. insidiosus* was not recorded in the release areas in Italy (Tavella *et al.*, 1994). Finally, *O. laevigatus* has been preferred to the other tested species because it seems the more adaptable to variable conditions. A strain collected in Sicily showed

no evident response to the photoperiod, indicating that predators can overwinter in quiescence or that their eventual diapause, if any, is weak (Tommasini and Nicoli, 1995; 1996). Therefore, it seems that strains collected at low latitudes can remain active also during the short-daylength period, if temperature is high enough (Chambers *et al.*, 1993). Moreover, the natural distribution of *O. laevigatus* in areas with marine influence (Péricart, 1972) and the good performances at high temperatures (Alauzet *et al.*, 1994) indirectly explain the effectiveness of this species also during the hot season in both the Mediterranean and northern Europe.

6. *Lysiphlebus testaceipes* (Cress.) (Hymenoptera Aphidiidae)

On a world-wide basis, *Aphis gossypii* Glover (Rhynchota Aphididae) is extremely polyphagous, as it has been recorded on more than 50 families of plants, and comprises an indefinite number of anholocyclic lines, some of which may have particular host-plant associations. *A. gossypii* is closely related to the European *Aphis* species of the *frangulae* group utilising *Frangula alnus* Miller as primary host, indicating a palaearctic origin. However, it been showed an holocyclic overwintering in USA, with *Catalpa bignonioides* Walt. and *Hibiscus syriacus* L. as primary hosts. Either separate nearctic and palaearctic species are confused under the same specific name or, more probably, *A. gossypii* has re-acquired its holocycle in North America utilising also new primary host plants (Blackman and Eastop, 1984). *A. gossypii* is the key pest of cucurbits in Europe and, in northern Italy, it overwinters on the primary host *H. syriacus* (Ferrari and Nicoli, 1994).

L. testaceipes is probably native to North and Central America (Mackauer and Starý, 1967); it has been introduced from Cuba (via Ceska Republic) to southern France in 1973/74 to control the exotic aphids *Toxoptera aurantii* (B.d.F) and *Aphis citricola* v.d.G., on which it exhibits incomplete parasitism (Starý *et al.*, 1988). It established in the Mediterranean France from where it spread at least along the European coast of the Mediterranean see in the Citrus growing areas; Tremblay *et al.* (1978) recorded the establishment of the exotic parasitoid in Italy in 1977. The presence of *L. testaceipes* in Spain since 1982-84 is believed to be due to the spread of this species along the sea shore from its release area in France; the parasitoid has reached the southern coast of Portugal in 1985 (Costa and Starý, 1988). The fast and powerful dispersal of *L. testaceipes* over the wide Mediterranean basin seems to be achieved by: i. the high genetic diversity that enables the parasitoid to develop on many new host species, becoming an important control agent of native pest too; ii. the ability to compete with indigenous parasitoids; iii. the capability to adapt to various habitats. Its host-range includes also most Aphidinae, *Myzus-Brachycaudus* spp., *Macrosiphum* and related genera. *L. testaceipes* was recently indicated as an effective natural enemy for the biocontrol of *A. gossypii* (van Steenis, 1995) and some producers started to mass-rear it. *L. testaceipes* is an exotic species, but the Mediterranean basin acts as a reserve of parasitoids and the seasonal host succession has enabled this natural enemy to occur throughout the season reaching often high densities.

7. Coleoptera Coccinellidae as natural control agents of aphids

Many species of Coccinellidae are voracious predators of aphids, but only the Asiatic species *Harmonia axyridis* (Pallas) is presently mass-produced in Europe for biological control of aphids in protected crops. The establishment of this exotic predator is highly probable but not yet confirmed in Italy (Ricci *et al.*, 1997).

On the other hand, several native Coccinellidae can play an effective role for the natural control of aphids in the Mediterranean agroecosystems. In northern Italy, for five years, these aphidophagous predators were sampled on both cultivated and uncultivated plants to know

their preferred sites for predation, reproduction and overwintering. Field experiments were carried out to evaluate the role of the wheat canopies for the reproduction (in springtime) of the overwintering females of some polyvoltine species and to verify when the newly-emerged adults (first generation) move from the development sites to colonise the summer crops infested by *A. gossypii* both in open field (such as watermelon and melon) and in plastic tunnel (melon and cucumber). These experiments showed that particularly *Adonia variegata* (Goeze) and *Propylaea 14-punctata* (L.) are very effective natural control agents, and the possibility of forecasting the flight period of the newly-emerged adults (from the wheat canopies, just before reaping) allowed to a new approach in the IPM for watermelon and melon in open field. For these crops, a strong reduction in the use of wide-spectrum insecticides for *A. gossypii* was obtained, limiting the chemical control to an eventual spray with a shortly-persistent insecticide to be used once at the beginning of the crop cycle to reduce the initial infestation, before the colonisation flight of the Coccinellidae. Thereafter the large amount of adults landing on the crops and their progeny can reduce very quickly the outbreaks under the economic threshold until the end of the crop cycle (Ferrari and Nicoli, 1994; Nicoli *et al.*, 1994; 1996). In some northern-Italy areas, an increasing number of growers started to grow melon and watermelon surrounded by unsprayed wheat canopies, in order to facilitate the colonisation of a large number of newly-emerged adults. In tunnel-grown crops, namely cucumber and melon, the colonisation of Coccinellidae appeared not so relevant, probably limited by the plastic film cover (Ferrari *et al.*, 1994). Bushes, hedges and grasslands proved to be important sites for sheltering (mainly when crops are harvested and no plants remain in the canopies) and overwintering, but less important for reproduction, due to the generally low aphid infestations recorded on these plants (Nicoli *et al.*, 1995).

Conclusions

In the past years, the rich Mediterranean biodiversity provided new natural enemies for biological/natural control of both native and exotic pests harmful to vegetable and ornamental crops, confirming the effectiveness of some new associations (Pimentel, 1963; Hokkanen and Pimentel, 1984; 1989). The Mediterranean area is also a natural source of established exotic natural enemies (as *L. testaceipes*) that can be collected and evaluated for mass-production and release by the 'seasonal inoculative' method, following the same procedures used for the native beneficial species.

In all the over-mentioned cases, the efficacy of predators and parasitoids was initially recorded by observing their natural colonisation of the infested crops (both in greenhouse and in open field), when the use of wide-spectrum insecticides was strongly reduced. Some palaearctic species were selected for mass-production and used for 'seasonal inoculative' releases also in the North-European glasshouses (mainly to control exotic pests) and it is highly probable that other natural enemies will be selected for the same purposes, in the future. But the natural enemies endemic in the Mediterranean can not be considered exotic for northern Europe: some of them (as *D. isaea* and *O. laevigatus*) can be found also at high latitudes in the palaearctic region and they should be considered as 'Mediterranean strains' of endemic species. In other cases, the beneficial species can not survive in the North because the climate is too cold (as *P. persimilis* and *M. caliginosus*) and/or neither the target host/prey can overwinter outdoor (as in the case of *Bemisia* spp. parasitized by *E. mundus*). It appears unrealistic to suppose that these Mediterranean species are not endemic in northern Europe because they were never imported from South, being in the same continent. Probably they were introduced several times (also accidentally), but they were unable to establish and no perturbation of the local entomophauna (and other arthropods) can be realistically feared.

The conservation of plant biodiversity in the Mediterranean agroecosystems, by improving the polyculture and the maintenance of areas with uncultivated plants (as hedges, woods, grasslands), can enhance natural control of harmful pests for which the release of natural enemies is too expensive or not yet available (as in the case of Coccinellidae preying *A. gossypii* on Cucurbits) or reliable and, more in general, can favour a natural colonisation of protected and open field crops by many natural enemies, including the species commercially mass-produced.

Acknowledgements

This work was supported by the Emilia-Romagna Regional Government (Agroecology Programme). Special thanks are due to Giuseppe Manzaroli, Marco Mosti, Maria Grazia Tommasini, Stefano Foschi (Biolab, Centrale Ortofrutticola, Cesena) and Roberto Ferrari (Centro Agricoltura e Ambiente, Crevalcore, Bologna) for useful information and comments.

References

ALAUZET, C., DARGAGNON, D. & MALAUSA, J.C., 1994. Bionomics of the polyphagous predator *Orius laevigatus* (Het.: Anthocoridae). Entomophaga, **39** (1): 33-40.

ALBAJES, R., ALOMAR, O., RIUDAVETS, J., CASTAÑÉ, C., ARNÓ, J. & GABARRA, R. 1996. The mirid bug Dicyphus tamaninii: an effective predator for vegetable crops. IOBC/WPRS Bull. **19**(1): 1-4.

ALOMAR, O., CASTAÑÉ, C., GABARRA, R., ARNÓ, J., ARIÑO, J. & ALBAJES, R., 1991. Conservation of native Mirid bugs for biological control in protected and outdoor tomato crops. Bull. IOBC/WPRS, **14** (5): 33-42.

ALOMAR, O., GOULA, M. & ALBAJES, R. 1994. Mirid bugs for biological control: identification, survey in non-cultivated winter plants, and colonization of tomato fields. IOBC/WPRS Bull. **17**(5): 217-223.

ARZONE, A., 1979. L'Agromizide neartico *Liriomyza trifolii* (Burgess) nuovo nemico di *Gerbera* in Italia. Inform. Fitopat., **29** (3): 3-6.

ARZONE, A., ALMA, A. & TAVELLA, L., 1990. Ruolo dei Miridi (Rhynchota Heteroptera) nella limitazione di *Trialeurodes vaporariorum* Westw. (Rhynchota Aleyrodidae). Boll. Zool. agr. Bachic., Ser. II, **22** (1): 43-51.

ATHIAS-HENRIOT, C., 1957. Phytoseiidae et Aceosejidae (Acarina, Gamasina) d'Algérie. I. Genres *Blattisocius* Keegan, *Iphiseius* Berlese, *Amblyseius* Berlese, *Phytoseius* Ribaga, *Phytoseiulus* Evans.- Bull. Soc. Hist. Nat. Afr. Nord, **48**: 319-352.

BELLOWS, T.S., PERRING, T.M., GILL, R.G. & HEADRICK, D.H., 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Am., **87** (2): 195-206.

BENUZZI, M., NICOLI G. & MANZAROLI G., 1990. Biological control of *Bemisia tabaci* (Genn.) and *Trialeurodes vaporariorum* (Westw.) by *Encarsia formosa* Gahan on poinsettia. Bull. IOBC/WPRS, **13** (5): 27-31.

BETHKE, J.A., PAINE, T.D. & NUESSLY, G.S., 1991. Comparative biology, morphology and development of two populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) on cotton and Poinsettia. Ann. Entomol. Soc. Am., **84** (4): 407-411.

BLACKMAN, R.L. & EASTOP, V.F., 1984. Aphids on the world's crops: an identification guide. John Wiley Sons, Chichester: pp.

BOISCLAIR, J., BRUEREN, G.J. & LENTEREN, J.C. Van, 1990. Can *Bemisia tabaci* be controlled with *Encarsia formosa*? Bull. IOBC/WPRS, 13 (5): 32-35.

BRØDSGAARD, H.F., 1994. Insecticide resistance in European and African strains of western flower thrips (Thysanoptera: Thripidae) tested in a new residue-on-glass test. J. Econ. Entomol., **87** (5): 1141-1146.

CELLI, G., NICOLI, G. & BENUZZI, M., 1987. Biological control in protected crops in northern Italy's Po valley. Bull. IOBC/WPRS, **10** (2): 37-40.

CHAMBERS, R.J., LONG, S. & HELYER, N.L., 1993. Effectiveness of *Orius laevigatus* (Hem.: Anthocoridae) for the control of *Frankliniella occidentalis* on cucumber and pepper in the UK. Biocontrol Science and Technology, **3**: 295-307.

COSTA, A. & STARÝ, P., 1988. *Lysiphlebus testaceipes*, an introduced aphid parasitoid in Portugal (Hym.: Aphidiidae). Entomophaga, **33** (4): 403-412.

COSTANZI, M. & PINI, S., 1991. Ruolo di due miridi predatori nella difesa delle colture ortofrutticole. Colture protette, **20** (11): 49-53.

CREDI, R., BETTI, L. & CANOVA, A., 1989. Association of a geminivirus with a severe disease of tomato in Sicily. Phytopath. Medit., **28** (3): 223-226.

DEL BENE, G., 1989. Nemici naturali di *Liriomyza trifolii* (Burgess), *Chromatomyia horticola* (Goureau) e *Chromatomyia syngenesiae* Hardy (Diptera Agromyzidae) in Toscana. Redia, **72** (2): 529-544.

DELRIO, G., FLORIS, I., LENTINI, A., LUCIANO, P. ORTU, S. & PROTA, R., 1991. Osservazioni sulla lotta biologica in colture protette di pomodoro in Sardegna. Proc. XVI Congr. Naz. Ital. Entomol., Bari-Martina Franca (TA), 23/28 Sept. 1991: 347-353.

DOSSE, G., 1958. Über einige neue Raubmilbenarten (Acar. *Phytoseiidae*). Pflanzenschutzber., **21**: 44-61.

DOSSE, G., 1967. Shadmilben des Libanons und ihre Prädatoren. Z. ang. Ent., **59**: 16-48.

EL-DESSOUKI, S.A., EL-KIFL, A.H. & HELAL, H.A., 1976. Life-cycle, host-plants and symptoms of damage of the tomato bug, *Nesidiocoris tenuis* Reut. (Hemiptera: Miridae), in Egypt. Zeit. Pflazensch., **83** (4): 204-220.

ENKEGAARD, A., 1993. *Encarsia formosa* parasitizing the poinsettia-strain of the cotton whitefly, *Bemisia tabaci*, on poinsettia: bionomics in relation to temperature. Entomol. exp. appl., **69**: 251-261.

FERRAGUT, F., GARCIA MARÍ, F. & MARZAL, C., 1983. Determinación y abundancia de los Fitoseidos (Acari: Phytoseiidae) en los agrios españoles. I Congreso Nacional de la Sociedad Española de Ciensas Horticolas, 28 noviembre-1diciembre 1983, Valencia, Spain: 299-308.

FERRARI, R., BURGIO, G. & NICOLI, G., 1994. Coccinellidae as natural control agents of aphids in vegetable crops. Bull. IOBC/WPRS, **17** (5): 181-183.

FERRARI, R. & NICOLI, G., 1994. Ciclo biologico e nemici naturali di *Aphis gossypii* Glover: prime osservazioni. Inform. Fitopat., **44** (5): 59-62.

GABARRA, R., CASTAÑÉ, C., BORDAS, E. & ALBAJES, R., 1988. *Dicyphus tamaninii* as beneficial insect and pest in tomato crops in Catalonia, Spain. Entomophaga, **33** (2): 219-228.

GALAZZI, D. & BAZZOCCHI, G., 1993. *Ceranisus menes*. Inform. Fitopat., **43** (3): 47-50.

GALAZZI, D. & NICOLI, G., 1996a. Comparative study of strains of *Phytoseiulus persimilis* Athias-Henriot (Acarina Phytoseiidae). I. Development and adult life. Boll. Ist. Ent. "Guido Grandi" Univ. Bologna, **50**: 215-231.

GALAZZI, D. & NICOLI, G., 1996b. Comparative study of strains of *Phytoseiulus persimilis* Athias-Henriot (Acarina Phytoseiidae). II. Influence of mass-rearing on population growth. Boll. Ist. Ent. "Guido Grandi" Univ. Bologna, **50**: 243-252.

GARCIA MARÍ, F., FERRAGUT, F., MARZAL, C., COSTA COMELLES, J. & LABORDA, R., 1986. Acaros que viven en las hojas de los citricos españoles. Inv. Agr.: Prod. Prot. Veg., **1** (2): 219-250.

GARCIA MARÍ, F., FERRAGUT, F., MARZAL, C., LABORDA, R., COSTA COMELLES, J., COSCOLLA, R. & SANCHEZ, J., 1987. Contribucion al conocimiento de los acaros fitoseidos y tetraniquidos en los viñedos Valencianos. Inv. Agr.: Prod. Prot. Veg., **2** (1): 89-95.

GENNADIUS, P., 1889. Disease of tobacco plantations in the Trikonia. The aleurodid of tobacco. Ellenike Georgia, **5**: 1-3 (in Greek).

HARGREAVES, E., 1915. The life-history and habits of the greenhouse white fly (*Aleyrodes vaporariorum* Westd.). Ann. Appl. Biol., **1**: 303-304.

HODDLE, M.S., DRIESCHE, R. Van & SANDERSON, J., 1996. Greenhouse trials of *Eretmocerus californicus* Howard (Hym.: Aphelinidae) for control of *Bemisia argentifolii* Bellows and Perring (Hom.: Aleyrodidae) on poinsettia in Northeastern U.S.A. Bull. IOBC/WPRS, **19** (1): 55-58.

HOKKANEN, H.T.M.L. & PIMENTEL, D., 1984. New approach for selecting biological control agents. Can. Ent., **116**: 1109-1121.

HOKKANEN, H.T.M.L. & PIMENTEL, D., 1989. New associations in biological control: theory and practice. Can. Ent., **121**: 829-840.

HUSSEY, N.W., 1985. History of biological control in protected culture. Western Europe. Biological pest control, the glasshouse experience. In: Hussey, N.W. & Scopes N. (Eds.). Blandford Press, Poole, Dorset: 11-22.

KENNETT, C.E. & CALTAGIRONE, L.E., 1968. Biosystematics of *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae). Acarologia, **10** (4): 563-577.

LENTEREN, J.C. Van, BENUZZI, M., NICOLI, G. & MAINI, S., 1992. Biological control in protected crops in Europe. In: Lenteren J.C. Van, Minks A.K., De Ponti O.M.B. (eds.). Biological control and integrated crop protection: towards environmentally safer agriculture. Pudoc Scientific Publ., Wageningen (NL): 77-89.

LINDEN, A. Van der, 1990. Prospects for the biological control of *Liriomyza huidobrensis* (Blanchard), a new leafminer for Europe. Bull. IOBC/WPRS, **13** (5): 100-103.

LIOTTA, G., MINEO, G. & RAGUSA, S., 1977. Sur l'état actuel des connaissances concernant certains arthropodes nuisibles aux agrumes en Sicile. Boll. Ist. Ent. agr. Oss. Fitopat. Palermo, Italia, **10**: 29-68.

LLOYD, L.L., 1922. The control of the greenhouse white fly (*Asterochiton vaporariorum*) with notes on its biology. Ann. Appl. Biol., **9**: 1-32.

LOMBARDINI, G., 1959. Acari nuovi. XXXVII. - Boll. Ist. Ent. agr. Oss. Fitopat. Palermo, Italia, **3**: 163-167.

LOOMANS, A. & LENTEREN, J.C. Van, 1995. Biological control of thrips pests: a review on thrips parasitoids. Wageningen Agric. Univ. Papers, **95.1**: 90-201.

LYON, J.P., 1985. *Liriomyza trifolii* Burgess. Les problèmes posé et les possibilités de contrôle biologique. La Défense des Végétaux, **234**: 6-11.

MACKAUER, M. & STARÝ, P., 1967. Hym. Ichneumonoidea. World Aphidiidae. In: Index of entomophagous insects. Delucchi, V. & Remaudiere, G. (Eds.). Le Francois Paris: 195 pp.

MALAUSA, J.C., DRESCHER, J. & FRANCO, E., 1987. Perspectives for the use of a predaceous bug *Macrolophus caliginosus* Wagner (Heteroptera Miridae) on glasshouses crops. Bull IOBC/WPRS, **10** (2): 106-107.

MALAUSA, J.C., 1989. Lutte intégrée sous serre: Les punaises prédatrices Mirides dans les cultures de Solanacées du sud-est de la France. P.H.M. Revue Horticole, **298**: 39-43.

MANZAROLI, G., TOMMASINI, M.G., MOSTI, M. & DRADI, D., 1997. Biological control of whitefly on poinsettia in Italy. Bull. IOBC/WPRS (same volume).

McMURTRY, J.A., 1977. Some predaceous mites [Phytoseiidae] on citrus in the Mediterranean region. Entomophaga, **22** (1): 19-30.

MEIRACKER, R.A.F. Van, 1994. Induction and termination of diapause in *Orius* predatory bugs. Entomol. Exp. Appl., **73**: 127-137.

MINKENBERG, P.J.M. & Van LENTEREN, J.C., 1986. The leafminers *Liriomyza bryoniae* and *L. trifolii* (Diptera: Agromyzidae), their parasites and host plants: a review. Agric. Univ. Wageningen papers, **86.2**: 50 pp.

NICOLI, G., FERRARI, R. & CAVAZZUTI, C., 1994. Ruolo delle Coccinelle nella lotta naturale contro *Aphis gossypii* su cocomero. L'Informatore Agrario, **50** (23): 61-64.

NICOLI, G. & PITRELLI, P., 1994. -Parasitization and predation of *Diglyphus isaea*.- *Proc. 7th Workshop "Quality Control of mass reared Arthropods"*, Nicoli G., Benuzzi M., Leppla N.C. (eds.), Rimini (Italy), September 13-16, 1993: 161-170.

NICOLI, G., LIMONTA, L., CAVAZZUTI, C. & POZZATI, M., 1995. -Il ruolo delle siepi nell'ecologia del campo coltivato. I. Prime indagini sui Coccinellidi predatori di afidi.- Inform. Fitopat., **45** (7-8): 58-64.

NICOLI, G., FERRARI, R., BURGIO, G., 1996. Seasonal distribution preying aphids in cultivated agroecosystem. XX International Congress of Entomology. Firenze, Italy, August 25-31, 1996: 659.

PÉRICART, J., 1972. Hémiptères Anthocoridae, Cimicidae, Microphysidae de l'Ouest-Paléarctique. Masson et Cie Ed., Paris: 406 pp.

PIMENTEL, D., 1963. Introducing parasites and predators to control native pests. Can. Ent., **95**: 785-792.

PRICE, J., SCHUSTER, D. & SHORT, D., 1987. Managing sweetpotato whitefly. Greenhouse Grower, Dec. 1987: 16 pp.

RAGUSA, S., 1965. Osservazioni preliminari sulla biologia dell'*Amblyseius tardi* Lomb. (Acarina-Phytoseiidae) predatore del *Tetranychus urticae* Koch. Boll. Ist. Ent. agr. Oss. Fitopat. Palermo, Italia, **6**: 199-206.

RAGUSA, S., 1974. Difesa del nocciolo dagli artropodi dannosi. VIII. Effetto del Lindano e dell'Azinphos-metile sugli acari Fitoseidi (*Acarina: Mesostigmata*). Boll. Ist. Ent. agr. Oss. Fitopat. Palermo, Italia, **8**: 203-214.

RAGUSA, S., 1977. Notes on phytoseiid mites in Sicily with a description of a new species of *Typhlodromus* (Acarina: Mesostigmata). Acarologia, **18** (3): 379-392.

RAGUSA, S., 1986. A five year study on population fluctuations of phytoseiid mites in a citrus orchard in Sicily. Acarologia, **27** (3): 193-201.

RAMBIER, A., 1972. Le *Phytoseiulus persimilis* Athias-Henriot dans le Midi de la France. Proc. VIIth European Mite Symp. "Advances in Agricultural Acarology in Europe", 1970, Warsaw; Zeszyty Probl. Post. Nauk. Rol., **129**: 89-91.

RICCI, C., PRIMAVERA, A. & CAPPELLETTI G., 1997. Predatori di insetti fitomizi in ambienti urbani. Primo Convegno Nazionale sulla Fauna Urbana. Roma, Italy, April 12, 1997: 38.

RIUDAVETS, J., 1995. Predators of *Frankliniella occidentalis* (Perg.) and *Thrips tabaci* Lind.: a review. Wageningen Agric. Univ. Papers, **95.1**: 43-87.

SCHÜTTE, C., HULSHOF, J., DIJKMAN, H. & DICKE, M., 1995. Change in foraging behaviour of the predatory mite *Phytoseiulus persimilis*: some characteristics of a mite population that does not respond to herbivore-induced synomones. Proc. Exp. Appl. Entomol., N.E.V. Amsterdam, **6**: 133-140.

SCHÜTTE, C., BAARLEN, P. VAN., DIJKMAN, H. & DICKE, M., 1996. How can predatory mites loose their response to plant signals?. Proc. Exp. Appl. Entomol., N.E.V. Amsterdam, **7**: 195-196.

SILVESTRI, F., 1939. Compendio di Entomologia Applicata. Vol. 1, Ed. Bellavista, Portici: 980 pp.

SPEYER, E.R., 1927. An important parasite of the greenhouse white-fly (*Trialeurodes vaporariorum*, Westwood). Bull. Ent. Res., **17**: 301-308.

STARÝ, P., LYON, J.P. & LECLANT, F., 1988. Biocontrol of aphids by the introduced *Lysiphlebus testaceipes* (Cress.) (Hym., Aphidiidae) in Mediterranean France. J. Appl. Ent., **105**: 74-87.

STEENIS, M. Van, 1995. Evaluation and application of parasitoids for biological control of *Aphis gossypii* in glasshouse cucumber crops. Thesis Wageningen: 217 pp.

SÜSS, L., 1991. Prima segnalazione in Italia di *Liriomyza huidobrensis* (Blanchard) (Diptera Agromyzidae). Boll. Zool. agr. Bachic., Ser. II, **23** (2): 197-202.

SWIRSKY, E. & AMITAI, S., 1968. Notes on phytoseiid mites (Acarina: Phytoseiidae) of Israel, with a description of one new species.- Israel J. Entomol., **3** (2): 95-108.

SWIRSKY, E. & RAGUSA, S., 1976. Notes on predacious mites of Greece, with a description of five new species (Mesostigmata: Phytoseiidae). Phytoparasitica, **4** (2): 101-122.

SWIRSKY, E. & RAGUSA, S., 1977. Some predacious mites of Greece, with a description of one new species (Mesostigmata: Phytoseiidae). Phytoparasitica, **5** (2): 75-84.

TAVELLA, L., ALMA, A. & ARZONE, A., 1994. Attività predatrice di *Orius* spp. (Anthocoridae) su *Frankliniella occidentalis* (Perg.) (Thripidae) in coltura protetta di peperone. Inform. Fitopat., **44** (1): 40-43.

TOMMASINI, M.G. & NICOLI, G., 1993. Adult activity of four *Orius* species reared on two preys. Bull. IOBC/WPRS, **16** (2): 181-184.

TOMMASINI, M.G. & NICOLI, G., 1994. Pre-imaginal activity of four *Orius* species reared on two preys. Bull. IOBC/WPRS, **17** (5): 237-241.

TOMMASINI, M.G. & MAINI, S., 1995. *Frankliniella occidentalis* and other thrips harmful to vegetable and ornamental crops in Europe. Wageningen Agric. Univ. Papers, **95.1**: 1-42.

TOMMASINI, M.G. & NICOLI, G., 1995. Evaluation of *Orius* spp. as biological control agents of thrips pests: Initial experiments on the existence of diapause in *Orius laevigatus*. Med. Fac. Landbouww. Univ. Gent, **60**/3a: 901-908.

TOMMASINI, M.G. & NICOLI, G., 1996. Evaluation of *Orius* spp. as biological control agents of thrips pests. Further experiments on the existence of diapause in *Orius laevigatus*. Bull. IOBC/WPRS, **19** (1): 183-186.

TREMBLAY, E. , BARBAGALLO, S., MICIELI DE BIASE, L., MONACO, R. & ORTU, S., 1978. Sulla presenza in Italia del *Lysiphlebus testaceipes* (Cr.) nemico naturale di afidi dannosi agli agrumi. (Hymenoptera Ichneumonoidea, Homoptera Aphidoidea). Boll. Lab. Ent. Agr. Portici, **35**: 169-179.

TROUVÉ, C., MARTINEZ, M., PHALIP, M. & MARTIN, C., 1991. Un nouveau ravageur en Europe, la mouche mineuse sud-americaine. Phytoma, **429**: 42-46.

VACANTE, V. & NUCIFORA, A., 1985. Gli acari degli agrumi in Italia. I. Specie rinvenute e chiave per il riconoscimento degli ordini, dei sottordini e delle famiglie. Boll. Zool. agr. Bachic., Ser. II, **18**: 115-166.

VACANTE, V. & NUCIFORA, A., 1986. A first list of the mites in citrus orchards in Italy. Proc. Expert's Meeting "Integrated pest control in citrus-groves", 26-29 March 1985, Acireale, Italy. Balkema, Rotterdam: 177-188.

VACANTE, V. & NUCIFORA, A., 1987a. Gli acari degli agrumi in Italia. II. I Gamasidi (*Phytoseiidae* Berlese e *Ascidae* Voigts, Oudemans): chiave per il riconoscimanto dei generi e delle specie e note di biologia. Boll. Zool. agr. Bachic., Ser. II, **19**: 145-184.

VACANTE, V. & NUCIFORA, A., 1987b. Possibilities and perspectives of the biological and integrated control of the two spotted spider mite in the Mediterranean greenhouse crops. Bull. IOBC/WPRS, **10** (2): 170-173.

VACANTE, V. & TROPEA GARZIA, G., 1994a. Indagini sul ruolo ecologico di *Nesidiocoris tenuis* (Reuter) nelle serre fredde di pomodoro nel Ragusano. Inform. Fitopat., **44** (9): 45-48.

VACANTE, V. & TROPEA GARZIA, G., 1994b. *Nesidiocoris tenuis*: antagonista naturale di aleurodidi. Inform. Fitopat., **44** (4): 23-28.

VET, L.E.M., LENTEREN, J.C. Van & WOETS, J., 1980. The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). IX. A review of the biological control of the greenhouse whitefly with suggestion for future research. Z. ang. Ent., **90**: 26-51.

VRIE, M., Van de, 1987. Explosieve aantasting en moeilijkebestrijding kenmerk Californische trips. Vakblad Bloem., **11**: 25-27.

WAGNER, E. & WEBER, H.H., 1964. Faune de France, Hétéroptères Miridae. Librairie Fac. Sciences, Paris, **67**: 592 pp

Tab.1: New entomophagous species native (or established) in the Mediterranean basin and selected for mass production and seasonal inoculative release to control arthropod pests in the European protected crops.

Natural enemy	**Origin of the natural enemy**	**Mass-production since**	**Target pest**	**Origin of the pest**	**Notes**
Diglyphus isaea	Palaearctic	1984	*Liriomyza trifolii* *Liriomyza huidobrensis*	North America South America	New associations
Phytoseiulus persimilis (Strains collected in the Mediterranean area)	Palaearctic (Mediterranean)	1991	*Tetranychus urticae*	Unknown (cosmopolitan)	*P. persimilis* was originally described as a new species with specimens collected in Algeria in 1955-57. Mass-production started in northern Europe in late 1960s using an initial stock found on orchids imported from Chile (Dosse, 1958).
Macrolophus caliginosus	Palaearctic (Mediterranean)	1992	*Trialeurodes vaporariorum* and other whiteflies	Tropical/sub-tropical America	New association
Orius laevigatus	Palaearctic	1993	*Frankliniella occidentalis*	North America	New association
Eretmocerus mundus	Palaearctic (Mediterranean)	1995	*Bemisia tabaci* *Bemisia argentifolii*	Probably Indian region Probably introduced from North America	In the Mediterranean area, *E. mundus* is known as a parasitoid of *Bemisia tabaci* from Silvestri (1939) and it adapted to *B. argentifolii* after the pest invaded Europe.
Lysiphlebus testaceipes	Nearctic, established in the Mediterranean	1996	*Aphis gossypii*	Probably palaearctic	*L. testaceipes* was imported in the Mediterranean basin in 1973/74 to control two Citrus aphids: *Toxoptera aurantii* and *Aphis citricola.*

INTEGRATED PEST MANAGEMENT ON TOMATOES IN GRAN CANARIA. (CANARY ISLANDS)

J. M. Rodríguez ; R. Rodríguez ; Ana Florido ; Rosa Hernández.

Sección de Fitopatología. Granja Agrícola Exptal. Cabildo Insular de Gran Canaria. Ctra. Gral. del Norte, Arucas, Gran Canaria. Islas Canarias.

Abstract

Tomato crops in Gran Canaria are developed on the flat coastal areas. After several experiences testing different materials nowadays tomatoes are grown under net cover protection. This kind of greenhouse is not very suitable to carry out IPM techniques as those known in some European countries.

There are also other added problems such as weeds control, seedling management free of pests, etc., due to Canarian climatic conditions.

Following the IPM lines we start a new trial in our conditions so as to introduce some modifications in order to obtain the best results : the nethouse was conditioned with double door entry and at the beginning cromoattractives traps were hung. The pollination was ameliorated with bumblebees.

The mainly problem which arose out were the attack of the whitefly, being necessary some fitosanitary applications. After the 7th *Encarsia* release together with the activity of the *Macrolophus* added together an important decrease of whitefly populations was observed.

The damage due to leafminer (*Liriomyza spp.)* has not been important due to auxiliar performance *Diglyphus isaea* since this parasite appears spontaneously and it is perfectly developed.

The thrips (*Frankliniella occidentalis)*, that at beginning affected the crop in a high percentage were controlled for the rest of the growth with only two abamectina applications.

Concerning caterpillar it was only necessary to give one *Bacillus* application.

The aphids were observed in very specific areas originating auxiliaries releases with very good results in control of them.

Other problems were solved with a few chemical applications.

Introduction

The Gran Canaria tomato farms are spread on the flat coastal area around the island, commonly integrated in cooperatives or belonging to large companies. From the 70's crops are indoor under protection (net o plastic cover) in order to avoid damage because of pests and wind induced in outdoors crops

After many trial with different materials, at present, tomatoes are grown under closed protection supported by galvanised pipe frame that sometimes take large surfaces (up to 2 Ha).

This type of plastichouse, increased by its surface, are not adaptable to the well known IPM techniques followed in greenhouses of European countries, perhaps it would be necessary to modify some plastichouse conditions according to the results obtained.

Another added problem observed, due the Canarian climatic condition, it is necessary, for a suitable IPM, to have a rigorous control on weeds, inside and outside plastichouses.

Following the IPM line started by the Granja Agrícola Experimental several years ago, from December 1996 and with the cooperation of Biobest Biological Systems (Belgium), supplier of auxiliar fauna, start a new trial in these terms.

Material and methods

The trial was carried out in an experimental nethouse of 1000 m^2 on soil crop with drip irrigation. The plant density was 2,5 plants/m^2 and "Daniela" as tomato commercial variety.

The plantation was carried out on February 2, 1997.

The nethouse was conditioned with two entry foyers (double door) to control unwished insects access, from outside. Also plates or cromoattractive traps were included at the time of plantation, in order to detect the presence of the pests, those which were withdrawn since the "releases" were begun.

Controls consist in weekly "counts", quantifying and testing the pest evolution and existing parasitism decisions being taken in agreement with Biobest.

Pollination, was ameliorated with bumblebee (*Bombus canariensis*).

All the incidences of fitosanitary treatments and auxiliar releases are included in the final table.

Results

The results of this study at the moment to edit this work (final of June 1997), are presented in the Graphics 1, 2, 3 and 4. Temperatures and humidities during the growth, in Graphic 5.

Discusion

The whitefly represents the greater pest problem in tomato cultivation in Gran Canaria and of highest cost in chemicals for its control, showing its evolution for this trial in the Graphic 1. We can observe two peaks of maximum adult population, that it should be to the massive entry of these aleurodides originated from the ended contiguous cultivation. This fact forced the application of some fitosanitary treatment to avoid a very labeled imbalance between *Encarsia formosa* and *Trialeurodes vaporariorum* populations, that prevented the correct performance of the auxiliary. It is considered that from the 7th *Encarsia* release is when an important decrease is proven of the population of adults, but this is produced mainly by the beginning activity of the *Macrolophus caliginosus* added in the release and the *Cyrtopeltis tenuis* that appear spontaneously in this crop.

In the first month and due to the existing whitefly populations, the percentage of plants with "white pupae" increases progressively (Graphic 2). In the same way it is proven from the 4th release the presence of "black pupas" increases, until the moment in which the number of "black pupas" were higher than "white pupae". It is then when a sharp pupa decrease is appreciated (black and white) due to *Miridae*, mostly *Cyrtopeltis tenuis*.

From the beginning of the cultivation the presence of *Lyriomiza spp* was confirmed (Graphic 3). for the existing of feeding spots in the leaves. But even although the damage produced by the "leafminer" have not been important due to auxiliar performance *Dygliphus isaea*, that from the first moment has avoided that it evolved in the galleries. In spite of having accomplished *Dygliphus* three release at the beginning of the crop, it was not necessary to continue with these since this parasite appears spontaneously and it is perfectly developed.

In Graphic 4 we have reflected the evolution of the remaining present pests during the crop. In first place we will make reference to the "thrips" (*Frankliniella occidentalis)* that at the beginning affected the crop in a high percentage, but the timely treatment with abamectina quickly reduced its population very soon, being maintained under control for the rest of the campaign.

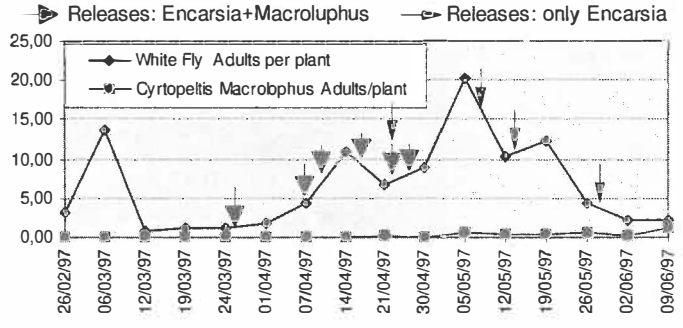
Concerning caterpillars (several species) have been present in the cultivation in low levels, with no economic damage, and it was only necessary to give one *Bacillus thuringiensis* application. .

The aphids were observed in very patchy areas, being present in two moments of high environmental humidity during March and April. Since then the aphid populations was controlled with *Aphidoletes aphidimiza* and *Aphielinus abdominalis* releases, observing thereinafter aphid dead and mummified.

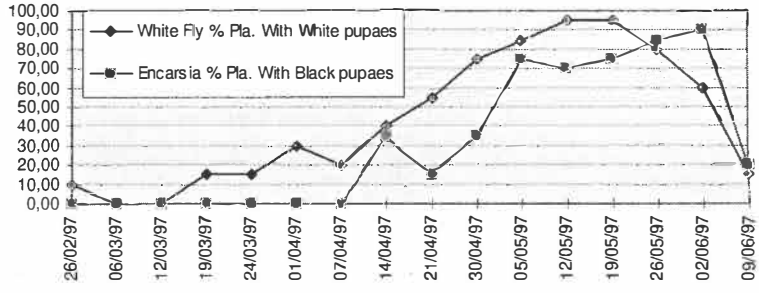
Finally to indicate a problem that can be serious on tomatoes crops in Gran Canaria, "the drying out of the tomato", caused by *Aculops lycopersici* and can be observed in the graphic when the temperatures rises and the environmental humidity decreases (May), a rapid chemical intervention is needed with bromopropilato and fenbutatin, with very good results.

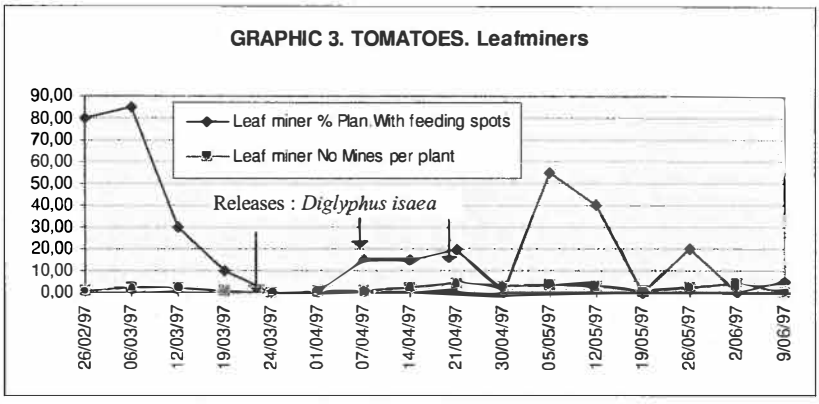
Once the project is finished and the crop period completed we will be able to obtain complete conclusions and we will able to assess the use of IPM in cultivation conditions of tomatoes in Gran Canaria.

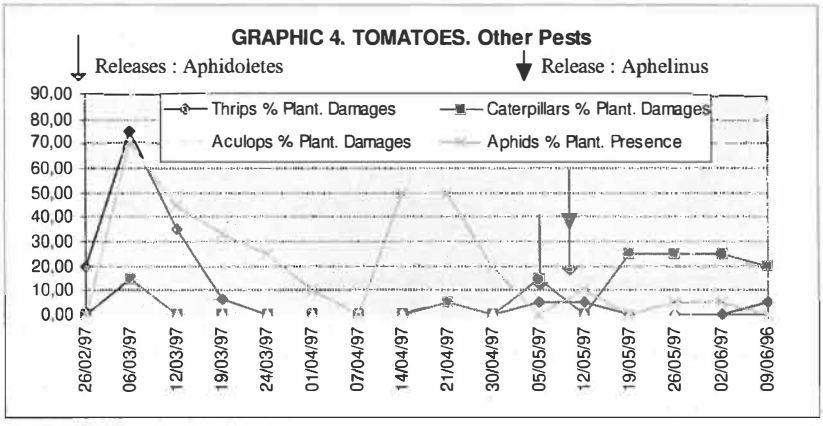
GRAPHIC 1. TOMATOES. White fly. *Trialeurodes*

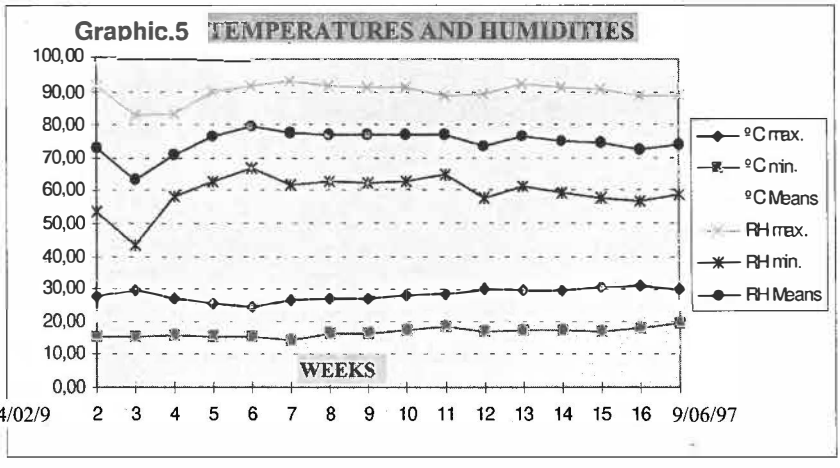


GRAPHIC 2. TOMATOES. *Trialeurodes: Encarsia*




GRAPHIC 3. TOMATOES. Leafminers
Leaf miner % Plan.With feeding spots
Leaf miner No Mines per plant
Releases : Diglyphus isaea
90,00
80,00
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
26/02/97
06/03/97
12/03/97
19/03/97
24/03/97
01/04/97
07/04/97
14/04/97
21/04/97
30/04/97
05/05/97
12/05/97
19/05/97
26/05/97
2/06/97
9/06/97


GRAPHIC 4. TOMATOES. Other Pests
Releases : Aphidoletes
Release : Aphelinus
Thrips % Plant. Damages
Caterpillars % Plant. Damages
Aculops % Plant. Damages
Aphids % Plant. Presence
90,00
80,00
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
26/02/97
06/03/97
12/03/97
19/03/97
24/03/97
01/04/97
07/04/97
14/04/97
21/04/97
30/04/97
05/05/97
12/05/97
19/05/97
26/05/97
02/06/97
09/06/96


Graphic.5 TEMPERATURES AND HUMIDITIES
ºC max.
ºC min.
ºC Means
RH max.
RH min.
RH Means
100,00
90,00
80,00
70,00
60,00
50,00
40,00
30,00
20,00
10,00
0,00
WEEKS
24/02/9
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
9/06/97

Integrated Pest Management for tomatoes

Project 1997

Dates	Treatments and Releases
20/02/97	abamectina (T)
23/02/97	buprofezin (WF)
07/03/97	piriproxifen (WF)
	cihexaestan (WF)
13/03/97	abamectina (T)
26/03/97	*Encarsia 5/m² (WF)*
	Macrolophus 0,5/m² (WF)
	Diglyphus 0,1/m² (LM)
03/04/97	miclobutanil (L)
07/04/97	*Encarsia 5/m² (WF)*
	Macrolophus 0,5/m² (WF)
11/04/97	*Encarsia 5/m² (WF)*
	Macrolophus 0,5/m² (WF)
	Diglyphus 0,1/m² (LM)
17/04/97	*Encarsia 5/m² (WF)*
	Macrolophus 0,5/m² (WF)
18/04/97	miclobutanil (L)
24/04/97	*Encarsia 5/m² (WF)*
	Macrolophus 0,5/m² (WF)
	Diglyphus 0,1/m² (LM)
24/04/97	*Encarsia 5/m² (WF)*
25/04/97	miclobutanil (L)
28/04/97	*Encarsia 5/m² (WF)*
	Macrolophus 0,5/m² (WF)
6/05/97	piriproxifen (WF)
8/05/97	*Encarsia 5/m² (WF)*
	Aphidoletes 1/m² (A)
12/05/97	bacillus thuringiensis (CA)
16/05/97	*Encarsia 5/m² (WF)*
	Aphidoletes 1/m² (A)
	Aphelinus 0,2/m² (A)
21/05/97	bupirimato (L) Soil drench
28/05/97	fenbutatin (AL)
29/05/97	*Encarsia 10/m² (WF)*
3/06/97	bromopropylato (AL)
	bupirimato (L) Soil drench
6/06/97	fenbutatin (AL)

Legends

(T) = Thrips (*Frankliniella occidentalis)*
(WF) = Whitefly (*Trialeurodes vaporariorum)*
(L) = *Levellulla taurica*
(LM) = Leaf Miners (*Liriomyza trifolii; L. huidobrensis)*
(O) = Oidium (*Sphaerotheca fuliginea*)
(CA) = Caterpillars (Several species)
(AL) = *Aculops lycopersici*
(A) = Aphids

References

ALOMAR, O., ARNÓ, J., 1995. Programa de Control Integrado para tomates de invernadero en Cataluña. III Jornadas Agrícolas Ganaderas de Canarias. Cabildo Insular de Gran Canaria.

BIOBEST BILOGICAL SYSTEMS. Side effects of Pesticides on Beneficial Organisms. Westerlo, Belgium.

CARNERO, A., *et al.,* 1988. La lucha integrada, una nueva estrategia para combatir las plagas. Hojas divulgadoras. 12/88. Ministerio de Agricultura Pesca y Alimentación. Madrid

DEL TORO, O., MARTÍN, R., ORTEGA, P., RODRÍGUEZ, J. M., RODRÍGUEZ, R., 1996. Experiencia de aplicación de un esquema de Control Integrado en cultivo de tomates bajo malla. Cuadernos de Fitopatología. XIII, 49 : 42-45.

FERRARI, M., MARCON, E., MENTA, A., 1995. Lotta Biológica. Edagricole Edizione. Roma.

GARIJO, C., *et al.,* 1990. I Curso de Control Integrado en cultivos hortícolas bajo invernaderos (tomates y pepinos). European Training in Integrated Crop Protection. Consejeria de Agricultura y Pesca. Junta de Amdalucia.

GOULA, M., ALOMAR, O., 1994. Míridos (*Heteroptera, Miridae)* de interes en el control integrado de plagas del tomate. Guía para su identificación. Bol.. San. Veg. Plagas, 20 : 131-143.

GRUPO DE TRABAJO DE CULTIVOS HORTÍCOLAS. 1991. Plagas del tomate. Bases para el control integrado. Ministerio de Agricultura, Pesca y Alimentación. Madrid.

MALAIS, M., RAVESBERG, W. J., 1992. The Biology of Glasshouse pest and their natural enemies. Knowing and Recognizing. Koppert Biological Systems, Koppert B. V. Berkel en Rodenrijs, The Netherlands.

RAMAKERS, P., 1995. Control Integrado de plagas en invernaderos, en el Norte de Europa. Ponencia. III Jornadas Agrícolas Ganaderas de Canarias. Cabildo Insular de Gran Canaria.

UNIVERSITY OF CALIFORNIA. 1990. Integrated Pest Management for Tomatoes

II. Fungal and bacterial diseases
Maladies fongiques et bactériennes

INTEGRATED MANAGEMENT OF SOIL-BORNE DISEASES IN THE MEDITERRANEAN PROTECTED VEGETABLE CULTIVATION

Mohamed Besri
Institut Agronomique et Vétérinaire Hassan II,Rabat,Morocco

Summary

In the Mediterranean area, the intensification of vegetable protected cultivation has created new optimal conditions for the development of soil borne diseases. Soil borne diseases problems were relatively simple in the early years,but they increased in importance as intensive cultivation continued. In many vegetables growing areas,the water salt content is very high.This salinity increases the susceptibility of vegetable crops to many diseases such as *Fusarium* and *Verticillium* wilts. Crop rotation and greenhouse rotation are not effective, because the alternative crops are often susceptible to the same diseases.In addition,many weeds act as alternative hosts to many soil borne diseases. Other cultural practices such as nursery management, elimination of crop residues,sowing and planting time,choice of the resistant varieties and of the plant spacing etc... are very important as components of an Integrated soil-borne diseases management program,but are not always properly adopted. Farmers are using certified seeds.However,many laboratory seed health tests have shown that these seeds could be infected by various seed borne pathogens such as *Clavibacter michiganensis subsp. michiganensis* and *Fusarium wilts* . The attitude that pesticides are the universal panaceas is still prevailing among many vegetable growers. In many cases,inappropriate applications of chemicals for soil disinfection fail in controlling soil-borne pathogens.

To control the major soil-borne vegetables diseases, an Integrated Disease Management Program should be implemented at the various step of the tomato production.

Introduction

In the last thirty years, protected vegetable cultivation has known an important development in the Mediterranean countries (Agrios 1988,Van Alebeek and Van Lenteren 1990). However, the intensification of protected crops production has created new optimal conditions for the development of many diseases. Soil-borne disease problems were relatively simple in the early years, but they increased in importance as intensive cultivation continued (Besri 1989, Besri 1990, Van Alebeek and Van Lenteren 1990). To avoid these problems,an integrated soil-borne diseases management program (IDM) should be implemented in each mediterranean country and region (Besri 1989, Van Alebeek and Van Lenteren 1990). These IDM programs should integrate all the chemical and non-chemical control methods in a compatible way to maintain the pathogen populations at levels below the economic injury level.

Main protected vegetables soil-borne diseases

The major soil-borne diseases of some protected vegetables crops are reported in table 1 (Besri 1990, Jones *et al.* 1991, Zitter *et al* 1996)

Table 1. Major soil-borne diseases of some protected vegetables crops

Crop	Pathogens	Diseases
Melon and Cucumber	*Fusarium oxysporum* f.sp. *melonis*	Fusarium wilts
	Fusarium oxysporum f.sp. *cucumis*	
	Fusarium solani	Fusarium collar and root rot
		Damping off
	Pythium spp, *Fusarium* spp.,	
	Rhizoctonia solani	White rot
	Sclerotinia sclerotiorum	
	Verticillium dahliae	Verticillium wilt
	Pseudomonas syringae pv. *lacrymans*	Angular leaf spot
	Meloidogyne spp.	Root knot nematodes
	Orobanche	Broomrape
Tomato	*Didymella lycopersici*	Stem canker
	Fusarium oxysporum f.sp. *radicis*	Fusarium root and collar rot
	Fusarium oxysporum f. sp. *lycopersici*	Fusarium wilt
	Phytophthora infestans	Late blight
	Phytophthora parasitica	Phytophthora root rot
	Pythium spp, *Fusarium* spp, *Rhizoctonia* spp.	Damping off
	Sclerotinia sclerotiorum	Sclerotinia stem rot
	Verticillium dahliae	Verticillium wilt
	Clavibacter michiganensis subsp.michiganensis	Bacterial canker
	Pseudomonas syringae pv. *tomato*	Bacterial speck
	Meloidogyne spp	Root knot
	Orobanche spp.	Broomrape
Eggplant	*Phytophthora capsici*	Late blight
	Pythium spp,	Damping off
	Phytophthora spp, Fusarium spp,	
	Sclerotinia sclerotiorum	Sclerotinia stem rot
	Verticillium dahliae	Verticillium wilt
	Fusarium	Verticillium wilt
	Pseudomonas syringae pv.*tomato*	Fusarium wilt
	Xanthomonas campestris vesicatoria	Bacterial speck
		Bacterial spot
	Meloidogyne spp	Root knot
	Orobanche spp.	Broomrape

Non chemical control methods

A- Cultural practices

Cultural practices have been used throughout history to create agricultural systems that can effectively suppress soil-borne diseases and pests (Agrios 1988).

1- Crop rotation

Crop rotation is a very important component of IDM.Crop rotation generally improves soil structure,maintains soil fertility and minimizes the need for pesticide.However, because of the presence of long-lasting viable such as microsclerotia of *Verticillium dahliae*,sclerotia of *Sclerotinia sclerotiorum* and chlamydospores of pathogenic *F.oxysporum* , the ability of some vegetables pathogens to subsist as saprophytes in competition with the soil flora and fauna or the extremely wide host range, including weeds, of most of the vegetable pathogens such as *Verticillium,Rhizoctonia, Phytophthora, Meloidogyne*, may limit the use of crop rotation as a control strategy (Agrios 1988, Besri 1991, Fry 1982). Crop rotation needs also time to be effective and the crop is often rotated with non-cash crops (Besri 1989, Besri 1991b).In addition, land and water costs are considered very high in protected vegetable production areas to adopt crop rotation.

The small spectrum of the main vegetables (tomato,cucumber, melon, pepper, eggplant),all belonging to only two plant families (Solanaceae and Cucurbitaceae) favor the spread of diseases from one crop to another.Therefore, crop rotation and greenhouse rotation are not effective in the control of most of vegetable soil-borne diseases (Besri 1991c,Cavallaro 1985).

2- Sanitation

Sanitation includes all activities aiming the elimination or the reduction of the inoculum present in a plant or field,and preventing the spread of the pathogen (Agrios 1988).Thus plowing under or removal and proper disposal of infected plant debris that may harbor the pathogen reduce the amount of inoculum. Washing the soil off farm equipment before moving it from one field to another may also help to avoid spreading any pathogen present in the soil .Since weeds can be infected by the same pathogen as the primary host, it is important to control their growth (Agrios 1988, Besri 1989, Cavallaro 1985, Shlevin *et al* 1994).

3- Improvement of plant growing conditions

Some cultural practices improve the vigor of the plants and consequently increase their resistance to pathogen attacks.Thus proper fertilization,drainage of fields,proper spacing of plants, weed control etc...improve the growth of plants and may have a direct or indirect effect on the control of a particular disease (Agrios 1988, Besri 1989, Besri 1991b).

Optimal irrigation is necessary to improve plant vigor and to reduce excessive air humidity.Current techniques involve furrow and drip irrigation.Furrow irrigation may cause oxygen deficiencies in the root system,increasing thereby the risks of pathogens attack.It increases humidity and the water is not used with efficiency.Drip irrigation is becoming more and more widespread, because of the economy and of the water efficiency.In combination with plastic mulching,drip irrigation reduces air humidity.Drip irrigation is also used for fertilizer and in some farms, for pesticides application (Besri 1981a, Cavallaro 1985, Fry 1982).

Soil and water salinity increase the susceptibility of tomato plants to many diseases and particularly to *Fusarium* and *Verticillium* wilts.Resistant varieties become susceptible when the irrigation water has a high salt content.Reducing the water salt content decreases the incidence and severity of these two pathogens (Besri 1981a, Besri 1981b, Besri 1991b).

4- Creating conditions unfavorable to the pathogen

Good soil drainage reduces the activity of certain tomato pathogens i.e. *Phytophthora,Pythium* and nematodes and may result in significant disease control.Appropriate choice of fertilizers or soil amendments may also lead to changes in the soil pH which may influence unfavorably the development of the pathogen (Besri 1981a, Besri 1981b, Van Alebeek and Van Lenteren 1990).

5- Soil-less culture

Soil-less culture using artificial substrates such as rockwool, rocks, clay granules and flexible polyurethane foamblocks, is a promising method to control protected vegetables soil borne pathogens particularly when the soil inoculum density is high (Braun and Supkoff 1994, Quarles Daar 1996, Schonfield *et al.*1995). However, although soil-less media are usually pathogen-free, infestation of these media by plant pathogenic micro-organisms such as *Phytophthora, Pythium, Rhizoctonia, Fusarium,* may occur in greenhouse if proper sanitation procedure are not followed (Walker 1981, Zinnen 1988).Soil-less media could be used in combination with biocontrol agents to selectively control some pathogens (Cook and Baker 1983, Gamliel and Katan 1991, Katan 1996).

6- Plant grafting

Resistant rootstocks,such as KNVF types,provide excellent control of many tomato diseases and particularly *Fusarium oxysporum f.sp.lycopersici,F.oxysporum f.sp.radicis and Meloidogyne spp.* (Jones *et al* 1991, Quarles and Daar 1996, Zitter *et al* 1996).

Grafting is used with an excellent efficacy in the control of many pathogens of cucurbits such as *Fusarium oxysporum f.spp.cucumerinum, F.oxysporum f.sp.melonis, Verticillium dahliae* and *Phomopsis sclerotiodes*.The main resistant rootstocks used are *Cucurbita ficifolia* and *Benincasa carifera* (Zitter *et al* 1996).

When grafting susceptible scions to resistant rootstocks,the scion root-system must be severed before transplanting the grafted plants (Jones *et al* 1991).Resistance may not be expressed when resistant cultivars are grown in soil infested with both *Fusarium* and root-knot nematodes because of physiological changes in the root induced by nematodes (Jones *et al* 1991).

7- Organic amendments

Use composted soft wood and hard wood barks give reproducible control of many soil borne diseases such as damping off caused by *Pythium ultimum* under greenhouse conditions. Fowl manure decreases the incidence of *Fusarium* wilt (Jones *et al* 1991). The addition of chitin into the soil suppresses *Rhizoctonia solani* and additionally may reduce nematodes .Chitin amendments to soil are also known to increase soil populations of actinomycetes (Hoitink and Fahy 1986). Compost appears also to improve soil water holding capacity, infiltration, aeration,permeability,soil aggregation and micronutrient levels and support microbial activity (Gamliel *et al* 1989, Gamliel et Stapleton 1993a, Hoitink and Fahy 1986, Hoitink *et al.* 1991, Rodriguez-Kabana and Morgan-Jones ,1987)).

8- Biofumigation with allelopathic plants

Biofumigation is the amendment of soil with organic matter for the purpose of releasing toxic gazes from the decomposition of the amendment to control soil borne pests .This technic may be combined with solarization (Katan 1996).

Fusarium crown and root rot of tomato can be controlled by growing lettuce between two successive tomato crops in the greenhouse or by planting dandelion beside tomato.Chemicals in lettuce,dandelion and allied plants such as endive and chicory, interfere with the growth of *F.oxysporum f.sp.radicis-lycopersici* in the soil (Jarvis 1989, Jarvis and Thorpe 1980).The active chemicals in this case are phenolic compounds wich retard the growth of the pathogen.

Residues of cabbage and other brassicas decompose in the soil,releasing volatile,sulfur-containing isothiocyanates and ammonia.The chemicals are toxic to several pathogens including *R.solani,F.oxysporum* and *Meloidogyne* spp.The toxic effects are enhanced when these metabolites are confined by plastic tarp,as during soil solarization (Jarvis and Thorpe 1980, Jones *et al* 1991, Katan 1996, Tjamos *et al* 1992).

The african marigold (*Tagetes erecta)* and the french marigold (*tagetes patula*) are inhibitory to nematodes.Their use may reduce root lesioning nematodes,root knot nematodes and virus transmission (Jarvis 1989, Jarvis and Thorpe 1980).The toxic compound secreted by marigolds are sulfur-containing polythienyls(Jarvis 1989, Jarvis and Thorpe 1980).

Tilled residues of some brassicas and various compositae give off volatile chemicals such as isothiocyanate and phenethyl isothiocyanate,which have herbicidal and nematocidal properties (Gamliel *et al* 1989, Hoitink and Fahy 1986).

9- Pathogen free seeds and transplants

The use of certified seeds is recommended,although there is no absolute guarantee that such seeds are disease free (Besri 1977, Besri 1978). Many seed health testing have shown that even certified tomato or melon seeds may be infected respectively by *Clavibacter michiganensis subsp.michiganensis , Fusarium oxysporum f.sp.lycopersici* and *Fusarium oxysporum f.sp.*melonis. Seed infection decreases the efficiency of soil fumigation and could introduce new races of some pathogens such as *Fusarium* in the soil.In general,when infected seeds are used, the disease incidence is higher in fumigated soils than in non fumigated ones (Besri 1977,Besri 1978, Besri 1990).Therefore,seeds should be tested by an acceptable and reliable method before their use

The main seed borne diseases of vegetables are reported in table 2

Sterilized soil,potting mix,and pots should be used in green house operations to avoid infection by soil borne pathogens (Jones *et al* 1991, Van Alebeek and Van Lenteren 1990).

B- Biological methods

Biological control of vegetables diseases can be achieved by selecting and breeding plants for resistance to particular pathogens or by using other microorganisms that are either antagonistic to the pathogen or paratize the pathogen itself (Agrios 1988, Cook 1993, Cook and Baker 1983).

1- Resistant varieties

Host plant resistance may contribute to the solution of many soil-borne and air-borne pests.The use of resistant varieties is the cheapest, easiest, safest and most effective means of

controlling tomato diseases .However, and particularly for soil-borne pathogens, because of the availability of broad spectrum and effective fumigants such as Methyl Bromide for the control of soil borne pests,the need for host resistance diminished and plant breeders spent more time and effort into the improvement of yield and quality .Most of the high yielding tomato varieties used at the moment are susceptible to nematodes.No tomato variety is resistant to *Verticillium* race 2. Resistant commercial cultivars of tomato to *F.oxysporum f.sp.radicis , Pyrenochaeta lycopersici* and to *F.oxysporum f.sp.lycopersici* race 3 are not available .The rise of new races particularly of *Fusarium* and *Verticillium* is a threat to the vegetable (Besri *et al* 1984, Besri 1990, Besri 1991b, Van Alebeek and Van Lenteren 1990,Walker 19821). Resistant commercial cultivars of cucurbits to *Acremonium,* bacterial wilt,root-knot nematodes, charcoal rot and to some *Fusarium* races are not available .Some newly released hybrids show a high level of resistance to *Macrophomina* (Zitter *et al* 1996).

Table 2. Main seed borne diseases of vegetables

Crop	Pathogen	Disease
Tomato	*Alternaria solani*	Early blight
	Didymella lycopersici	Stem canker
	Fusarium oxysporum f.sp. *lycopersici*	Fusarium wilt
	Clavibacter michiganense	Bacterial canker
	Pseudomonas syringae p.v. *tomato*	Bacterial speck
Melon	*Fusarium oxysporum* f. sp. *melonis*	Fusarium wilt of melon
Cucumber	*Fusarium oxysporum* f. sp. *cucumis*	Fusarium wilt of cucumber
	Cucumber Mosaic Virus	CMV
	Pseudomonas syringae pv. *lacrymans*	Angular leaf spot
	Alternaria cucumerina	Alternaria blight
Pepper	*Alternaria solani*	Alternaria blight
	Fusarium oxysporum	Fusarium wilt
	Xanthomonas campestris pv. *vesicatoria*	Bacterial spot

2- Suppressive soils

Suppressive soils to several soil borne pathogens (*Fusarium oxysporum, Rhizoctonia solani, Pythium ultimum, Phytophthora spp,* etc..) have been reported in many agricultural regions of the world. Soil borne diseases develops well in some soils (conducive soils) while they develop less and cause much milder diseases in other soils (suppressive soils) (Cook and Baker 1983, Schneider 1992). Planting susceptible vegetables crops but high yielding cultivars to one or more pathogens in resistant soil may be used as a control method (Greenberger *et al* 1987, Katan 1996, Schneider 1992).

3- Bio-control agents.

Many antagonistic microorganisms to soil-borne pathogens (*Trichoderma harzianum, Bacillus penetrans, Gliocladium* spp.) are naturally present in crop soils and exert a certain degree of biological control over one or many soil-borne pathogens.Plant pathologists have been

attempting to increase the effectiveness of antagonists either by introducing new and larger populations of antagonists or by adding soil amendments that stimulate their growth and activity (Chang *et al* 1986, Cook 1993, Cook and Baker 1983, Locke *et al.* 1985, Shtienberg and Elad 1997, Tjamos *et al.* 1992).

Biological control methods have the potential to control diseases and increase crop yield without adverse effects to the environment.*Trichoderma,Penicillium,Fusarium,Pachybasidium, Bacillus subtilis* have been tried with some encouraging success to control *Fusarium wilts* when incorporated into planting material (Cook 1993). Unfortunately,while biological control is effective in the laboratory and in the greenhouse,neither have been successful in the field.Therefore,uptill now,this control method is not available and therefore can not be used as a control method to soil borne pathogens .

C- Physical soil disinfection

1- Solarization

a- Soil solarization

Soil solarization is a promising technique,which may have an important future in many mediterranean countries,particularly when the methyl bromide use will be stopped according to the Montreal protocol (Anonymous 1995).The advantages of soil solarization are that this technique is relatively cheap, effective, poses little health risks, and changes the soil micro-organism composition in such a way that production is improved for more than 3 years (Katan 1996).

Soil solarization is such a successful new appropriate technology that it spread to over 40 countries within 15 years of its introduction.Though initially used only in hot regions during the summer,technological advances are extending the range of solarization to cooler areas and cooler seasons (Garibaldi and Tamietti 1989).Many green house and nursery crops worldwide,now utilize solarization. There are numerous variations of traditional soil solarization: double layers of plastic to increase soil heating,use of old plastic tunnel cover,spray on biodegradable mulches ,addition of biomass amendments producing biofumigation (Ben-Yephet *et al* 1987, Calabretta *et al* 1991,Gamliel and Katan 1991, Garibaldi and Tamietti 1989, Greenberger *et al.*1987, Katan 1996)

Soil solarization controls many pathogens such as *Fusarium,Verticillium, Phytophthora, Pseudomonas,* and many weeds. However, many others such as *Meloidogyne* spp. are not controlled by this method (Grinstein and Hertzoni 1991,Katan 1996).

b- Agricultural material solarization

Solarization of tomato supports is a successful control method for some diseases such as *Didymella* stem canker. Solarization of tomato stakes could be achieved by storing these agricultural material in empty plastic greenhouses during the hot months of the year.This technic is applied with great success by many farmers in Morocco (Besri 1982a, Besri 1982b, Besri 1983,Besri 1991a).

In intensive greenhouse vegetable production, populations of soil borne pathogens accumulate in the container mixes. Solarization may be used both to disinfest new and to recycle used growth media (Gamliel *et al* 1989).

c- Space solarization

Intensive agriculture in greenhouse can result in a build up of plant pathogens such as *Fusarium oxysporum f.sp.radicis lycopersici* both in the soil and on greenhouse structures. In warmer areas,pathogens can be killed by closing a greenhouse in the off-season,and allowing sunlight to disinfest the greenhouse. In Morocco and many other mediterranean countries,space solarization is a summer IPM tactic used between tomato and other vegetables greenhouse crops.Closing the greenhouse during the period between crops raises air temperature to pathogen lethal temperatures (Shlevin *et al* 1994a, Shlevin *et al* 1994b).

2- Steam treatment

Steam at 80°-100° C control most tomato soil-borne diseases and pest weeds (Jones *et al* 1991,Quarles and Daar 1996,). However,steam is very expansive and needs a large quantity of water which is very difficult to obtain particularly in the mediterranean countries where water is lacking. Uptill now,this physical control method have never been used in North Africa and in the Middle East region.However,many new steaming systems have been recently developed by industrialized countries (Holland, Germany, USA) .These systems seems to be economic and use a smaller quantities of water (Quarles and Daar 1996).

Steam effectively controls most tomato soil borne pathogens and weeds.However,steam is very expensive (Agrios 1988, Jones *et al* 1991, Walker 1981) and the plant growth could be suppressed, possibly due to the release of toxic compounds (high level of ammonia,manganese and soluble salts) and/or killing of beneficial fungi such as the mycorrhizal fungi (Anonymous 1995, Quarles and Daar 1996).Soil steaming leaves as do most fumigants,a biological "vacuum" suitable for re-infestation by plant pathogens (Anonymous 1995, Agrios 1988, Quarles and Daar 1996).

IV- Chemical control

Many chemicals for soil disinfestation are available in the market .These chemicals and their uses are represented in table 3.

The most efficient chemical is the methyl bromide (MB).The other chemicals have never been as efficient as MB to control soil-borne pathogens (Klein 1996).In general, these chemicals do not have the penetration capacity and do not disperse as well as MB in the soil.Their efficiency depends also on proper application which includes thorough mixing with soil to desired depth (Munnecke and Gundy 1979).

These pesticides are also hazardous to human health and to the environment.They have the ability to leach through soil and contaminate the ground water (Braun and Supkoff 1994).

A- Fumigants

1- Methyl bromide

MB is widely used for protected vegetables crops soil disinfection.The utilization rate varies from 750 kg\ha and 1000 kg\ha (Klein 1996)

For soil fumigation,MB is used in combination with chloropicrin (respectively 98% and 2%).Chloropicrin is used as a detector because MB is an odorless gas (Klein 1996).

MB controls soil borne pathogens,insects,nematodes,bacteria and weeds.Preplant application of the fumigant permits the soil to be replanted within a short waiting period with the

Table 3. Examples of pesticides used for soil disinfection in protected cultivation

Pesticides	Pathogens
Aldicarb (TerniK) Caduzophos (Rugby) Carbofuran (Furadan) Chloropicrin * Dazomet (Basamid) ** 1,3 Dichloropropène (Telone)* Ethoprop (Mocap) Fenamiphos (Nemacur) Isazophos (miral) Metam sodium (Vapam) ** Methyl Bromide * Oxamyl (Vydate) Terbufos (Counter)	*Alternaria solani* *Didymella lycopersici* *Fusarium oxysporum* f.sp. *radicis* *Fusarium oxysporum* f. sp. *lycopersici* *Phytophthora infestans* *Phytophthora parasitica* *Pythium* spp, *Fusarium* spp, *Rhizoctonia* spp. *Sclerotinia sclerotiorum* *Verticillium dahliae* *Corynebacterium michiganensis* *Pseudomonas syringae* pv. *tomato* *Meloidogyne* spp

** : Fumigants (MITC generators)
* : Fumigants (non MITC generators)

same crop and on the same year.With MB soil fumigation,it is possible to have two tomato crops per year for export.The chemical is injected into the soil,which is covered by a plastic tarp to contain the pesticide.In the mediterranean conditions,in general, only the plant rows are fumigated.The tarps are then used as mulch for the plant and are removed only at the end of the season (Klein 1996).

As a broad spectrum biocide,MB sterilizes the soil and kills a full range of pests and pathogens as well as beneficial organisms. MB leaves a biological "vacuum" suitable for reinfestation by plant pathogens.When pathogens such as *Fusarium oxysporum f.sp.lycopersici,F.oxysporum f.sp.radicis lycopersici and Meloidogyne spp.* are introduced into the soil, the incidence of the diseases is higher in fumigated soil than in non fumigated one (Besri 1978, Besri 1989, Besri 1991).

MB is one of the most widely used fumigants in the world because it is relatively inexpensive and kills all organisms with which it comes into contact.However,MB is also powerful ozone depleter.This fumigant was listed in 1993 by the parties of the Montreal Protocol as an ozone-depleting compound (Anonymous 1995, Braun and Supkoff 1994, Schonfield *et al.* 1995).

Soil fumigation with Methyl bromide to control soil borne pathogens,nematodes,some bacteria and weeds is considered as one of the main factors for the success of protected crops production (Besri 1990,Klein 1996). The fumigant is applied by specialized companies or by the farmers themselves according to the country legislation. (Braun and Supkoff 1994, Clark *et al* 1994, Spreen *et al* 1995).

2- Chloropicrin

Chloropicrin (CP) is a very effective fungicide for the control of vegetables soil-borne fungi, but not for weed and nematodes control compared to MB. After application, the dispersion of CP into soil and evaporation from soil occurs much slower than MB.Therefore,a longer period for CP is required before planting to prevent damage due to phytotoxicity than BM. In many countries the product is used only as a warning agent for Methyl Bromide at the concentration of 2 % (Braun and Supkoff 1994, Quarles and Daar 1996, Schonfield *et al* 1995, Spreen *et al* 1995)

3- 1,3 Dichloropropene (Telone)

Telone is as efficacious as MB in controlling nematodes,but does not control fungi or insects.At high rates,1,3-D has some efficacy against few weeds.Telone has been found to contaminate ground water in some areas (Braun and Supkoff 1994, Clark *et al* 1994).

4- Dazomet (Basamid) and Metam-sodium (Vapam)

Dazomet (Basamid), Metam-sodium (Vapam),applied to moist soil,decompose to Methyl Isothiocyanate (MITC) which is the biocidal agent.These chemicals do not provide control of soil-borne pathogens as consistent and comparable as to MB.The conventional methods of applications of these chemicals do not allow a uniform distribution of the pesticides in the soil (Braun and Supkoff 1994, Clark *et al* 1994).

B- Non fumigants

The most important non-fumigant soil pesticides are: Aldicarb (Temik), Caduzophos (Rugby), Carbofuran (furadan), Ethothrop (Mocap), Fenamiphos (Nemacur), Isazophos (miral), Oxamyl (Vydate) and Terbufos (counter).

The efficacy of these compounds is not comparable to fumigants and particularly to MB for nematode control.They also not effectively control weeds and soil-borne fungi.Control of pests deeply in soil cannot be adequately controlled by these compounds.A loss of efficacy due to microbial degradation is reported for some of these pesticides (Carbofuran) (Braun and Supkoff 1994,Clark *et al*. 1994, Quarles and Daar 1996).

V- Conclusion

To control soil-borne diseases of protected vegetables crops ,an integrated disease management program based on the known control methods and on local research results should be implemented with farmers collaboration (Cavallaro 1985, Fry 1982, Van Alebeek and Van Lenteren 1990). The IDM program should integrates all the suitable technics and methods in a compatible way to maintain the soil pest populations at levels below the economic injury level.The IDM program should be implemented at the various steps of the crop production (Besri 1991b, Fry 1982, Van Alebeek and Van Lenteren 1990):

- Pest management of the land: choice of the field and measures to be taken to reduce the pathogen populations (soil resistance, soil preparation,organic amendments,rotations, sanitation, solarization,chemical disinfection...)
- Pest management in the seed beds: Resistant cultivars to be grown, seed and seedling quality ,plant grafting, sowing and planting dates, seeding and planting densities,....

- Pest management in the field: irrigation, fertilization, chemical control, weed control,drainage,roguing...

References

ANONYMOUS 1995. Montreal protocol on substances that deplete the ozone layer. Report of the methyl bromide technical options committee options

AGRIOS G.N. 1988. Plant Pathology,Academic Press,New.York,803 pp.

BEN-YEPHET Y.,STAPLETON J.J.,WAKEMAN R.J.and De VAY J.E.,1987. Comparative effects of soil solarization with single and double layers of polyethylene film on survival of *Fusarium oxysporum f.sp.vasinfectum.* Phytoparasitica,15,3,181-185

BESRI M. 1977. La phase séminale du *Fusarium oxysporum f. sp. lycopersici.* Travaux dédiés à G. Viennot-Bourgin. Société française de Phytopathologie, 416 pp., 19-25.

BESRI M. 1978. Phases de la transmission de *F. oxysporum f.sp. lycopersici* et de *V. dahliae* par les semences de quelques variétés de tomate. Phytopathol. Z, 93, 148-163.

BESRI M. 1981a. Qualité des sols et des eaux d'irrigation et manifestation des trachéomycoses de la tomate au Maroc. Phytopathologia Mediterranea, 20, 107-111.

BESRI M. 1981b. Influence de la salinité du sol et des eaux d'irrigation sur la population de *Fusarium oxysporum f. sp. lycopersici*. Phytopathologia mediterranea, 20, 101-106.

BESRI M. 1982a. Conservation de *Didymella lycopersici* dans les cultures de tomate par les tuteurs. Phytopath. Z. 105, 1-1O.

BESRI M. 1982b. Solar heating (solarization) of tomato supports for control of *Didymella lycopersici* Kleb. stem. canker. Phytopathology. 7, 939.

BESRI M. 1983. Lutte contre le chancre *Didymella lycopersici* de la tomate par chauffage solaire (solarization) des tuteurs. Phytopath. Z., 108, 333-340.

BESRI M., M. ZROURI et I. BEYE. 1984. Appartenance raciale et pathogénie comparée de quelques isolats de *Verticillium dahliae* (Kleb.) obtenus à partir de tomates résistantes au Maroc. Phytopathol. Z., 109, 289-294.

BESRI M. 1989. Integrated management of some tomato diseases in Morocco. Proceedings of the "89 integrated pest management in tropical and subtropical cropping systems", Bad Durkheim, R.F.A. (February 8-15), 275-280;

BESRI M. 1990. Maladies et ravageurs des cultures maraichéres sous abris plastiques au Maroc. Rapport FAO, 27 pp.

BESRI M. 1991a. Solarization of soil and agricultural materials for control of *Verticillium* wilt and *Didymella* stem canker in Morocco. In: Soil solarization, eds J. Katan and J.
E.Devay. CRS press, pp 237-243.

BESRI M. 1991b. Lutte integrée contre les maladies cryptogamiques de la tomate au Maroc.Proceedings of the working group "Integrated control in protected crops under mediterranean climate" Alassio, Italy, September 29-October 2,WPRS bulletin, 187-191

BESRI M. 1991c. La Verticilliose de la tomate cultivée sous abris plastique au Maroc. Acta horticulturae,287,355-360

BRAUN A.L. and SUPKOFF D.M.,1994. Options to methyl bromide for the control of soil borne diseases and pests in California with reference to Netherlands.Pest management analysis and planning program.Sacramento,California,52 pp.

CALABRETTA C.,COLOMBO A.,COSENTINO S., SCHILIRO E. and SORTINO O., 1991. Effects of soil solarization on larvae of *Meloidogyne* spp.in soil and roots of tomato"Novi F1"in cold greenhouses.Bull.SROP 14,5,164-171

CAVALLARO R. 1985. Integrated and biological control in protected crops. Proceedings of a meeting of the EC experts' group. Heraklion, 24-26 April 1985, A.A. Balkema, Rotterdam, 251 pp.

CHANG Y.C.,BAKER R.,KLEIFELD O. and CHET I.,1986.Inceased growth of plants in the presence of biological control agent *Trichoderma harzianum*. Plant Dis.,70,145-148

CLARK C.,LYONS G.,PETERSEN M.,SAUNDERS L.,STERN R.,TAN E., and SCHONFIELD A.,1994. Southern exposure.The phaseout of methyl bromide in developing countries.Pesticide Action Network,North American Regional Center,35 pp.

COOK R.J. 1993.Making greater use of introduced microorganisms for biological control of plant pathogens. Ann. Rev.Phytopath. 31,53-80

COOK R.J.and BAKER K.F.,1983. The nature and practice of biological control of plant pathogens. American Phytopathological Society,St Paul,MN,539 pp.

FRY W.E., 1982. Principles of plant disease management. Academic Press, New-York, 378 pp.

GAMLIEL A.,KATAN J.,CHEN Y.and GRINSTEIN A.,1989. Solarization for the recycling of container media.Acta Horticulturae,255,1781-1787.

GAMLIEL A. and KATAN J.,1991.Involvement of fluorescent *Pseudomonads* and other microorganisms in increased growth response of plants in solarized soils.Phytopathol.,81,494-502

GAMLIEL A. and STAPLETON J.J.,1993a. Effect of chicken compost or ammonium phosphate and solarization on pathogen control,rhizosphere microorganisms and lettuce growth.Plant Dis.,77,886-891

GAMLIEL A. and STAPLETON J.J.,1993b. Characterization of antifungal volatile compounds from solarized soil amendments with cabbage residues.Phytopathology,83,899-905.

GARIBALDI A. and TAMIETTI G.,1989.Solar heating:Recent results obtained in Northern Italy. Acta Horticulturae,255,125-129

GREENBERGER A.,YOGEV A. and KATAN J.,1987,Induced suppressiveness in solarized soils.Phytopathol.,77,1663-1667.

GRINSTEIN A. and HETZRONI A.,1991.The technology of soil solarization (Katan J. and De Vay J.E.,eds).CRC Press,Boca Raton,USA,159-170

HOITINK H.A.J.and FAHY P.C.,1986.Basis for the control of soil borne pathogens with composts.Ann.Rev.Phytopathol.,24,93-114.

HOITINK H.A.J,INBAR Y. and BOEHM M.J.,1991.Status of compost-amended potting mixes naturally suppressive to soil borne diseases of floricultural crops.Plant Disease,75,869-873.

JARVIS W.R.,1989. Allelopathic control of *Fusarium oxysporum f.sp.radicis lycopersici*.Pages 479-486 in C.H.Beckman and E.C.Tjamos eds.,Vascular wilt diseases in plants.NATO ASI,Springer-Verlag,Heidelberg.590 pp

JARVIS W.R. and THORPE H.J., 1980.Control of Fusarium foot rot and root rot by soil amendments with lettuce residues.Can.J.Plant Pathol.3,159-162

JONES J.B., JONES J.P.,STALL R.E. and ZITTER T.A., 1991. Compendium of Tomato Diseases.APS Press.The American Phytopathological Society,St Paul,Minnesota,73pp.

KATAN J.,1996. Soil solarization:Integrated control aspects. In: Principles and practice of managing soilborne plant pathogens. R.Hall,eds.,APS Press,250-278

KLEIN L.,1996.Methyl Bromide as a soil fumigant.In Bell H., Price N. and Chakrabarti B.: The Methyl Bromide issue, John Wiley & Sons Ltd, 191-235

LOCKE J.C.,MAROIS J.J.,and PAPAVIZAS G.C.,1985. Biological control of Fusarium wilt of greenhouse grown chrysanthemums.PLant Dis.,69,167-169

MUNNECKE D.E. and GUNDY S.D.V.,1979. Movement of fumigants in soil,dosage responses,and differential effects.Annual Review of Phytopathology,17,405-429

QUARLES W. and DAAR S.,1996.IMP alternatives to Methyl bromide.A compilation of articles from the IPM Practitioner, Bio-Integral Resources Center,Berkeley,USA

RODRIGUEZ-KABANA R. and MORGAN-JONES G.,1987.Biological control of nematodes:Soil amendments and microbial antagonists.Plant and Soil,100,237-247

SCHNEIDER R.W.,1992. Suppressive soils and plant diseases. APS,St Paul,MN,88 pp.

SCHONFIELD A.,WAMUKONYA L. and GLENDENING S.1995.Under African Skies.Methyl bromide use and alternatives in sub-saharan Africa.Pesticide Action Network,North American Regional Center,21 pp.

SHLEVIN E.,KATAN J;,MAHRER Y.,and KRITZMAN G., 1994a. Sanitation of inocula plant pathogens in the greenhouse structure by space solarization,Phytoparasitica,22,1,156

SHLEVIN E.,KATAN J.,MAHRER Y.,and KRITZMAN G.1994b. Space solarization of the greenhouse structure for the elimination of surviving soil borne pathogens.Phytoparasitica,22,1,82

SHTIENBERG D. and ELAD Y.,1997. Incorporation of weather forecasting in integrated,biological-chemical management of *Botrytis cinerea*, Phytopathology,87,332-340

SPREEN T.H.,VAN SICKLE J.J.,MOSELEY A.E.,DEEPAK M.S,and MATHERS L.,1995. Use of methyl bromide and the economic impact of its ban on the Florida fresh fruit and vegetable industry.University of Florida,Agricultural Experimental Station,Bulletin 898,204 pp.

TJAMOS E.S.,PAPAVIZAS G.C.and COOK R.J.,eds,1992.Biological control of plant diseases,Plenum Press,NY,462 pp.

VAN ALEBEEK F.A.N. and VAN LENTEREN J.C., 1990. Integrated pest management for vegetables grown under protected cultivation in the Near East. FAO, part I and II.

WALKER J.C.,1981. Fusarium wilt of tomato. Am.Phytopathol. Soc. Monogr., 6, 56 pp.

ZINNEN T.M.,1988.Assessment of plant diseases in hydroponic culture. Plant Disease,72,96-99

ZITTER T.A., HOPKINS D.L. and THOMAS C.E., 1996. Compendium of Cucurbit diseases. The American Phytopathological Society,APS Press,St Paul,Minnesota,87 pp.

LUTTE BIOLOGIQUE CONTRE *Fusarium oxysporum* f. sp. *radicis-lycopersici* CHEZ LA TOMATE EN SERRE NON CHAUFFÉE.

V.A.Bourbos[1],G. Michalopoulos[2] et M. T. Skoudridakis[3]

1.Fondation Nationale de Recherche Agricole.Institut des Plantes Subtropicales et de l'Olivier.Laboratoire de Parhologie Végétale,Agrokipio,Chania ,Crète, GRECE.
2.ZENECA HELLAS SA,Syngrou 231,17121 Athènes,GRECE.
3.Direction d' Agriculture de Chania,Sphakion 26,73100,Chania, Crète,GRECE.

RÉSUMÉ

Fusarium oxysporum f.sp.*radicis-lycopersici* Jarvis et Thorpe provoque des dégâts importants chez la tomate en serre. Dans ce travail on a essayé d' étudier la possibilité de lutter biologiquement contre le dit pathogène en utilisant le produit connu sous le nom commercial Promot. Ce produit qui contient les antagonistes *Trichoderma harzianum* Rifai et *T.koningii* Oudem a été appliqué à la dose de 0.15 g/m^2.

Les essais ont été effectués pendant deux années consécutives dans des serres en plastique et non chauffées où le potentiel de la maladie était élevé. On a utilisé le cultivar de tomate Early pack no 7 et comme produit de référence le fongicide quintozene (46.8%) + etridiazol (11.5%) commercialisé sous le nom Terraclor super X à la dose de 35 g /hl. Les deux produits ont été appliqués en arrossage et en pulverisation de la surface du sòl juste ,1,3 et 4 mois après la transplantation.

Dans les parcelles expérimentales des produits utilisés on a constaté une diminution sans différence significative de la présence quantitative du pathogène de l' ordre de 85.8-86.7%. Chez les parcelles-témoin a été, au contraire, observée une augmentation d' inocculum de 11.1%. Pour les deux produits utilisés le taux de brunissement de la surface de la racine principale et de plantes avec des symptômes sur le collet est arrivé a 3.8 et 4.3 % pour le produit biologique et 2.8 et 6.2% pour le produit de référence, tandis que chez le témoin a 61.4 et 65.7%. La production par plante dans le cas du produit biologique était la plus élevée (7.269-7.295 Kg).

INTRODUCTION

La pourriture du système racinaire et la nécrose du collet connue en aglais comme Grown Root Rot et due au *Fusarium oxysporum* f. sp.*radicis-lycopersici* peut sous certaines conditions provoquer des dégâts sévères dans les regions serricoles de tomate en Grèce. Cette maladie se manifeste par une chlorose des plantes a la récolte des premiers boutons et en principe par des nombreuses nécroses brunes sur le système racinaire secondaitre, la racine principale et le collet. Une obstruction gommeuse des vaisseaux extendue à une longueur de 30 cm maximum du niveau du sòl, peut très souvent être présente.

L' agent pathogène a été décrit pour la première fois par Jarvis et Shoemanker en 1978 (Jarvis et Shoemaker, 1978). Il attaque la plupart des cultivars de tomate et surtout Early Pack (Daskalaki *et al.*, 1992). L' aubergine et le poivron (Yamamoto *et al.*, 1974). Il peut également heberger l' arachide (Rowe, 1978). En Grèce la maladie a été signalée pour la première fois en Crète dans les serres de la région de Rethymnon en 1980 chez les cultivars GC 204 et Dombo (Malathrakis *et al.*, 1980; Malathrakis, 1985) et plus tard en 1981 à la Canée chez le GC204 (Bourbos, 1983).

La lutte contre ce pathogène a fait l'objet des nombreuses études dans le monde entier. Elle est orientée vers les tecniques culturales (Bourbos et Skoudridakis, 1989), des mesures prophylactiques et des méthodes chimiques (Jarvis, 1988) et biologiques.

Dans le domaine du contrôle biologique des mélanges d' antagonistes, des engrais verts, des cultivars résistants ou tolerants et la solarisation du sòl ont été fréquement utilisés. Les antagonistes *Trichoderma harzianum* (Sivan *et al.*, 1987), *Fusarium oxysporum* saprophyte (Louter et Edgington; 1985), *Glomus intraradices* en mycorrhization (Caron *et al*, 1986), *Aspergillus ochraceus+ Penicillium funiculosum+ Trichoderma harzianum* (Marois et Mitchell, 1981), *Aspergillus alutaceus+ Penicillium chrysogenum+ P.funiculosum+ Trichoderma harzianum* (Vourdoubas *et al.*, 1992), donnent des résultats satisfaisants. Les engrais verts de laitue diminuent rémarquablement le potentiel infectieux du pathogène (Jarvis et Thorpe, 1981). La solarisation du sòl appliquée seule ou avec des biorégulateurs de la microflore tellurique, des matières organiques et des antagonistes contrôlent efficacement la maladie (Bourbos et Skoudridakis, 1993). Des cultivars résistants ou tolerants au pathogène destinés à la culture en serre ou en plein air ont été actuellement créés et utilisés en pratique agricole (Thiboteau, 1981; Brisson *et al.*, 1985).

MATERIEL ET METHODES

Les essais ont été effectués pendant deux périodes culturales consecutives (1994-1995 et 1995-1996) dans les serres en plastique et non chauffées où le potentiel de la maladie était très élevé.

On a utilisé le cultivar de tomate Early Pack No 7. très sensible au pathogène.

Le produit biologique testé, connu sous le nom commercial Promot, contient les antagonistes *Trichoderma harzianum* et *T. koningii* . Il a été appliqué à la dose de 0.15 g /m^2.

Comme produit de référence on a utilisé le fongicide quintozene (46.8%) + etridiazol (11.5%) (nom commercial Terraclor super X) à la dose de 35 g /hl.

Les deux produits ont été appliqués par arrossage et par pulvérisation de la surface du sòl juste 1, 3 et 4 mois après la transplantation.

Le protocol experimental comprenait en 5 répétitions les traitements: Témoin, produit biologique et produit de référence. La surface de chaque parcelle- serre était 25 m^2 .

L' estimation de l 'efficacité des produits utilisés a été basée sur l analyse de la présence quantitative du pathogène dans la rhizosphère avant les traitements et après la récolte et sur le calcul, après la récolte, du taux de brunissement de la surface de la racine principale et du nombre de plantes avec des symptômes.

RÉSULTATS

Dans les parcelles témoin on a observé une augmentation de l' inocculum rhizospférique du pathogène pendant les deux périodes culturales de l' ordre de 11.11%. Au contraire, chez les parcelles -serres traitées par le produit biologique et chimique on a constaté une forte diminution de la présence quantitative du pathogène, sans différence statistique, arrivant à 85.00 -86.67% et 86.67-88.00% respectivement (Tableau 1).

Dans les cas de deux produits utilisés le taux de brunissement de la surface de la racine proncipale a varié du 3.82 au 4.33% pour le produit biologique et du 2.67 au 2.85 % pour le produit chimique , tandis que chez le témoin du 61.31au 61.46% . Le pourcentage de plantes avec des symptômes au collet était de 4.2- 4.,33% (produit biologique), de 6.17-6.26 (produit chimique) et de 65.40- 65.96 (témoin) (Tableau 2).

Traitements	Colonies par g X100			
	Période culturale 1994-1995		Période culturale 1995-1996	
	Avant transplantation	Après récolte	Avant transplantation	Après récolte
Témoin	0.40	0.50 a	0.50	0.50 a
Produit biologique (à base de *Trichoderma harzianum* et *T.koningii*)	0.30	0.04 b	0.40	0.06 b
Quintozène (46.80%) + etridiazol (11.50%)	0.30	0.04 b	0.50	0.06 b

Tableau 1.Présence quantitative du *Fusarium oxysporum* f.sp. *radicis-lycopersici* dans la rhizosphère de tomate en serre. Les chiffres avec la même lettre ne différent pas statistiquement (P=0.05).

Traitements	Période culturale 1994-1995		Période culturale 1995-1996	
	% Surface de racine principa- le avec brunissement	% Plantes avec des symptômes	% Surface de racine principa- le avec brunissement	%Plantes avec des symptômes
Témoin	61.31 a	65.40 a	61.46 a	65.96 a
Produit biologique (à base de *Trichoderma harzianum* et *T.koningii*)	3.82 b	4.33 b	3.81 b	4.20 b
Quintozène (46.80%) + etridiazol (11.50%)	2.85 b	6.26 b	2.67 b	6.17 b

Tableau 2. Taux de brunissement de la surface de racine principale et de plantes avec des symptômes au collet, après la récolte. Les chiffres avec la même lettre ne différent pas statistiquement (P=0.05).

La production des parcelles serres traitées par le produit biologique était la plus élevée arrivant à 7.295 Kg par plante pour la période 1994-1995 et à 7.269 en 1995-1996.

Traitements	Période culturale 1994-1995	Période culturale 1995-1996
	Production (kg/plante)	
Témoin	4.731 a	4.689 a
Produit biologique (a base de *Trichoderma harzianum* et *T.koningii*)	7.295 b	7.269 b
Quintozene (46.80%) + etridiazol (11.50%)	7.269 b	7.245 b

Table 3. Action des traitements sur la production totale de la tomate. Les chiffres avec la même lettre ne diffèrent pas statistiquement (P=0.05).

DISCUSSION

Il est bien connu que les champignons du genre *Trichoderma* ont été fréquement utilisés avec une réussite interessante pour lutter contre les divers pathogènes fongiques au niveau du sòl (Chet,1987). Parmi ces pathogènes ont été décrits aussi des différents espèces du genre *Fusarium* (Wang *et al.*,1996). Les antagonistes *Trichoderma* spp.exercent une action en même temps competitive pour le milieu de la croissance, mycoparasitique et stimulatrice pour le développement des plantes (Windham *et al.*, 1986; Sivan et Chet, 1987).

La pourriture des racines et la nécrose du collet est une maladie très difficile à contrôler. Même la désinfection du sòl par les bioctones ou par la vapeur est inefficace à cause de la récolonisation rapide du sòl désinfecté par le pathogène.

D'après nos résultats, le produit biologique testé a pu contrôler, sous les conditions de ces essais, très efficacement le pathogène *Fusarium oxysporum* f.sp.*radicis lycopersici* responsable de la pourriture des racines et de la nécrose du collet chez la tomate en serre. Il a démontré la même efficacité que le produit chimique de référence. En plus, les parcelles-serres traitées par le produit biologique ont donné une production très élevée.

On peut alors conclure que ce produit pourrait jouer un rôle important dans les programmes de phytoprotection de la tomate en serre vis à vis de ce pathogène. Il serrait aussi nécessaire d' étudier la possibilité d'utilisation de ce produit en combinaison avec la solarisation du sòl , puisque cette méthode s' applique actuellement très souvant dans la pratique pour lutter contre d'autres pathogènes telluriques de la tomate.

Réferences

BOURBOS, V.A.,1983.Contribution à l' étude de la mycoflore du sòl en culture de tomate en serre non chauffée.Thèse Ing.Doct.Paris VI,108 p.

BOURBOS,V.A., et SKOUDRIDAKIS,M.T.,1989.(En grec).Ennemis et maladies de la tomate en serre.Ed.Ekdotiki agrotechniki,vol.I,160 p.

BOURBOS,V.A. et SKOUDRIDAKIS,M.T., 1993. Lutte contre *Fusarium oxysporum* f.sp.*radicis- lycopersici* par l'utilisation de la solarisation du sol et des champignons antagonistes.3ème Congrés de la Soc.Fran.Phytop.,Dijon,France.

BOURBOS,V.A. et SKOUDRIDAKIS,M.T.,1993.Lutte contre *Fusarium oxysporum* f.sp. *radicis-lycopersici* par l' utilisation de la solarisation du sol et de la chitine.6th Intern.Congress of Plant Pathology, Montreal, Canada, 275.

BRISSON,J.D., DOUCET,R., et THIBODEAU,P.O., 1987. Evaluation de croisements de tomates de serre a fruits roses en regard de la résistance au ToMV et au Fusarium. Research Report Canadian Horticultural Council,154.

CARON,M. RICHARD,C., et FORTIN,A.,1986. Effect of preinfestation of the soil by a vesicular-arbuscular mycorrhizal fungus,*Glomus intraracides*,on Fusarium crown and root rot of tomatoes.Phytoprotection,**67**:15-19.

CHET,I.,1987.*Trichoderma* -application,mode of action and potential as biocontrol agent of soilborn plant pathogenic fungi.In Innovative Approaches of Plant Diseases Control.Ed.Wiley J. and sons,New york,137-160.

DASKALAKI, A.,BOURBOS,V.A., et SKOUDRIDAKIS,M.T.,1992.Behaviour of some greenhouse tomato hybrids to the fungus *Fusarium oxysporum* f.sp.*radicis-lycopersici*. 6th Panellinic Phytopathological congress,Patra,Greece,28.

JARVIS,W.R.,1988.Fusarium crown and root rot of tomatooes.Phytoprotection,**69**:49-64.

JARVIS, W.R. et SHOEMAKER R.A.,1978. Taxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato.Phytopathology,**68**: 1679-1680.

JARVIS,W.R., et THORPE,H.J.,1981.Control of Fusarium foot and root rot of tomato by soil amendement with lettuce residues.Can.Plant Pathol.,**3**:159-162.

LOUTER, J.H.,et EDGINGTON,1985.Cross protection of tomatoes against Fusarium crown and root rot.Can.J.Plant Pathol.,**7**:445-446.

MALATHRAKIS,N.E., XAPHIS, C.T., et AYGELIS;A.D.,1980.Tomato diseases in Crete.Phytopthol.Mediterr.,**19**:71.

MALATHRAKIS, N.E., 1985.Tomato crown and root rotcaused by *Fusarium oxysporum* f.sp. *radicis-lycopersici*.Inf.Fitopatol.,**11**:59-61.

MAROIS,J.J.,et MITCHELL,D.J.,1981.Effects of fungal communities on the pathogenic and saprophytic activities of *Fusarium oxysporum* f.sp.*radicis-lycopersici*.Phytopathology, **71**:1251-1256.

ROWE, R.C.,1978.Comparative pathogenicity of *Fusarium oxysporum* isolates causing crown and root rot of greenhouse and winter-grown tomatoes.Phytopathology News,**12**:222.

SIVAN,A. et CHET,I.1987.Possible mechanisms for control of *Fusarium* spp.by *Trichoderma harzianum*.Brit.Crop ProtectionConference-Pests and Diseases,2:865-872.

SIVAN,A.UCKO,O.,etCHET,T.,1987.Biological control of Fusarium crown and root rot by *Trichoderma harzianum* under field conditions.Plant Diseases,**71**:587-592.

THIBODEAU,P.O.,1980.Evaluation de la résistance de cultivars de tomate de serre a la purriture fusarienne des racines et du collet.Research Report Canadian Horticultural Council,165.

VOURDOUBAS,P.,BOURBOS, V.A., et SKOUDRIDAKIS,M.T.,1992.Control possibility of *Fusarium oxysporum* f.sp. *radicis-lycopersici* by using antagonistic fungi. 6th Panellinic Phytopathological congress,Patra,Greece,45.

WANG,W.,ZHAO,Q. et YANG W.1996.Antagonism of Trichoderma viride T2 against soil-borne *Fusarium* pathogens.In Advances in Biological Control of Plant Diseases.Ed.China Agricultural University Press,Beijing,China,113-115.

WINDHAM,M.T.,ELAD,Y. et BAKER R.,1986.A mecanism for increased plant growth induced by *Trichoderma* spp.Phytopthology,76:518521

YAMAMOTO,I.,KOMADA,H. , KUNIYASY, K.,SAITO,M.,et EZUCA,A.,1974.A new race of *Fusarium oxysporum* f.sp. *radicis- lycopersici* inducing root rot of tomato.Proc.Kansai Plant Prot. Soc.(Japan),**16**:17-29.

BIOLOGICAL CONTROL OF FUSARIUM WILT OF TOMATO

A. De Cal, S. Pascual, R. García-Lepe, and P. Melgarejo
Dept. de Protección Vegetal. CIT-INIA, Crtra. de La Coruña km. 7. 28040 Madrid. SPAIN

Summary

Penicillium oxalicum reduced disease induced by *Fusarium oxysporum* f.sp. *lycopersici* on different cultivars of tomato, both in hydroponic and soil systems, with different kinds of inocula. In both, hydroponic and soil systems, treatments with PO did not result in a reduction of FOL propagules in the rizosphere of tomato. *P. oxalicum* colonized tomato rhizosphere along the experiments but it was not detected inside stems, demonstrating that *P. oxalicum* and *F. oxysporum* f.sp. *lycopersici* remained spatially separated. These results suggest the ability of *P. oxalicum* to trigger defence mechanisms in the plant. Biological control was observed both in sensitive and "resistant" cultivars, indicating the role of a general resistance mechanism. Therefore *P. oxalicum* can also be a suitable biocontrol agent for cases of cultivar resistance failure.

Introduction

Fusarium oxysporum f. sp. *lycopersici* (Sacc.) Snyder & H.N. Hansen (FOL) causes vascular wilt of tomato. Both field and glasshouse crops are affected and losses may be severe unless control is effective (IMI Description). Disease control measures combine resistant cultivars, soil fumigation, avoidance of infected seed or planting material, careful management of soil condition and crop rotation (Besri *et al*, 1984). Resistant cultivars is the most important means of control (Beckman, 1987) but new pathogenic races may arise that overcome plant resistance. Soil fumigation reduces partially wilt incidence where applications are allowed by economic and legislative restrictions, which are increasing now because of the ban of methyl bromide applications.

Biological control can be an alternative to use in a disease management programme. Different biocontrol agents have been reported to control *Fusarium* wilt of tomato such as fungi (Phillips *et al*., 1967; Tamietti *et al*., 1993) or bacteria (Fuchs and Défago, 1991; Lemanceau and Alabouvette, 1993). In a previous study, we screened some fungal antagonists to FOL and we obtained a *Penicillium oxalicum* Thom (PO) strain that reduce *Fusarium* wilt of tomato (De Cal *et al*, 1995). We reported here the control of FOL obtained in several tomato cultivars grown both in hydroponic culture in growth chambers and in soil systems in the greenhouse.

Materials and Methods

Cultures

An isolate of *Fusarium oxysporum* f. sp. *lycopersici* race 2 (FOL) was kindly provided by Dr. Tello (Universidad de Almeria, Spain). It was originally isolated from tomato in southern Spain. The FOL culture was stored in sterile sand tubes at 4°C and grown on Czapek-Dox agar (CDA) (Difco) in darkness at 25 °C for mycelial production.

To obtain microconidial inoculum of FOL, flasks (250 ml) containing 150 ml sterile Czapek-Dox broth (Difco) were inoculated with three mycelial plugs of FOL from 7-day-old cultures on CDA as described by De Cal *et al.* (1995). To obtain chlamydospores of FOL for inoculum the method described by De Cal *et al* (in press) was used. Three 0.5 mm diam. mycelial plugs from 7-day old FOL cultures in Czapek-Dox agar (Difco) were used to inoculate 250 ml erlenmeyer flasks containing 150 ml of Czapek-Dox broth. After 5 days of incubation in an

orbital shaker (150 rpm and 20-25 °C), the culture was filtered through sterile glass wool and the suspension was diluted to 10^6 microconidia/ ml. Sixty ml of the conidial suspension were poured on pots (22 x 22 x 9 cm) containing a sterile mixture of soil:peat:sand (1:1:1, v:v:v) and mixed throughoutly. Pots were maintained in a growth chamber with 16 h photoperiod at 25-21 °C (lightness-darkness) until use.

The isolate of *P. oxalicum* (PO) was kindly provided by Dr. F. Reyes (Consejo Superior de Investigaciones Científicas, Spain). PO was stored in PDA slants at 4°C and grown in PDA plates in darkness at 20-25 °C. Conidia from 7-day-old PDA colonies were gently dislodged in water + 0.1 % Tween 80, and the conidial suspension was adjusted to 10^7 conidia ml^{-1}.

Plant material

The tomato cultivars used were: Super Roma, Precodor, VFN, Novy, Lorena and Ramon. Super Roma, Precodor and VFN are susceptible to race 1 and 2 of FOL. Novy and Lorena are susceptible to race 2 of FOL but resistant to race 1. Ramon is resistant to both races of FOL. Tomato seeds were sown in trays containing an autoclaved mixture of vermiculite-peat (1:1, v:v) and maintained in a chamber at 22-28°C with fluorescent light (100mE $m^{-2}s^{-1}$, 16 h photoperiod) and 80-100% humidity for about 3 weeks. Tomato seedlings in seedbeds were treated 7 days before transplanting with a conidial suspension of PO (10^7 conidia ml^{-1} in sterile water + 0.1 % Tween 80. The seedbeds were watered with 60 ml of the conidial suspension per litre of substrate.

Experiments in hydroponic systems

An hydroponic system described elsewhere (De Cal *et al.*, in press) was used. Super Roma, VFN, Precodor and Ramon seedlings from seedbeds with two true leaves were transplanted into 100 ml flasks containing 125 sterile Hoagland nº 2 solution (Hoagland and Arnon, 1950). Seedlings were held by a sterile screen attached to the flask neck. Flasks were infested with a microconidial suspension of FOL (10^4 microconidia ml^{-1}) in water just before transplant. Control treatments were: i) plants inoculated only with FOL and untreated with PO, ii) plants uninoculated with FOL but treated with PO, and iii) plants uninoculated with FOL and untreated with PO. Five replicate flasks, each containing four plants, were used per treatment and cultivar. The flasks were placed in a randomized block design in a growth chamber at 22-28 °C with fluorescent light (100mE $m^{-2}s^{-1}$, 16 h photoperiod) and 80-100% humidity for about 3 weeks. When the initial nutrient was consumed in any flask (always in those containing uninoculated plants, usually at 8 days after transplanting) fresh solution was added to all flasks to give a final volume of 100 ml and the consumption of solution per flask was recorded. This proccess was repeated each time that any flask became emptied of solution, usually every 3-5 days. Disease severity induced by FOL was assessed each time that the flasks were replenished using the disease index scale described by De Cal *et al.* (1995): 1 healthy plants; 2 lower leaves yellow; 3 lower leaves dead and upper leaves yellow; 4 lower leaves dead and upper leaves wilted; 5 dead plant. Number of leaves, weight of root and aerial parts, populations of FOL and PO in the rhizosphere (estimated as colony forming units g^{-1} of fresh root weight (cfu), as described in De Cal *et al.* (1995) were recordered per planta at the end of the experiment. Reduction in plant size and solution consumption were calculated at the end of the experiment as a percentage by comparison with untreated and uninoculated plants.

Experiments in soil systems

Experiment 1: Novy and Ramon seedlings with 2-4 leaves were transplanted into pots (20x20x9 cm, four plants per pot) containing sterile peat and placed in the greenhouse. Plants were inoculated with the pathogen 7 days after transplanting. A longitudinal wound in the stem, 2-

3 cm above the soil surface, approximately 0.5 cm in length was made with a scalpel. A CDA block (0.5 cm diameter) containing a 7-day-old culture of FOL was placed in each wound. The inoculation site of stem was then wrapped in moist cotton wool and aluminum foil, which were removed after 5-7 days. Control treatments were plants inoculated with FOL and untreated with PO, plants uninoculated with FOL and treated with PO and plants uninoculated with FOL and untreated with PO. Each treatment was applied to both cultivars in a randomized block design with 10 blocks and four plants per treatment and block.

Rhizosphere population of PO was determined 24 h and 60 days after transplanting (estimated as cfu g^{-1} fresh root weight) as described in De Cal *et al* (1995). The extent of FOL and PO colonization of stems was determined at the end of each experiment by cutting stems into 5-cm-length pieces, and plating on, respectively, selective media for FOL and PO (De Cal *et al.* 1997).

Severity of disease was recordered 20, 30, 40, 50 and 69 days after transplanting. Disease severity was estimated by using the disease index scale described above (De Cal *et al.* 1995). All the plants were placed in humid chambers at the end of the experiment and the presence/absence of the pathogen in the crown was recorded after 48 h incubation. The complete experiment was repeated twice.

Experiment 2: Lorena seedlings with 2-4 leaves were transplanted into pots (20x20x9 cm, four plants per pot) containing sterile peat or peat infested with chlamydospores as described in De Cal *et al*, in press) and placed in the greenhouse. Control treatments were plants inoculated with FOL and untreated with PO and plants uninoculated with FOL and untreated with PO. Pots were placed in a glasshouse at 15-30 °C in a randomised block design with 10 blocks and four plants per treatment and block.

Disease severity were assessed 60 days after transplanting. Disease severity was evaluated using the disease index scale described above (De Cal *et al.*, 1995) and the presence/absence of the pathogen in the crown was recorded. The number of plants showing stunting (size smaller than 90% size of control plants) was also determined.

Rhizosphere population of FOL was determined 60 days after transplanting as cfu g^{-1} fresh root weight as described in De Cal *et al* (1995).

Statistical analysis

Population density data were subjected to log transformation. Disease severity and percentage of reduction in plant size and solution consumption were converted to ln (1/(1-x)) or arcsin. All data were then analyzed using analysis of variance (Sokal & Rolhf, 1981). When F test was significant at P=0.05, means were compared by Least Significant Difference (LSD) test. Data on incidence and isolation of the pathogen from crown were analyzed by a chi square test (P=0.05).

Results

Experiments in hydroponic systems

All susceptible cultivars inoculated with FOL showed old leaves drooped and curved downwards, and some of them turned yellow. The plants frequently wilted and died. Stunting was evident as a decrease in the number of leaves and weight of the aerial part. The cultivars Super Roma, and Precodor were the most susceptible to FOL. Ramon plants showed no symptoms.

In relation to the different parameters tested, FOL reduced the solution consumption (Table 1) as well as the size and weight of aerial parts and roots in inoculated plants. When FOL-inoculated Precodor plants were treated with PO the reduction in solution consumption and the

size and weight of plants was significantly decreased (P=0.05). A decrease in the reduction of the solution comsumption was recorded also for PO-treated Ramon plants (Table 1).

Cultivar	Treatment*	Reduction of solution consumption#(%)	Disease severity+(%)
Precodor	- / -	18.5 (0.21)	0.0 (0.00)
	- / FOL	76.3 (1.46)	45.0 (0.60)
	PO / FOL	21.6 (0.25)	6.2 (0.06)
	PO / -	0.0 (0.00)	0.0 (0.00)
	LSD (P=0.05)	(0.22)	
Ramon	- / -	9.3 (0.10)	0.0 (0.00)
	- / FOL	20.6 (0.23)	0.0 (0.00)
	PO / FOL	5.7 (0.07)	0.0 (0.00)
	PO / -	0.0 (0.00)	
	LSD (P=0.05)	(0.05)	
VFN	- / -	0.0 (0.00)	0.0 (0.00)
	- / FOL	35.7 (0.49)	32.8 (0.40)
	PO / FOL	36.3 (0.46)	15.6 (0.17)
	PO / -	0.0 (0.00)	0.0 (0.00)
	LSD (p=0.05)	(0.38)	
Super Roma	- / -	0.0 (0.00)	0.0 (0.00)
	- / FOL	44.8 (0.66)	40.6 (0.57)
	PO / FOL	36.2 (0.49)	35.0 (0.43)
	PO / -	0.0 (0.00)	0.0 (0.00)
	LSD (p=0.05)	(0.46)	
LSD			(0.13)

* PO means plants treated with *P. oxalicum*, FOL means plants inoculated with *F. oxysporum* f. sp *lycopersici* and - means plants uninoculated or untreated.
# Data in parentheses are transformed by ln(1/(1-x)) before analysis.
+ Data in parentheses are transformed by arcsin before analysis.

Table 1: Reduction of solution consumption (%) and disease severity (%) induced by *Fusarium oxysporum* f. sp *lycopersici* in different cultivars of tomato treated with *Penicillium oxalicum*

Disease severity was significantly reduced in all the cultivars when treated with PO, except in Ramon plants where no symptoms were observed (Table 1).

Uninoculated plants treated with PO did not show any disease symptoms and consumed as much nutrient solution as uninoculated and untreated plants (Table 1)

Treatment with PO did not affect rhizosphere population of FOL, regardless of the tomato cultivar used. Values of FOL population in the tomato rhizosphere ranged from 30.000 to 300.000 cfu g^{-1} fresh root weight.

Population of PO in the rhizosphere of tomato plants was not affected by the presence of FOL. Rhizosphere population of PO ranged from 10^6 to 3 x 10^6 cfu g^{-1} fresh root weight.

Experiments in soil systems

Experiment 1: PO treatment reduced severity of disease induced by FOL inoculated into stems of tomato plants. (Table 2). Although tomato plants cv. Ramon are marketed as resistant to Fusarium wilt, they showed disease symptoms in both of the experiments carried out. Disease severity was the same in both Ramon (resistant) and Novy (sensitive) cultivars (Table 2).

Percentage isolation of FOL from crown of plants cv. Ramon was reduced by PO treatment, while no reduction was observed in the case of cv. Novy (Table 2).

Cultivar	Treatment*	Disease severity (%)#	Pathogen isolation (%)+
Novy	- / -	25.5 (0.05)	13.5 b
	- / FOL	45.8 (0.10)	78.4 a
	PO / FOL	32.4 (0.07)	72.9 a
	PO / -	28.7 (0.06)	16.2 b
	LSD	(0.01)	
Ramon	- / -	26.7 (0.05)	2.7 c
	- / FOL	51.0 (0.09)	81.1 a
	PO / FOL	42.4 (0.07)	51.3 b
	PO / -	28.5 (0.05)	8.1 c
	LSD	(0.01)	

* PO means plants treated with *P. oxalicum*, FOL means plants inoculated with *F. oxysporum* f. sp *lycopersici* and - means plants uninoculated or untreated. # Data in parentheses are transformed by ln(1/(1-x)) before analysis.

+ Data were analysed with a chi-square test (P=0.05)

Table 2: Disease severity induced by *Fusarium oxysporum* f. sp *lycopersici*, and pathogen isolation from crowns, 69 days after inoculation of tomato plants cv. Novy and Ramon treated with *Penicillium oxalicum*.

At the end of the experiment, PO population densities in the rhizosphere were significantly (P=0.05) higher in plants treated with PO and uninoculated with FOL (10^6 cfu g^{-1} fresh root weight) than in plants treated with PO and inoculated with the pathogen (10^3 cfu g^{-1} fresh root weight).

Stem colonization by FOL in plants treated and untreated with PO ranged between 7-11cm and was not significantly different. In plants treated with PO, the antagonist was never isolated from inside the stems.

Experiment 2: PO treatment reduced disease severity induced by FOL in tomato plants cv. Lorena when chlamydospores of the pathogen were present in soil (Table 3). Stunting in tomato plants caused by FOL was reduced by PO treatment. However, crown isolation of FOL in treated and untreated plants was not significantly different (P=0.05) (Table 3).

FOL population densities 60 days after transplanting were 10^4 cfu g^{-1} fresh root weight in both plants treated and untreated with PO. PO population density in the rhizosphere of treated plants, at the end of the assay was 10^4 cfu g^{-1} fresh root weight.

Treatment*	Disease severity (%)#	Stunting (%)
PO / FOL	41.4 (0.17)	3.74
- / FOL	51.9 (0.22)	21.65*
- / -	14.7 (0.06)	-
LSD	(0.03)	

* PO means plants treated with *P. oxalicum*, FOL means plants inoculated with *F. oxysporum* f. sp *lycopersici* and - means plants uninoculated or untreated. # Data in parentheses are transformed by arcsin before analysis.
* Significantly different from uninoculated plants (P=0.05).

Table 3: Disease severity and stunting induced by *Fusarium oxysporum* f. sp *lycopersici,* 60 days after inoculation of tomato plants cv. Novy and Ramon treated with *Penicillium oxalicum*

Discussion

P. oxalicum reduced disease induced by *F. oxysporum* f.sp. *lycopersici* on different cultivars of tomato both in hydroponic and soil systems with different kinds of inocula. Reduction of disease was greater in the most susceptible cultivars than in the more resistant ones. In the stem inoculation experiment disease severity was similar in both resistant (Ramon) and susceptible (Novy) cultivars. Jones and Crill (1974) found that many cultivars carrying the I gene conferring resistance to race 1 of *F. oxysporum* f.sp. *lycopersici* behaved as susceptible, and concluded that these cultivars were tolerant but certainly not "highly resistant" or "immune". Also the artificial method of stem inoculation could be at least in part responsible for disease induction on the resistant cultivar. It is possible that using a less aggressive inoculation method would allow the cultivar Ramon to show its resistance.

Disease severity induced by *F. oxysporum* f.sp. *lycopersici* on stem inoculated plants was reduced by *P. oxalicum* treatment applied to roots. Control of disease observed may thus be attributed to mechanisms of induced resistance as was reported previously by De Cal *et al* (1997).

Treatment with *P. oxalicum* reduced disease severity in both susceptible and resistant cultivars. Previous work demonstrated that induced resistance is not cultivar specific (Hammerschmidt *et al*, 1976). Van Peer *et al* (1991) demonstrated induction of resistance by a strain of *Pseudomonas* sp. in a moderately resistant cultivar of carnation stem-inoculated with *F. oxysporum* f. sp. *dianthi.*

The delay in *F. oxysporum* f.sp. *lycopersici* disease development did not appear to be related to a delayed movement of the pathogen within *P. oxalicum* treated plants; stem colonization by *F. oxysporum* f.sp. *lycopersici* was similar in treated and untreated plants. However Liu *et al* (1995) observed that some PGPR reduced the fusarium movement in cucumber plants. Furthermore, PO was never detected in the tomato stem, which indicates that the antagonist and the pathogen remained spatially separated during the experiments and the antagonism did not occur inside the plant.

P. oxalicum treated and *F. oxysporum* f. sp *lycopersici* infested tomato plants consumed more nutrient solution than inoculated and untreated plants. These results suggest that *P. oxalicum* partially prevents blocking or collapse of xylem vessels in infected plants. In cultivars resistant to *Fusarium*, different mechanisms have been described which may be responsible for blocking avoidance: formation of barriers preventing progress of the pathogen (Beckman, 1966), confinement of the pathogen to primary xylem (Beckman *et al*, 1972; Beckman *et al*, 1962; Scheffer & Walker, 1953; Scheffer & Walker, 1954), proliferation of pathogen-free vessels (Edgington *et al*, 1961; Scheffer & Walker, 1954), inhibition of pathogen growth (Gao *et al*, 1995), and decrease of embolism risk, all of them allowing fluid movement in xylem vessels. Future experiments are in progress out to find out the physiological mechanisms responsible for

the observed diminished reduction of solution consumption in *P. oxalicum* treated plants infested with *F. oxysporum* f. sp *lycopersici*.

Acknowledgements
This research was supported by a grant from INIA (SC-95-098). We thanks to C. Simon her assistant support.

References

BECKMAN CH, 1966. Cell irritability and localization of vascular infections in plants. *Phytopathology* **56**:821-4.

BECKMAN CH, 1987. *The Nature of wilt Diseases of Plants*. St. Paul, Minnesota: The American Phytopathological Society. APS Press.

BECKMAN CH, Halmos S, Mace ME, 1962. The interaction of host, pathogen, and soil temperature in relation to susceptibility to Fusarium wilt of bananas. *Phytopathology* **52**:134-40.

BECKMAN CH, Elgersma DM, MacHardy WE, 1972. The localization of fusarial infections in the vascular tissue of single-dominant-gene resistant tomato. *Phytopathology* **62**:1256-60.

BESRI M, ZROURI M AND BEYE I 1984 Comparative race classification and pathogenity of some isolates of *Verticillium dahliae* (Kleb.) from resistant tomatoes in Marocco. Phytopathol-Z-J-Phytopathol. 109, 289-294.

DE CAL A, PASCUAL S, MELGAREJO P, 1995. Biological control of *Fusarium oxysporum* f.sp.*lycopersici*. *Plant Pathology* **44**, 909-14.

DE CAL A, PASCUAL S, MELGAREJO P, 1997. Involvement of resistance induction by *Penicillium oxalicum* in the bicontrol of tomato wilt. *Plant Pathology* **46**:72-80.

EDGINGTON LV, CORDEN ME, DIMOND AE, 1961. The role of pectic substances in chemically induced resistance to Fusarium wilt of tomato. *Phytopathology* **51**:179-82.

FUCHS J, DÉFAGO G, 1991. Protection of tomatoes against *Fusarium oxysporum* f.sp. *lycopersici* by combining a non-pathogenic Fusarium with different bacteria in un-treated soil. In: Keel C, Koller B, Défago G, eds. *Plant Growth-promoting Rhizobacteria, Progress and Prospects*. IOBC/WPRS, 51-6.

GAO H, BECKMAN CH, MUELLER WC, 1995. The rate of vascular colonization as a measure of genotypic interaction between various cultivars of tomato and various formae or races of *Fusarium oxysporum*. *Physiological Molecular Plant Patholology* **46**:29-43.

HAMMERSCHMIDT R, ACRES S, KÚC J, 1976. Protection of cucumber against *Colletotrichum lagenarium* and *Cladosporium cucumerinum*. *Phytopathology* **66**, 790-3.

HOAGLAND DR, ARNON DI, 1950. The water-culture method for growing plants without soil. *Calif. Agric. Exp. Stn. Circ.* 347.

IMI Descriptions of Fungi and Bacteria No. 1117. 1992 *Fusarium oxysporum* f.sp. *lycopersici*. Mycopathologia 118, 51-53.

JONES JP, CRILL P, 1974. Susceptibility of "resistant" tomato cultivars to *Fusarium* wilt. *Phytopathology* **64**, 1507-10.

LEMANCEAU P, ALABOUVETTE C, 1993, Suppression of Fusarium wilts by fluorescent Pseudomonads:mechanisms and applications. *Biocontrol Science and Technology* **3**, 219-34.

LIU L, KLOPPER JW, TUZUN S, 1995. Induction of systemic resistance in cucumber against fusarium wilt by plant growth-promoting rhizobacteria. *Phytopathology* **85**, 1064-8.

PHILLIPS DV, LEBEN C, ALLISON CC, 1967. A mechanisms for the reduction of Fusarium wilt by a *Cephalosporium* species. *Phytopathology* **57**, 916-9.

SCHEFFER RP, WALKER JC, 1953. The physiology of fusarium wilt of tomato. *Phytopathology* **43**:116-25.

SCHEFFER RP, WALKER JC, 1954. Distribution and nature of fusarium resistance in the tomato plant. *Phytopathology* **44**:94-101.

SOKAL RR, ROHLF FJ, 1981. *Biometry*. New York: W.H. Freeman and Company.

TAMIETTI G, FERRARIS L, MATTA A, ABBATTISTA GENTILE I, 1993. Physiological responses of tomato plants grown in Fusarium suppressive soil. *Journal of Phytopathology* **138**, 66-76.

VAN PEER R, NIEMANN GJ, SCHIPPERS B, 1991. Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r. *Phytopathology* **81**, 728-34.

INTEGRATED MANAGEMENT OF FOLIAR DISEASES IN GREENHOUSE VEGETABLES ACCORDING TO PRINCIPLES OF A DECISION SUPPORT SYSTEM - GREENMAN

Y. Elad and D. Shtienberg

Dept. of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250, Israel

Summary

Several fungal plant diseases such as gray, white and leaf molds and downy mildew are promoted in vegetable greenhouses under conditions of humidity saturated atmosphere. Manipulation of the greenhouse environment to avoid high humidity-promoted pathogens is practiced by farmers for the management of these diseases. However, in many greenhouses this is not enough for adequate suppression of the diseases. The biocontrol preparation TRICHODEX (*Trichoderma harzianum* T39) and chemical fungicides are other measures to be used during development of epidemics. The integration of biocontrol and chemicals in the greenhouse is aided by the use of a decision support system named greenhouse disease management (GREENMAN). Decision which control agent (biological or chemical) should be used in the greenhouse is taken before each spray. The factors taken into consideration are weather (four days forecast including the amount and duration of rain, maximal and minimal temperatures and cloudiness), the nature of the crop and the greenhouse regarding conduciveness to development of epidemics and the severity of the various diseases. In this system cultural measures and biocontrol are the most important tool and chemical control is implemented only occasionally, as necessary. The potential effectiveness of the fungicides to be used towards the prevailing pathogens, the actual resistance of pathogen population in the greenhouse and the compatibility of chemical fungicides to be used with the biocontrol agent are also considered. As a result of spraying according to GREENMAN guidelines, gray mold (*Botrytis cinerea*) on cucumber and tomato, white mold (*Sclerotinia sclerotiorum*) on cucumber and leaf mold (*Fulvia fulva* = *Cladosporium fulvum*) on tomato, were controlled effectively and a ca 60% reduction in the use of chemical fungicides was achieved.

Introduction

Manipulation of the greenhouse environment to avoid water-dependent pathogens can be practiced by farmers but this is expensive. Unfortunately, growers of greenhouse crops may rely heavily on fungicides to protect their crops because of the prevalence of epidemics of plant diseases. The high disease pressure and high crop value call for frequent applications of the most effective fungicides, which result in the selection and predominance of resistant pathogen strains (demonstrated for *B. cinerea*) in a rather short time. Hence, alternative control methods are urgently needed, either for conventional application or for integration with existing methods. Plastic covered vegetable greenhouses in Mediterranean or desert areas are either not heated or partially heated during the colder winter months (especially at nights). Restricted ventilation and low light intensity, especially on the lower plant parts result in saturated atmosphere for too long periods each day and promote several fungal plant diseases such as gray, white and leaf molds and downy mildew. There are no resistant plant cultivars available in most of the systems.

Humidity enhanced greenhouse diseases

In greenhouses, plants and their pathogens can develop during seasons which restrict their development in the open. As a consequence, the behavior of plant diseases differs according

to the type of greenhouse. However, high humidity, free leaf moisture, reduced intensity and quality of light, and temperatures higher than outdoors, at least during part of the day, promote the development of plant diseases. This is often amplified by the development of a luxurious plant canopy, which reduces aeration and illumination and facilitates development of diseases. Patterns of the same disease on the same host may vary in different types of greenhouses. Greenhouse factors that affect the varying development of diseases comprise specific systems of heating, the architecture of the greenhouse and the covering material, systems of ventilation and irrigation, the growth medium, the general crop management, and factors which condition the interaction between pathogens and their hosts. For instance, ventilation or, conversely, restricted air movement and the concomitant increase in humidity, in addition to direct effects on disease, may affect the plant's development, its reproduction and yield, all of which may affect the disease indirectly.

Our work in integrated management of diseases is presently concentrated in high humidity promoted diseases. Diseases caused by *Botrytis cinerea* (gray mold), *Sclerotinia sclerotiorum* (white mold) and *Fulvia (Cladosporium) fulvum* (leaf mold) are very common in greenhouses under a variety of growing conditions. *B. cinerea* is a ubiquitous pathogen which causes severe losses in many fruit, vegetable and ornamental crops and which can be especially important in greenhouse production. The pathogen infects the leaves, stems, flowers and fruits; in greenhouse vegetables. During severe epidemics the entire foliage may be destroyed. *F. fulva* and *S. sclerotiorum* are similarly promoted by high RH. The first pathogen only infects leaves of tomato whereas the second infects all plant parts and is common on various crops. Downy mildew (*Pseudoperonospora cubensis*) is promoted by leaf moisture on cucumber plants and powdery mildews of cucumber and tomato crops are also common.

Growers rely heavily on fungicides to control these diseases. However, it has been shown that *B. cinerea* may develop resistance against specific fungicides within a relatively short time, and resistance against benzimidazoles, dicarboximides, diethofencarb and two sterol biosynthesis inhibitors has been found (Elad *et al.*, 1991). One of the alternative methods to control these diseases currently practiced) in greenhouses is prevention of canopy wetness by intensive heating and ventilating (Morgan, 1984; Winspear *et al.*, 1970). This is in general effective against infection of leaves, flowers and fruits, but not against stem infections. However, the current heating regimes in some countries are very energy-intensive and expensive (Elad & Shtienberg, 1995).

Integrated control of foliar diseases to minimize use of chemical fungicides

a. Biological control

The first biocontrol agent commercialized and registered for greenhouse crops *is Trichoderma harzianum T39*. Intensive biocontrol work with *Trichoderma* spp. under commercial conditions has been carried out and some significant achievements have been obtained on greenhouse crops (Elad & Shtienberg, 1995; Elad *et al.*, 1994). Isolate T39 of *T. harzianum* (commercially available as TRICHODEX 20P, Makhteshim Ltd., Be'er Sheva, Israel), effectively controls *Botrytis* diseases in greenhouse crops (Elad, 1994; Elad *et al.*, 1994). The commercial biocontrol of other foliar diseases was much less studied as compared with the detailed researches mentioned above in regard to gray mold. The reason for that is perhaps the availability of effective chemicals and the availability of resistant cultivars (against *F. fulva* in some countries. TRICHODEX (*T. harzianum* T39) is also effective against white mold, and gray mold of cucumber. It can be seen in the figure that the biocontrol agent alone suppressed the disease significantly. Thus it can be incorporated as the biological entity in IPM of diseases in cucumber greenhouses. It can also be incorporated in

tomato IPM in greenhouses since it controls gray mold of this crop (Shtienberg & Elad, 1997) and leaf mold. However, the compatibility of the chemical agents aimed at other diseases with the biocontrol agent should be taken into consideration.

b. GREENMAN - Integration of biocontrol in a general disease control system

The moderate effectiveness of the currently studied BCAs against *B. cinerea* calls for their integration with other means of control. A new approach based on a decision support system named BOTMAN was developed by for the control of *B. cinerea* (Shtienberg & Elad, 1997). It was later widened in order to take care of other greenhouse diseases. The outcome is a system named GREENMAN.

According to the instructions obtained by the GREENMAN system chemical fungicides or the biocontrol agent TRICHODEX are applied during the growth season. The decision whether to apply a biological or a chemical agent is taken before each spray according to the following criteria: The suitability of weather conditions for disease development (Four days future weather forecast), the severity of the diseases, the susceptibility of the pathogen to fungicides and their relative efficacy, the nature of the greenhouse and the crop. When weather is expected to be extremely promotive to the pathogens (heavy rains, optimal temperatures), the greenhouse is poorly ventilated and the inoculum density is high, then the use of highly effective chemicals is recommended. Under other conditions, moderately effective fungicides are used and when conditions are expected to be moderate to the pathogen, the use of the biocontrol agent is recommended.

The GREENMAN version of the decision support system was tested in ten commercial greenhouse experiments in the seasons of 1996-1997. On average, ca. 11 sprays were applied in each experiment in the standard treatments (alternation of various fungicides on calendar basis) for tomatoes and cucumbers. In plots treated according to GREENMAN four chemical sprays and six biological sprays were applied. Respective percentages of control of white mold, leaf mold and gray mold were 55, 65 and 70% in the standard treatment and 63, 60 and 64% in treatments when GREENMAN recommendations were followed. The difference between these treatments were insignificant. TRICHODEX significantly contributed to the control achieved by the fungicides in GREENMAN. Relying on this system for management of diseases saved 60% of the chemical sprays.

In the treatments applied according to GREENMAN, out of the average 11 sprays with either chemical or biological agents in greenhouses, 60% of the sprays in programs according to GREENMAN were carried out with the biocontrol preparation TRICHODEX. The performance of GREENMAN is now examined further for other diseases. It is expected to enable growers to reduce the application of chemicals and rely more on biocontrol.

It can be seen in the figure that sprays according to GREENMAN achieved good control of both white and gray molds on cucumber fruits. It was better than the treatment with only the chemicals (no biocontrol agent), thus the TRICHODEX was found to be an important entity in the system contributing not only to increased efficacy but also to drastic reduction of chemicals used.

c. Environmental control

The integration between biological and chemical control achieves better suppression of plant diseases when applied in greenhouses where all other factors that promote diseases are taken care. The factors which affect disease development in the greenhouse are temperatures of air, vapor pressure deficit (VPD), dew, soil water content, and light (quality, day length and intensity). All this can be controlled to a certain extent depending on the facilities. However, it should be noted that there are interactions among air temperature, VPD, dew deposition on

the canopy, physiological status of the host, saprophytic micro-flora and aggressiveness of the population of the pathogen, in their effect on disease. Interplay among these factors affects sporulation, dispersal, germination of conidia, penetration of the germ tubes, and lesion development.

IR repellent covers raise the crop temperature and decrease the leaf moisture. An example of such environmental control is given by cucumber gray mold development in non-heated polyethylene greenhouses covered with various infra-red (IR)-repellent covers compared with a cover with no IR repellence (Elad et al., 1989). Non-persistence of dew on the foliage was the limiting factor in disease development in a relatively dry winter. Disease severity under the different IR sheets was correlated with the duration of dew.

Conditions in the greenhouse influence the physiological status of the host organs and thereby affect the later's susceptibility to infection. Low night temperatures and high RH in the greenhouse predispose cucumbers to the disease, and optimal climatic conditions for crop production during the winter favor the disease. In general, the thermal polyethylene covers (IR) reduce the duration of dew on plants but extend the duration of temperatures favorable for epidemics.

On the basis of disease records and micro-climatic parameters, a model was developed for predicting outbreaks of gray mold in greenhouses. Outbreaks of gray mold occurred when on a weekly average the wetting period exceeded 7 h/day and the duration of 9-21°C temperatures during the night (18:00-08:00) exceeded 9.5 h/day. It was suggested that the potential for outbreaks of gray mold epidemics could be reduced by measures which restrict the wetting period (Yunis et al., 1994). This problem is now overcome by additives added to the polyethylene, which act as anti-fog and anti-dripping, thereby leading to drier plants in the greenhouse.

There are other potential non-chemical methods to decrease disease damages in greenhouses. These include: (a) drip irrigation to reduce soil and foliage wetting; (b) soil mulching by black polyethylene sheets to minimize water evaporation to the air and to extend the intervals between watering; (c) installation of a thermal screen which prevents infra-red irradiation from escaping into the atmosphere on bright nights and thus increasing the temperature of the canopy and minimizing the differences in temperature between the plant organs and the surrounding air; (d) growing plants in soil-less media or hydroponics systems; (e) filtering and sterilizing the irrigation water, mainly to avoid pathogenic bacteria; (f) installing nets which can prevent penetration of vector insects into the greenhouse, in order to prevent virus infection (nets are dangerous because they reduce ventilation and may increase high-humidity diseases, and should therefore be removed when not needed); and (i) insects, covering the soil with shiny and reflective covers (aluminum foil or polyethylene) to deter but the mechanism is not yet known.

Sporulation of *B. cinerea* is known to be inhibited by blue and red light and stimulated by the near-UV and far-red regions of the spectrum (Tan, 1975). Lesion development in cucumber downy mildew is suppressed by limiting light exposure through decreasing photosynthetic activity of the host plant (Inaba and Kajiwara, 1971). Commercially available greenhouse covers filter selectively the penetrating light. For instance, PVC, glass and UV-absorbing-polyethylene all filter UV irradiation. New custom-made polyethylene films with blockers in the far red region of the spectrum were tested for their effect on sporulation of *B. cinerea*. A green-pigmented sheet partially screened it mainly in the range of 570-800 nm, with lowest transmission at 620-670 nm. The films screened the UV radiation up to 380 nm. The use in commercial greenhouses, of the green-pigmented polyethylene reduced gray mold by 35-75%, correlated with reduction of conidial load. Retarding white mold (*S. sclerotiourum*), powdery mildew (*Spaerotheca fuliginea*) and downy mildew (*P. cubensis*)

was recorded in a greenhouse covered with this polyethylene film. Similarly, leaf mold (*F. fulva*) was significantly reduced on tomato plants grown under the green pigmented film (Elad, 1997). It was speculated that an indirect effect on the host plant may be important in reducing the sporulation. This is because some sporulation of *B. cinerea* occurred in the dark, because the effect of disease suppression was obtained in greenhouses where only one bay was covered with the screening film and because light could penetrate from the sides of the covered greenhouse. Induced resistance mechanism may explain the

Conclusions

The integration of cultural, chemical and biological means result in drastic reduction in the damages inflicted by foliar pathogens in greenhouses. The incorporation of GREENMAN criteria with considerations relevant for the management of other diseases and pests will probably result in the future in intensive reduction of chemical pesticides use.

References

ELAD, Y. 1994. Biological control of grape grey mould by *Trichoderma harzianum*. Crop Protec. **13**:35-38.

ELAD, Y. 1997. Effect of filtration of solar light on the production of conidia by field isolates of *Botrytis cinerea* and on several diseases of greenhouse-grown vegetables. Crop Prot. In press.

ELAD, Y. & SHTIENBERG, D. 1995. *Botrytis cinerea* in greenhouse vegetables; chemical, cultural, physiological and biological controls and their integration. Integ. Pest Manag. Rev. **1**:15-29.

ELAD, Y., SHTIENBERG, D. & NIV, A. 1994. *Trichoderma harzianum* T39 integrated with fungicides: Improved biocontrol of grey mould. Brighton Crop Prot. Conf. Pests Diseases pp. 1109-1113.

ELAD, Y., YUNIS, H. & KATAN, T. 1991. Multiple resistance to benzimidazoles, dicarboximides and diethofencarb in field isolates of *Botrytis cinerea* in Israel. Plant Pathol. **41**: 41-46.

ELAD, Y., YUNIS, H. & MAHRER, Y. 1989. Effect of climatic conditions in polyethylene-covered structures on gray mould disease of winter cucumber. Appl. agric. Res. **3**:243-247.

INABA, T. & KAJIWARA, T. 1971. Effect of light on lesion development in cucumber downy mildew. Ann. Phytopathol. Soc. Jpn., **37**:340-347.

MORGAN, W.M. 1984. The effect of night temperature and glasshouse ventilation on the incidence of *Botrytis cinerea* in a late planted tomato crop. Crop Prot. **3**: 243-251.

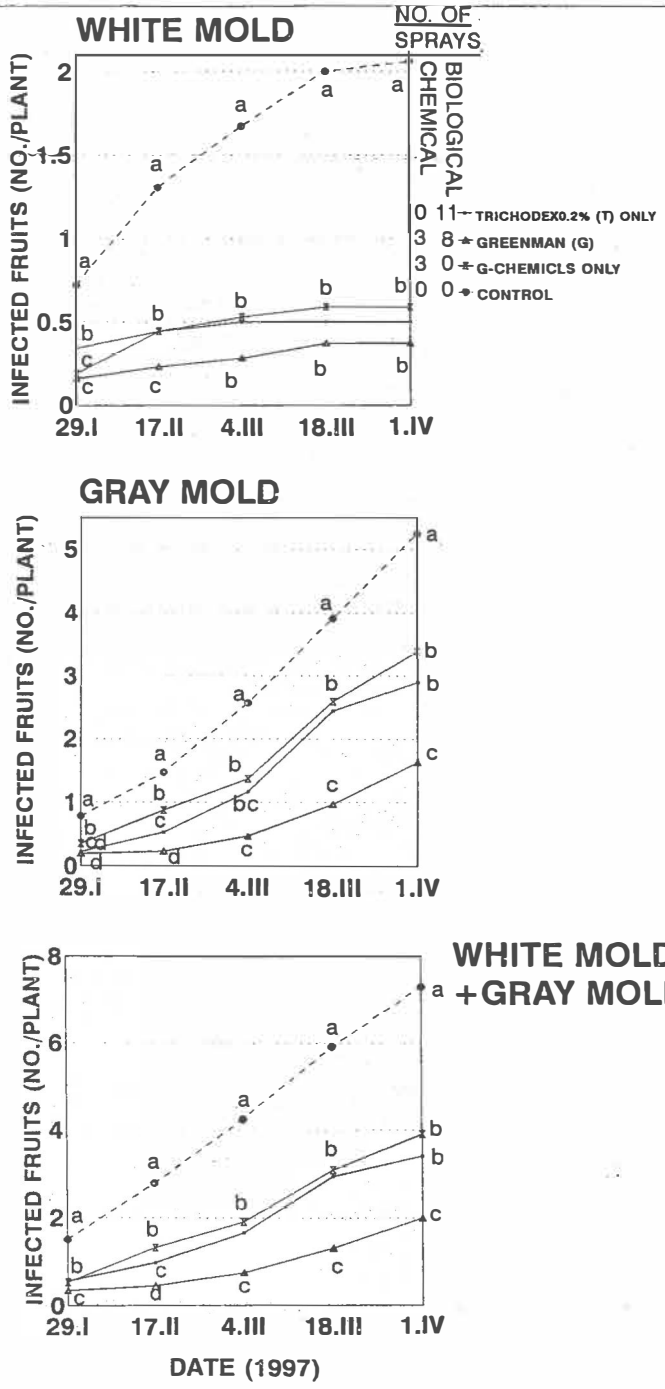
SHTIENBERG, D. & ELAD, Y. 1997. Incorporation of weather forecasting in integrated, biological-chemical management of Botrytis cinerea. Phytopathology **87**: 332-340.

TAN, K.K. 1975. Interaction of near-ultraviolet, blue, red, and far-red in sporulation of *Botrytis cinerea*. Trans. Brit. mycol. Soc., **64**: 215-222.

WINSPEAR K.W., POSTLETHWAITE, J.D. & COTTON, R.F. 1970 The restriction of *Cladosporium fulvum* and *Botrytis cinerea*, attacking glasshouse tomatoes, by automatic humidity control. Ann. Appl. Biol. **65**: 75-83.

YUNIS, H., SHTIENBERG, D., ELAD, Y. & MAHRER, Y. 1994. Qualitative approach for modeling outbreaks of gray mold epidemics in non-heated cucumber greenhouses. Crop Prot. **13**: 99-104.

CONTROL OF CUCUMBER DISEASES, YAMA 1997



A BACTERIAL DISEASE OF TOMATO FRUITS CAUSED BY *PSEUDOMONAS VIRIDIFLAVA* IN CRETE

D.E. Goumas & A.K. Chatzaki

National Agricultural Research Foundation, Plant Protection Institute
P.O. Box 1802, 711 10 Heraklio, Crete, Greece
Fax: 081-245858, E-mail : dgoumas@cnet.gr

Abstract

A new bacterial disease of tomato fruits recorded during January 1994 in outdoor crops at Antiskari and Arvi areas of Heraklion Pronince. The causal damage to commercial production was up to 30%. The symptoms of the disease were restricted on fruits; on middle sise immature fruits, bacterial spot appear on the upper surface becoming progressively larger with the increase of fruit size. Those are round or elongated, superficial and lightly sunken, 1,5-2cm. The spot centre dries becoming progressively grey,broun to black-brown with more intense coloured margin. Soft rot was never observed on those infections. On the basis of morphplogical, physiological and biochemical characters of eleven isolates, the fluorescent bacterium *Pseudomonas viridiflava* was identified as the causal agent. Koch's postulates were fulfilled. To our knowledge these symptoms have never been previously described on tomato fruits.

Introduction

Symptoms of an unknown bacterial disease have been frequently recorded throughout the island of Crete on tomato (*Lycopercicon esculentum* Mill.) plants grown in greenhouses and outdoors. The disease was firstly recorded during January 1994 in outdoor crops at Antiskari area of Heraklion Province. At this region the causing damage to commercial production was up to 30%. Initial streak-plate isolations made on King's medium B, from the affected plants, consistently resulted in essentially pure cutlures of a fluorescent bacterium.

The symptoms of the disease were mainly restricted on fruits; spontaneously plants bearing heavily infected fruits indicated silver discolouration on the underside leaf surface. On middle size fruits, bacterial spots appear on the upper surface becoming progressively larger with the increase of fruit size. These are round or elongated, supperficial and lightly sunken 1,5-2 cm in diameter. The spot centre dries becoming progressively grey, brown to black-brown with more intense coloured margin. Soft rot was never observed on those infections in the field. Under favourable conditions, the enlarged spots usally coalesce to cover a large area of fruit, but never result necrosis of the whole fruit.

The objectives of the present work were to characterize the causal agent of this unreported disease in Greece and to suggest a possible control of the disease.

Materials and methods

Isolation and identification of isolates

Small pieces taken from the margin of the lesions on tomato fruits, were triturated in a few drops of sterile distilled water. Loopfuls of the suspensions were streaked onto plates of NDA (Nutrient Dextrose Agar) and King's B medium [King *et al.*, 1954].

Plates were incubated for 48h at 30°C. Single colonies were subcultured, checked for purity and stored as slant cultures at 4°C on NDA.

Isolation on King's B medium [King *et al.*, 1954] indicated that the isolated bacteria were fluorescent pseudomonads. Thus, many isolates were initially tested accordingly to the LOPAT tests [Lelliott *et al.*, 1966] and eleven representative isolates were used for further characterisation using the differential tests presented in Table 2. All methods had been previously described by Malathrakis and Goumas[1987]. Reference bacterial strains, are presented also in Table 1. Each test was repeated at least twice. For further chatacterization following additional tests were performed : Gram stain [Schaad, 1988], glusose fermentation in Hugh and Leifson [1953] medium, â-glucosidase on arbutin hydrolysis medium [Crosse and Garrett, 1963]. The differential capacity of *P. viridiflava, P. syringae* pv *syringae and P. syringae* pv *tomato* strains to fluoresce on iron-deficient Misaghi & Grogan's medium [1969] containing sucrose, erythritol or DL-lactate as single carbon source, was also tested as described by Jones *et al.* [1986].

Pathogenicity tests

In preliminary studies all isolates were screened for their ability to induce a hypersensitive reaction on tobaco leaves and cause rot of potato slices by methods previously described [Malathrakis and Goumas, 1987]. In pathogenicity tests, immature detached tomato fruits were used, which were shabed with 70% ethanol and then washed twice with sterile water. Inoculations were made as described by Lelliott and Stead (1987), by deposition of 0,02 ml of a appropriate bacterial suspension on tthe upper surface of tomato fruits previously picked with a sterile needle at six sites. Inocula were prepared from 48 hr-old King's medium B cultures [King *et al.*, 1954] suspended in sterile distilled water and adjusted to approximately 10^8 cfu ml $^{-1}$ · Serial tenfold dilutions within 10^6 and 10^4 cfu ml $^{-1}$ were used. Three isolates of the bacterium (TKK 542, TKK 5a, TKK 615), two others obtained from tomato with bacterial soft rot (PV 442) and cucumber with bacterial necrosis (PV 400), previously characterized as *P. viridiflava* [Malathrakis and Goumas, 1987; Goumas and Malathrakis, 1987], *Pseudomonas syrigae* pv *tomato* (Pst 11) and *Pseudomonas syrigae* pv *syrigae* (Pss 5) respectively were used comparatively. After inoculation, tomato fruits were held in closed trasparent boxes lined with damp blotting papper, either at incubators (10^0C and 28^0C) or laboratory conditions (12 -28 ^{0}C) with 16h photoperiod. Controls were similarly treated with sterile water and all were assesed daily for ten days to record disease symproms. The same methothology was used for inoculations of immature fruit on tomato plants growing in greenhouse during winter (9 -28 ^{0}C). After inoculation tomato fruits were maintained in closed clear polyethylene bags for three days. Disease symptoms were recorded within 15 days. Pathogens were reisolated on King's medium B [King *et al.*, 1954].

The same isolates were used for stem inoculation of tomato plants at the 4-5 true leaf stage by stabbing with the tip of a sterile toothpick with an appropriate culture into the stem of each plant above first true leaves. Inoculated sites were covered with parafilm and plants were placed in a mist chamber at 22^0C and 16h photoperiod until the record of disease symptoms ten days later. Controls were similarly treated with sterile toothpicks.

Inoculations on snap bean (*Phaseolus vulgaris* L.) pods were also conducted as

described by Cheng *et al.* [1989] and on immature lemon and pear fruits as proposed by Lelliott and Stead (1987).

Results

Isolation and characterization of the pathogen

All isolations carried out on King's medium B, revealed consistently the presence, in essentially pure culture, of a fluorescent bacterium of the genus *Pseudomonas*. The colonies of *Pseudomonas,* on King' s medium B, appeared opaque, convex, shiny, semifluid and produced a bright green-blue diffusible fluorescent pigment. On NDA medium the colonies were convex, smooth and whitish yellow. After five to ten days incubation on 5% sucrose nutrient agar the centre of the colonies became greenish [Lelliot and Stead, 1987]. In LOPAT tests [Lelliot *et al.*, 1966] all tested isolates, together with reference stains of *P. viridiflava* from cucumber and tomato gave similar results. Results of identifications tests of isolates are presented in Table 1. These are in agreement with those obtained with the reference strains of *P. viridiflava*. Other reference strains used in this work gave results which were consistent with their designated classification [Malathrakis and Goumas, 1987; Hildebrand *et al* ,1988].

Quick identification of isolates as *P. viridiflava* was successeful by using the pattern of Jones *et al.* [1986] for fluorescence on single carbon source media (Table 3).

On the basis of their morphological, physiologiacal biochemical and pathological characteristics, the eleven representative isolates of *Pseudomonas* spp were identified as *P. viridiflava* accordingly to the determinative schemes proposed by Lelliott *et al.* [1966], Sand *et al.* [1970] and Billing [1970].

Pathogenicity tests

All isolates of *P. viridiflava* from tomato fruits were pathogenic. The symptoms induced, were similar to those due to natural infections without differences on fruits either detached or on the plant. On tomato fruit the disease started as a water-soaked spot which developed in 3-4 days into small or large irregular lesions. The centre of the lesions later became dry and tan to black in colour.

Differentiation was observed on the evolution of symptoms with the strains of *P. viridiflava* isolated from bacterial soft rot of tomato and bacterial necrosis of cucumber. At the begining induced symptoms were similar to those produced by tomato isolates but within 10 days the infection in many times resulted in a soft rot. The soft rot symptom was obtained only in a few cases at incubation temperature of 10^0C with the isolates from tomato fruits. Also, all strains after stab inoculation of the stem, developed bacterial soft-rot on tomato [Malathrakis & Goumas, 1987]. Reisolations made from the artificially infected fruits and plants yielded, pure cultures of *P. viridiflava*. Bacterial identification was confirmed by LOPAT tests.

Finally, all isolates of *P. viridiflava* and reference strains of the bacterium, caused rust-coloured lesions within 48 hr on excised pods. Strains of *P. syringae* (pv *syringae*, pv *tomato*) caused a necrotic reaction. Isolates of *P. viridiflava* do not produce any symptom on lemon or pear detached fruits. Strain of *Pseudomonas syrigae* pv *syringae* reproduce the black pit symptom on lemon and a watersoaked necrotic spot on pear fruit.

Discussion

Results of this study suggest that the bacterium consistently isolated from diseased

tomato fruits is *P. viridiflava*, which has been characterized as an opportunistic [Billing, 1970; Burkholder, 1930; Wilkie *et al.,* 1973] pathogen to a wide variety of plants (Brandbury, 1986].

In Greece *P. viridiflava* has been often found to cause severe problems on tomatoes and cucumbers grown in plastic houses or outdoors. The bacterium has been reported as the causal agent of pith necrosis of tomato [Alivizatos, 1986] ; bacterial soft rot of tomato [Malathrakis and Goumas, 1987], bacterial rot of eggplant [Goumas and Malathrakis, 1985] and bacterial blight of cucumber [Goumas and Malathrakis, 1987]. To our knowledge this is the first record of the bacterium *P. viridiflava* causing these symptoms on tomato fruits [Brandbury, 1986].

The outbreak of these diseases, each year since 1985, has been associated with favourable conditions, probably predisposing plants to the invasion of the pathogen. Cool and humid conditions in greenhouses or outdoors during winter, the succulence and vigour of the plants, due to the high nitrogen fertilization, prolonged wet conditions, low night temperatures (<15^{o}C) and reduced illumination, combined with the absence of copper compound applications, create ideal conditions to initiate the disease. This is done by increased inoculum levels remaining from the preceding infected crops or from the pathogen which epiphytically colonizes the plant tissue. The epiphytic survival of the bacterium has been also well documented [Billing,1970 ; Mariano and McCarter,1993].

The occurence of the disease on many different hosts, its reapparence in the same field, the favourable conditions predisposing infection during winter season in Crete and the results of our inoculation experiments under similar conditions, support the view of the majority of previous reports [Gitaitis *et al.*, 1991 ; Hunter and Cigna, 1981 ; Jones *et al.*, 1984 ; Shakya and Vinther, 1989] which refer to *P. viridiflava* as an oppurtunistic pathogen. Also, observations made throughtout recent years in Crete show that efficient aeration of the greenhouse and protective applications of copper compounds can result in effective control of the diseases caused by the bacterium.

References

ALIVIZATOS, A.S., 1986. Tomato pith necrosis caused by *Pseudomonas viridiflava. Annls Inst. Phytopath. Benaki* (N.S.) **15**: 41-47

BILLING, E., 1970. *Pseudomonas viridiflava* (Burkholder, 1930; Clara, 1934). *Journal of Applied Bacteriology* **33**: 492-500

BRANBURY, J.F. 1986. *Guide to Plant Pathogenic Bacteria* CAB International, Farnham Royal.

BURKHOLDER, W.H. 1930. The bacterial disease of the bean. A comparative study. *Cornell University Agricultural Experiment Station Memoir* **127**: 1-88

CHENG, G.Y., LEGARD, D.E., HUNTER, J.E. & BURR, T.J. 1989. Modified bean pod assay to betect strains of *Pseudomonas syringae* pv. *syringae* that cause bacterial brown spot on snap bean. *Plant Disease* **73**: 419-423

CROSSE, J.E. & GARRETT, C.M.E. 1963. Studies on the bacteriophagy of *Pseudomonas mors - prunorum*, *P. syringae* and related organisms. *Journal of Applied Bacteriology* **26**: 159-177

GITAITIS, R.D., BAIRD, R.E., BEAVER, R.W., SUMNER,, D.R., GAY, J.D. & SMITTLE, D.A. 1991. Bacterial blight of sweet onion caused by *Pseudomonas viridiflava* in Vidalia, Georgia. *Plant Disease* **75**: 1180-1182

GOUMAS, D.E. & MALATHRAKIS, N.E,. 1985. Bacterial rot of eggplant in plastic

greenhouses in Crete. 3rd National Phytopathological Conference, 1985. Summaries of invited and research paper, Greece, Volos , p 23 (abstract.)

GOUMAS, D.E. & MALATHRAKIS, N.E., 1987. A new bacterial disease of cucumber grown in greenhouses in Greece. 4th National Phytopathological Conference, 1987. Summaries of invited and research paper, Greece, Athens, p 57 (abstract.)

HILDEBRAND, D.C., SCHROTH, M.N. & SANDS, D.C., 1988. *Pseudomonas*. In: Schaad ND (ed.) Laboratory Guide for Identification of Plant Pathogenic Bacteria (pp 60-80). The American Phytopathological Society, St Paul, Minesota, USA

HUGH, R. & LEIFSON, E., 1953. The taxonomic significance of King fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. *Journal of Bacteriology* **66**: 24-26

HUNTER, J.E. & CIGNA, J.A., 1981. Bacterial blight incited in parsnip by *Pseudomonas marginalis* and *Pseudomonas viridiflava*. *Phytopathology* **71**: 1238-1241

JONES, J.B., JONES, J.P., MCCARTER, S.M. & STALL, R.E., 1984. *Pseudomonas viridiflava*: Causal agent of bacterial leaf blight of tomato . *Plant Disease* **68**: 341-342

JONES, J.B., GITAITIS, R.D. & MCCARTER, S.M. 1986. Fluorescence on single-carbon sources for separation of *Pseudomonas syringae* pv *syingae, P. syringae* pv *tomato* and *P. viridiflava* on tomato transplants. *Plant Disease* **70**: 151

KING, E.O., WARD, M.K. & RANEY, D.E., 1954. Two simple media for the demonstration of pyocyanine and fluorescin. *Journal of Laboratory and Clinical Medicine* **44**: 301-307

LELLIOTT, R.A., BILLING, E. & HAYWARD, A.C. 1966. A determinative scheme for fluorescent plant pathogenic pseudomonads. *Journal of Applied Bacteriology* **29**: 470-489

LELLIOT, R.A. & STEAD, D.E., 1987. Methods for the Diagnosis of bacterial Diseases of Plants. Blackwell Scientific Publications, Oxford.

MALATHRAKIS, N.E. & GOUMAS, D.E., 1987. Bacterial soft rot of tomato in plastic greenhouses in Crete. *Annals of Applied Biology* **111:** 115-123

MARIANO, R.L.R. & MCCARTER, S.M., 1993. Survival of *Pseudomonas viridiflava* on tomato and selected weed species. *Microbial Ecology* **26**: 47-58

MISAGHI, I. & GROGAN, R.C. 1969. Nurtitional and biochemical comparisons of plant- pathogenic and saprophytic fluorescent pseudomonas. *Phytopathology* **59**: 1436-1450

WILKIE, J.P., DYE, D.W. & WATSON, D.R.M., 1973. Further hosts of *Pseudomonas viridiflava*. *New Zealand Journal of Agricultural Research* **16:** 315-322.

SANDS, D.C., SCHROTH, M.N. & HILDERBRAND, D.C., 1970. Taxonomy of phytopathogenic pseudomonads. *Journal of Bacteriology* **101**: 9-23.

SCHAAD, N.W. 1988. Initial identification of common genera In: Schaad ND (ed.) Laboratory Guide for Identification of Plant Pathogenic Bacteria (pp 1-11). The American Phytopathological Society, St Paul, Minesota USA .

SHAKYA, D.D. & VINTHER, F., 1989. Occurrence of *Pseudomonas viridiflava* in seedling of radish. *Journal of Phytopathology* **124:** 123-127.

Table 1. Bacterial strains used in this study

References strains	Collection no	Host	Origin
Pseudomonas viridiflava	Pv400, Pv401	*Cucumis sativus*	Mesara Crete
	Pv441, Pv442	*Lycopercicon esculentum*	Tymbaki Crete
	NCPPB 1249	*Chrysanthenum morifolium*	England (1962)
Pseudomonas syringae pv *syringae*	Pss3, Pss5	*Citrus sinensis*	Fodele Crete
	Pss 11	*Citrus lemon*	Fodele Crete
	NCPPB[1] 2778	*Pyrus communis*	France (1965)
pv *tomato*	Pst 18, Pst 29	*Lycopercicon esculentum*	Antiscari Crete
	Pst 30		Tymbaki Crete
pv *lachrymans*	Psl 110, Psl 119	C. sativus	Ierapetra Crete
	Psl 102	C. melo	Lasithi Crete
	NCPPB 541	C. sativus	Canada 1951

[1] NCPPB, National Collection of Plant Pathogenic Bacteria.

[2] INRA-V, Institut Nationale de la Recherche Agronomique - Versailles

Table 3. Differential capacity of *P. viridiflava, P. syringae* pv *syringae and P. syringae* pv *tomato* strains to grow and fluoresce on a mineral base medium containing sucrose, erythritol or DL-lactate as single carbon source [1].

Bacteria	No. of strains tested	Carbon source		
		Sucrose	Erythritol	DL- Lactate
Isolates from tomato fruits	11	-[2]	+[3]	+
Pseudomonas viridiflava	5	-	+	+
Pseudomonas syrigae pv *syrigae*	4	+	+	+
Pseudomonas syrigae pv *tomato*	3	+	-	-

[1] As described by Jones *et al.* [1985], on iron-deficient Misaghi & Grogan's medium [1969]. No growth was occured on basal medium without carbon source

[2] - : Neither growth nor fluorescence

[3] + : Growth and fluorescence

Table 2. Comparison of isolates from tomato fruits with strains of *Pseudomonas vidiflava* and *Pseudomonas syringae* pathovar

Tests	Isolates from: tomato fruits (11)	*Pseudomonas viridiflava* (5)	*Pseudomonas syringae* pathovar *syringae* (4)	*lachrymans* (3)	*tomato* (3)
Levan	-[1]	-	+	+	+
Oxidase	-	-	-	-	-
Potato rot	+	+	-	-	-
Arginine dihydrolase	-	-	-	-	-
Hypersensitivity	+	+	+	+	+
Nitrate reduction	-	-	-	-	-
Fluorescent pigment	+	+	+	+	+
Gelatin hydrolysis	+	V	+	NT	-
Pectate gel pitting	+[2]	+	+	NT	NT
Lipases	+	+	+	NT	NT
2- Ketogluconate	-	-	-	-	-
Use for growth					
D(-) Mannitol	+	+	+	+	+
D(+) Cellobiose	-	-	-	-	-
D(-) Sorbitol	+	+	+	+	+
D(+) Trechalose	-	-	-	-	-
D(+) Sucrose	-	-	+	+	+
i-Inositol	+	+	+	+	+
L(-) Rhamnose	-	-	-	-	-
D(-) Arabinose	-	-	-	-	-
Erythritol	+	+	+	+	+
Betaine	+	+	+	-	+
Adonitol	-	-	-	-	-
DL-Lactate	+	+	+	+	+
L(-) Lactate	+	+	+	-	-
L(+) Tartrate	-	-	-	-	-
D(-) Tartrate	+	+	-	-	+
Malonate	+	+	+	+	+
Anthranilate	-	-	-	-	-
L-Valine	-	-	-	-	-
â-Alanine	-	-	-	-	-

1 - : Negative reaction, + : Positive reaction, NT : not tested, V : Variable reaction.

2 Pectate gel pitting occurs at pH 7 and 8,3 for *Pseudomonas viridiflava*.

ALTERNATIVE STRATEGIES TO CONTROL ROSE POWDERY MILDEW*

C.Pasini[1], F. D'Aquila[1], P. Curir[1], M.L. Gullino[2]

[1] Istituto Sperimentale per la Floricoltura di Sanremo, Italy
[2] Di.Va.P.R.A. Patologia Vegetale, Università di Torino, Italy

Summary

Weekly sprays of the mycoparasite *Ampelomyces quisqualis* (AQ10 Biofungicide), of the mineral salt KH_2PO_4, of potassium salts of fatty acids (MYX F 4020 and MYX F 403.02-050), of a canola oil (Synertrol), of wine vinegar, of neem extract (FU-3 Trifolio M) and a petroleum oil (JMS Stylet-Oil) provided good control of rose powdery mildew (*Sphaerotheca pannosa*), being as effective as the standard fungicide dodemorph, in two separate experiments carried out under greenhouse conditions. An aqueous formulation of concentrated extracts from leaves of *Reynoutria sachalinensis* (Milsana) did not provide adequate control. The various types of combination of the above mentioned antifungal compounds tested were all effective. The availability of a number of effective antifungal compounds permitted to reduce the total number of dodemorph applications.

1. Introduction

Powdery mildew, incited by *Sphaerotheca pannosa* var. *rosae*, is the most widespread and economically important disease in commercial production of cut roses. In order to prevent its damage on leaves, stems and flowerbuds, during the growing season up to 20 sprays are frequently carried out (Gullino and Garibaldi, 1996): dodemorph and other ergosterol biosynthesis inhibiting fungicides (EBI) are widely applied. However, technical and ecological problems, as a consequence of their frequent applications, may originate, including phytotoxicity, development of fungicide resistance and re-entry problems for growers. Alternatives to conventional fungicides are at present being investigated: they include mineral salts, mineral and vegetable oils, plant extracts and biocontrol agents. The management of rose powdery mildew by repeated applications of several antifungal compounds of different origin, sprayed either singly or in different sequences, proved effective (Horst *et al.*, 1992; Pasini *et al.*, 1997). In this work, the results further obtained in two recent experimental trials are reported.

2. Materials and Methods

Two experimental trials were carried out at the Floriculture Institute of Sanremo on roses (cv Micol) cultivated in a greenhouse maintained under normal production conditions. Roses were grown in benches and arranged in a randomized block design with four replicates (20 plants/replicate). Sprays were applied to runoff with a knapsack sprayer, at 7 day intervals, between 30 May and 4 July 1996 during the fist trial, and between 18 March and 13 May 1997 in the second one. A slight powdery mildew infection was present on young leaves at the time of the first application in both trials. The products compared were: dodemorph (Mehltaumittel, BASF, 40% of a.i.), KH_2PO_4 (Sigma Chemical Co.), *Ampelomyces quisqualis* (strain AQ 10 E10 containing 10^{10}CFU/g, Ecogen Inc.), potassium salts of fatty acids (MYX-F 4020 and MYX-F 403.2.050, Mycogen Co.), JMS Stylet-Oil or

* This work was supported by a grant from Regione Liguria

ADDQ (Sun Refining and Marketing Co.), emulsified canola oil (Synertrol fungicide, Organic Crop Protectants Pty Ltd), wine vinegar with 6% acidity (Aceto di vino rosso, Cirio), neem FU-3 (Trifolio-M GmbH) and an aqueous formulation of extracts from leaves of *Reynoutria sachalinensis* (Milsana, BASF). In both experiments, the severity of foliar infection was estimated 3-4 days after the last application on 40 leaves (5-foliate) per plot (amounting to 200 leaflets/plot), randomly collected on the terminal portion of flowering stems. The percentage of infected area was evaluated by eye on individual leaflets as previously described (Pasini *et al.*, 1997). Data were submitted to ANOVA statistical analysis.

3. Results

In 1996 trial, all treatments applied six times alone or in different rotations significantly reduced the severity of powdery mildew in comparison to untreated roses. Under a severe disease pressure, the mycoparasite AQ 10 and JMS Stylet-oil provided the best control (89% and 86% disease reduction respectively in comparison with the control plots), followed by Synertrol. All the rotations tested resulted effective. Stylet-oil and Myx F 4020, when applied on a stand alone basis, caused some phytotoxicity.

In 1997, nine applications were carried out: all treatments significantly and equivalently reduced the severity of rose powdery mildew, with Synertrol being the most effective. All the rotation tested were effective, including those without fungicide sprays.

This present study confirms that the salt KH_2PO_4, the fungicidal soap Myx F 403.2.050, the Neem oil FU3, the canola oil Synertrol, the wine vinegar and the biofungicide AQ10 can control rose powdery mildew as well as dodemorph. No differences in fungicidal activity were observed when the various antifungal compounds were sprayed singly or in different successions, with or without the fungicide dodemorph. Milsana confirmed the poor activity already observed against rose powdery mildew (Pasini *et al.*, 1997). The results obtained confirm that there are novel means of rose powdery mildew control, which permit to reduce the use of synthetic fungicides.

4. References

GULLINO M.L., GARIBALDI A., 1996. Disease of roses: evolution of problems and new approaches for their control. Acta Horticulturae, n. **424**: 195-201.

HORST R.K., KAWAMOTO, S.O., PORTER L.L. (1992) Effect of sodium bicarbonate and oils on the control of powdery mildew and black spot of roses. Plant Disease, **76**, 247-251.

PASINI C., D'AQUILA F., CURIR P., GULLINO M.L., 1997. Effectiveness of antifungal compounds against rose powdery mildew (*Sphaerotheca pannosa* var. *rosae*) in glasshouses. Crop Protection **16** : 251-256.

Table 1. Effect of multispray program of different products against rose powdery mildew in trial 1 (1996).

Treatment	Amount (x 100 L)	N° sprays °	Leaf area infected (%)
single application			
•Unsprayed control			66.2 c°°
•Dodemorph	80.0 g	6	23.3 ab
•Milsana	2.0 L	6	38.9 b
•KH_2PO_4	0.35 Kg	6	33.8 ab
•Myx F 4020	1.0 L	6	27.9 ab
•Myx F 403.2.050	1.0 L	6	19.1 ab
•Stylet-Oil	0.5 L	6	8.8 a
•Neem FU3	0.5 L	6	20.5 ab
•Synertrol	0.35 L	6	10.1 ab
•Wine vinegar	3.5 L	6	23.7 ab
•AQ 10 -E10	5.0 g	6	6.8 a
application in rotation			
•Wine vinegar	3.5 L	2	
Stylet-Oil	0.5 L	2	23.6 ab
Dodemorph	80.0 g	2	
•Wine vinegar	3.5 L	2	
AQ 10	5.0 g	2	15.9 ab
Dodemorph	80.0	2	
•KH_2PO_4	0.35 Kg	2	
Dodemorph	80.0 g	2	
AQ 10	5.0 g	1	35.9 b
Stylet-Oil	0.5 L	1	

° carried out at 7 day intervals

°° Means in the column followed by the same letters are not different for P=0.05, according to the S-N-K test. The data were transformed in arcsin before the Anova.

Table 2. Effect of multispray program of different products against rose powdery mildew in trial 2 (1997).

Treatment	Amount (x 100 L)	N° sprays °	Leaf area infected (%)
single application			
•Unsprayed control			73.7 b °°
•Dodemorph	80.0 g	9	25.6 a
•KH_2PO_4	0.5 Kg	9	27.8 a
•Myx F 403.2.050	1.0 L	9	18.6 a
•Neem FU3	0.5 L	9	22.0 a
•Synertrol	0.5 L	9	5.7 a
•Wine vinegar	5.0 L	9	26.9 a
•AQ 10 -E10	5.0 g	9	19.1 a
application in rotation			
•Dodemorph	80.0 g	3	
Wine vinegar	5.0 L	2	14.3 a
Neem FU3	0.5 L	2	
AQ 10	0.5 g	2	
•Dodemorph	80.0 g	3	
KH_2PO_4	0.5 Kg	2	11.6 a
AQ 10	5.0 g	2	
Wine vinegar	5.0 L	2	
•Dodemorph	80.0 g	3	
AQ 10	5.0 g	2	16.4 a
AQ 10	5.0 g	2	
Wine vinegar	5.0 L	2	
•Wine winegar	5.0 L	3	
Neem FU3	0.5 L	3	22.0 a
Dodemorph	80.0 g	3	
•Wine winegar	5.0 L	3	
Synertrol	0.5 L	3	17.6 a
AQ 10	5.0 g	3	
•Wine winegar	5.0 L	3	
Myx 403.2.050	1.0 L	2	
AQ 10	5.0 g	2	13.8 a
Synertrol	0.5 L	2	

° and °° see table 1

III. Nematodes

WEED HOSTS TO *MELOIDOGYNE* SPP. ASSOCIATED WITH VEGETABLE CROPS IN NORTHEAST SPAIN.

Barcelo, P.[1], Sorribas, F. J.[1], Ornat, C.[2], Verdejo-Lucas, S.[2]

[1]Departamento Agronomía, ESAB, Comte d'Urgell, 187, 08036 Barcelona. [2]Departamento Patologia Vegetal, IRTA. Crta de Cabrils s/n. 08348 Cabrils, Barcelona. Spain.

Abstract

Root-knot nematodes, *Meloidogyne* spp., have a wide range of plant hosts including weeds and they can reproduce on them. To determine the host status of weeds to *Meloidogyne* in coastal areas of northeast Spain, 50 sites were sampled during the growing season. Sites were selected because they were known to be infested by *M. incognita*, *M. arenaria* or *M. javanica* as shown in previous surveys. *Meloidogyne* spp. were found infecting 46 plant species of weeds belonging to 20 botanical families. Root galls (> 2 mm) were easily observed on weed species of Amaranthaceae, Chenopodiaceae, Compositae, Portulacaceae, and Solanaceae, although they were not as obvious on the other weeds such as on species of Gramineae. Volunteer plants from the previous crops, particularly tomato and cucumber, acted as weeds and they showed galled roots frequently. In general, galls in deep rooted perennial weeds were inconspicuous although they hosted numerous females in their rhizomes or tubers. Weeds are contributing to the persistence of root-knot nematodes on vegetable crops, specially perennial weeds since they are very difficult to control.

Introduction

Root-knot nematodes, *Meloidogyne* spp, are the most important plant-parasitic nematodes damaging vegetable crops in Spain. These nematodes have an extensive host range including cultivated and noncultivated crops (Sasser, 1979), and a widespread distribution in the country (Belda *et al.*, 1994, Esparrago & Navas, 1995, Rodriguez Rodriguez, 1990, Sorribas & Verdejo-Lucas, 1994). Alternate hosts of root-knot nematodes that might influence its development need to be identified, thereby enabling management strategies to be developed.

Weeds are present in many agricultural ecosystems and they have long been recognized as competitors to cultivated plants for energy sources and as pest and pathogen reservoirs including nematodes. Weeds that support nematode development and reproduction can reduce the beneficial effects of crop rotation with non-host or nematode-resistant crops. Also, the effectiveness of fallow periods would be reduced if weeds are present. In coastal areas of northeast Spain, weeds associated to vegetable crops include at least 147 plant species (Izquierdo *et al.*, 1996). The majority of these weeds have a worldwide distribution and are also associated to other crops. The identification of weeds that host root-knot nematodes in a given production area will be a useful tool for pest management since the persistance of weeds growing among vegetables or between two successive crops is an important source of nematode survival and infestation.

In this paper, weeds that are host of *Meloidogyne* spp in coastal areas of northeast Spain are reported.

Materials and methods.

A survey was conducted to determine the host status of weeds to root-knot nematodes. Fifty sites were sampled along the growing season from 1993 to 1996. Sites were selected because they were known to be infested by *M. incognita*, *M. arenaria* and *M. javanica* as shown in previous surveys (Ornat & Verdejo-Lucas 1994, Sorribas & Verdejo-Lucas, 1994). Sites were located in Maresme and Baix Llobregat counties, Barcelona, Spain. Plants were collected from patches of poor plant growth and from the edges of the fields. Each sample consisted of the aerial part of the plant and the corresponding roots with the adhering soil carefully collected fron the 5-20 cm depth. Plants were identified to species according to Bolos *et al* (1993), and Tutin *et al.*, (1993). Roots were washed fred of soil and observed with a stereomicroscope to detect the presence of galls and egg masses on the root. When galls were not obvious, roots were stained (Bridge *et al.*, 1982), and they were checked for the presence of juveniles or other stages of the nematode life cycle under a compound microscope at 100X. The species of *Meloidogyne* were identified based on the perineal patterns of the females (Eisenback *et al.*, 1981). A weed was considered as a host of *Meloidogyne* spp. when infection occurred.

Results and Discussion.

Fortysix weed species belonging to 20 botanical families were found to be host of *Meloidogyne* spp. (Table 1). The presence of the nematode associated with roots of so many weeds is consistent with its poliphagic description. Typical symtoms of *Meloidogyne* infestation (galled roots) were observed in many weeds but they were more evident in members of the family Amaranthaceae, Compositae, Chenopodiaceae, Portulacaceae, and Solanaceae. Weeds belonging to Amaranthaceae and Compositae have been described as good hosts of root-knot nematodes (Quénéhervé *et al.* 1995). Inconspicuous galls were observed on rhizomes of perennial weeds such as *Cirsium arvense, Convolvulus arvensis,* and *Lepidium draba*, although they harbored numerous females. Tubers of *Cyperus rotundu,* did not show symtoms of infection but females were detected. Rhizomes and tubers can provide established foci from which the nematode spread to crops. In most Gramineae, galls were not noticiable, despite nematode infection and reproduction.

Meloidogyne incognita was the species most frequently found in this study followed by *M. javanica.* Apparently, lower number of weeds were host of *M. arenaria* than *M. incognita* or *M. javanica* but fewer sites were infested by *M. arenaria* than by the other root-knot nematode species. Six weed species were hosts of the three more common *Meloidogyne* species that occur in vegetable crops in northeast Spain (Table 2). *Portulaca oleracea*, *Stellaria media* and *Sonchus* spp (*S. oleraceus* and *S. tenerrimus*) are among the ten most frequent weeds found along the season. Especial attention should be paid to these weeds that are present most of the growing season and hosted the three species of *Meloidogyne.* In contrast, *Urtica urens,* a common autunm-winter weed, was infected only by *M. incognita* in one of the sites sampled. On the other hand, differences in host suitability have been reported at the race level. For exemple, *Chenopodium album* was a good host of *M. arenaria* race 2 but a moderate host of *M. incognita* race 3 (Tedford & Fortum, 1988). Weed host status is apparently dependent on genetic differences in both the nematode population as well as weed biotypes (Griffin, 1982)

Table 1. Weed host species of *Meloidogyne incognita* (Mi), *M. javanica* (Mj) and *M. arenaria* (Ma) in northeast Spain.

Botanical family	Plant species	*Meloidogyne* species
AMARANTHACEAE	*Amaranthus albus* L.	Mi, Mj
	A. blitum L.	Mj
	A. graecizans L. ssp. *sylvestris* (Vill.)	Mi
	A hybridus L.	Mi
	A. retroflexus L.	Ma, Mj
CARYOPHYLLACEAE	*Stellaria media* (L.) Vill.	Mi, Ma, Mj
CHENOPODIACEAE	*Atriplex patula* L.	Mi
	Chenopodium album L.	Mi, Mj
	Chenopodium murale L.	Mj
COMPOSITAE	*Cirsium arvense* (L.) Scop.	Mj
	Erigeron L. spp.	Mi, Mj
	Galinsoga parviflora Cav.	Mi
	Senecio vulgaris L.	Mi, Mj
	Sonchus oleraceus L.	Mi, Mj
	Sonchus tenerrimus L.	Mi, Ma, Mj
	Xanthium strumarium L.	Mi
CONVOLVULACEAE	*Convolvulus arvensis* L.	Mi, Mj
CRUCIFERAE	*Capsella bursa-pastoris* (L.) Medicus	Mi, Ma, Mj
	Coronopus didymus (L.) Sm.	Mi, Mj
	Lepidium draba L.	Mj
CYPERACEAE	*Cyperus rotundus* L.	Mi
EUPHORBIACEAE	*Mercurialis annua* L.	Mj
GERANIACEAE	*Geranium molle* L.	Mi
GRAMINEAE	*Bromus wildenowii* Kunth.	Mi
	Cynodon dactylon (L.) Pers.	Mi
	Digitaria sanguinalis (L.) Scop.	Mi, Ma, Mj
	Echinochloa crus-galli (L.) Beauv.	Mi, Mj
	Lolium perenne L.	Mi
	Poa annua L.	Mi, Ma
	Setaria verticillata (L.) Beauv.	Mi, Ma, Mj
	Sorghum halepense (L.) Pers.	Mi
LABIATAE	*Lamium amplexicaule* L.	Mi
	Mentha L. spp.	Mj
LEGUMINOSAE	*Medicago arabica* (L.) Hudson	Ma, Mj
	Trifolium L. spp.	Mi, Mj
	Vicia sativa L.	Mi
OXALIDADCEAE	*Oxalis corniculata* L.	Mj
	Oxalis corymbosa DC.	Ma
POLYGONACEAE	*Polygonum aviculare* L.	Mi
	Rumex crispus L.	Ma, Mj
PORTULACACEAE	*Portulaca oleracea* L.	Mi, Mj
PRIMULACEAE	*Anagallis arvensis* L.	Ma
ROSACEAE	*Potentilla reptans* L.	Mi
SCROPHULARIACEAE	*Veronica hederifolia* L.	Mi
SOLANACEAE	*Solanum nigrum* L.	Mi, Mj
URTICACEAE	*Urtica urens* L.	Mi

Weeds compete with crops for nutrients, moisture, sunlight and can harbor pathogens that damage crops. Fields heavily infested by *Meloidogyne* had patches of poor plant growth and plants showed less dense foliage which allowed profuse weed development. These weeds were invaded by the nematode and showed galled roots. Also, root-knot nematode infected weeds were located at the edges of the fields where they escape biocidal treatments. Volunteer plants from the previous crops, particularly tomato and cucumber, may act as carry-over hosts of the nematode since they showed galled roots frequently. These plants are better host of *Meloidogyne* than weeds and they should be removed soon after they germinate. On the other hand, volunteer plants could act as catch crops since nematodes are attracted to them but they should be removed before the nematode completes its life cycle.

Table 2. Seasonal distribution of the most frequent weeds infected with *Meloidogyne incognita*, *M. arenaria* and *M. javanica* in northeast Spain.

Plant species	Spring	Summer	Autumn	Winter
Portulaca oleracea	**	**	**	
Stellaria media	**		**	**
Sonchus spp (*S. oleraceum*, *S. tenerrimus*)	**	**	**	**
Capsella bursa-pastoris			**	**
Setaria verticilata		**		
Digtiaria sanguinalis		**		

Some widespread weeds found in this study such as *Portulaca oleracea*, *Sonchus* spp., and *Amaranthus* spp. could be considered as bioindicators of root-knot nematode infestations in fallowed fields or prior to cultivation of vegetable crops in Spain. Weed control can help to control root-knot nematode damage to crops, especially in intensive vegetable production systems where most rotation crops are nematode susceptible and short time is left between crops.

References.

BELDA, J., CASADO, E., GOMEZ, V., RODRIGUEZ, M. D. & SAEZ, E. 1994. Plagas y enfermedades de los cultivos hortícolas intensivos. Almeria, 1994. Phytoma España 57: 9-39.

BOLOS, O., VIGO, J., MASALLES, R. M., NINOT, J. M. 1993. Flora manual dels Països Catalans. Editorial Pòrtic S. A., Barcelona. 1247 pp.

BRIDGE, J., PAGE, S. & JORDAN, S. 1986. An improved method for staining nematodes in roots. In SOUTHEY, J. F (Ed.). 1970. Laboratory methods for work with plant and soil nematodes. Ministry of Agriculture, Fisheries and Food. Her Majesty's Stationery Office, London. Pp 82.

EISENBACK, J. D., HIRSCHMANN, J. N., SASSER, J. N. & TRIANTAPHYLLOU, A. C. 1981. A guide to the four most common species of root-knot nematodes (*Meloidogyne* species) with a pictorial key. Raleigh: North Carolina State University Graphics.

ESPARRAGO, G. & NAVAS, A. 1995. Nematofauna fitoparásita asociada a cultivos hortícolas y tabaco en regadíos de Extremadura. Bol. San. Veg. Plagas 21 (3): 303-317.

GRIFFIN, G. D. 1982. Differences in the response of certain weed hosts populations to *Heterodera schachtii*. J. Nematol. 14: 147-182.

IZQUIERDO, J. BOSQUE, J.LL. COLOMER, M. 1996. Distribució i fenologia de les males herbes dels cultius hortícoles del litoral catalá (1995-96): Primers resultats. I Jornada sobre Protecció Vegetal ICEA: 113-118.
ORNAT, C., VERDEJO-LUCAS. 1994. Detección del nematodo *Meloidogyne* en cultivos de tomate en el Maresme (Barcelona). Abstr VII Congreso de la Sociedad Española de Fitopatología. Sitges, Barcelona.
QUÉNÉHERVÉ, P., DROB, F. TOPART, P. 1995. Host status of some weeds to *Meloidogyne* spp., *Pratylenchus* spp., *Helicotylenchus* spp., and *Rotylenchus reniformis* associated with vegetable cultivated in polytunnels in Martinique. Nematropica 25: 149-157.
RODRIGUEZ RODRIGUEZ, R. 1989. Los nematodos del género *Meloidogyne* en hortícolas: biología, daños y control. Cuadernos Fitopat. 20: 95-101.
SASSER, J. N. 1979. Pathogenicity, host ranges and variability in Meloidogyne species. In F. Lamberti & C. E. Taylor eds. Root-knot nematodes (*Meloidogyne* species). Systematics, biology and control Pp. 257-268. London. Academic Press.
SORRIBAS, F. J., S. VERDEJO-LUCAS. 1994. Survey of *Meloidogyne* spp. in tomatofield of the Baix Llobregat county, Spain. J. Nematol. 26: 731-736.
TEDFORD, E. C. & FORTUM B. A. 1988. Weed hosts of *Meloidogyne arenaria* and *M. incognita* common in tobacco fields in South Carolina. Ann. Appl. Nematol. 2: 102-105.
TUTIN, T. G., HEYWOOD, V. H., BURGES, N. A., MOORE, D. M., VALENTINE, D. H., WALTERS, S. M., WEBB, D. A. (Eds.). 1993. Flora Europea. Vol. 5. Cambridge University Press.

Aknowledgement
This work has been partially financed by the Instituto Nacional de Investigaciones Agrarias grant no. SC95-049.

MANAGEMENT OF ROOT-KNOT NEMATODES IN PROTECTED CROPS OF NORTHEAST SPAIN.

S. Verdejo-Lucas, C. Ornat, and F. J. Sorribas

Departamento Patología Vegetal, IRTA, Crta de Cabrils s/n, 08348 Cabrils, Barcelona, Spain, and Departamento Agronomía, ESAB, Comte d'Urgell 187, 08036 Barcelona, Spain.

Abstract

Root-knot nematodes are the most important plant-parasitic nematodes affecting horticultural crops in Spain. A survey of vegetable crops grown intensively in plastic polytunnel greenhouses of coastal areas of northeast Spain showed that 50% of the 66 sites sampled were infested with the nematode. *Meloidogyne incognita*, *M. arenaria*, and *M. javanica* were the species detected. Control of root-knot nematodes in greenhouses heavily rely on the use of preplant soil fumigants and methyl bromide is the chemical most widely used. Despite the broad use of methyl bromide the nematode is detected usually at harvest of the first or second crop after the fumigant application. Nonfumigant nematicides (oxamyl, phenamiphos and ethoprophos) did not provide sufficient level of nematode control. Greenhouse tests have shown that most resistant tomato cultivars available commercially in Spain are highly resistant to *M. incognita* or *M. arenaria* but they are moderately resistant to *M. javanica*. The infectivity and reproductive potential of the *M. javanica* populations examined were higher than those of *M. incognita* and *M. arenaria* on Mi-resistant tomato cultivars.

Introduction

Root-knot nematodes, *Meloidogyne* spp., are the most important plant-parasitic nematodes affecting vegetable crops in Spain due to their widespread distribution and high frequency of infestation (Andreu Lopez *et al.*, 1986, Rodriguez Rodriguez, 1984, Sorribas and Verdejo-Lucas, 1994). Management of root-knot nematodes on vegetable crops has been based heavily on the use of nematicides (Rodriguez Rodriguez, 1989). The use of resistant plants to root-knot nematodes could be an effective, economic, and safe method for nematode management since they considerably reduce nematode reproduction. However, the existance of pathogenic variavility in the nematode may limit the usefulness of the resistant cultivars.

Vegetable crops are grown intensively for fresh market in coastal areas of northeast Spain. These intensive production systems are possible because the climate is characterized by mild winters and warm summers. Growers have developed specialized crop sequences that provide marketable crops economically attractive. Two to three crops are usually cultivated in the same site from spring to winter, and in general, little time is left between crops. The land is family operated and the average size of the greenhouses is 2,000 m^2. Tomato, cucumber and lettuce or french bean constitute the crop sequence more commonly cultivated in plastic polytunnel greenhouses during a growing season. The cultivation of tomato, the most economically important annual crop, occupies the land for about six month of the year, and it is cultivated as an early crop from February to July.

In this paper, we report the result of a survey of tomato fields to detect *Meloidogyne* spp in greenhouses of coastal areas of northeast Spain, the agricultural practices most common in these areas, and the reproductive potential of local populations of the root-knot nematode on resistant and susceptible tomato cultivars under greenhouse conditions.

Materials and Methods

Survey of tomato fields. During the 1991 and 1994 growing seasons, 16 and 50 sites were sampled for *Meloidogyne* in Baix Llobregat and Maresme county, respectively. Sites to be planted with tomato, as the first crop of the season, were identified through direct grower contacts. Composite soil and root samples were collected from plastic polytunnel greenhouses. Samples were taken from the top 30 cm soil layer with a soil auger at pre-plant and at harvest. Samples were sieved to separate root from soil, and nematodes in a 250 cm^3 soil subsample were extracted by the centrifugation-flotation method (Jenkins, 1964). Data on previous crops, chemical treatments and agricultural production practices were obtained from interviews with growers. *Meloidogyne* species were identifided based on morphological characteristics of the head of adult males and perineal patterns of females (Eisenback, 1985), and on their esterase phenotype (Davies and Beadle, 1995).

Greenhouse tests. In experiment 1, the response of seven *M. incognita*-resistant cultivars and one susceptible tomato cultivar to one isolate of *M. incognita*, one of *M. arenaria* and one of *M. javanica* was tested under greenhouse conditions. The resistant tomato cultivars tested were Carmelo, Carpy, Rambo, Mina, Bermuda, Luxor and Empire. The susceptible tomato Precodor was also tested for comparison. Tomato seedlings were transplanted to pots containing steam-sterilized sand 4 weeks after germination. Plants were inoculated with 2 eggs per cm^3 of soil of each nematode isolate. Each isolate-cultivar combination was replicated seven times and plants were maintained in a greenhouse. Nematode reproduction was assessed when the nematode had completed the first generation (7-8 weeks after inoculation). Roots were separated from soil and eggs were extracted from the entire root system in a 0.5% NaOCl solution for 10 minutes. The reproductive potential was calculated as the relationship between the number of eggs per plant at harvest (final population) divided by the number of egg inoculum (initial population).

For experiment 2, two resistant tomato cultivars, Carmelo and Luxor, and a susceptible ultivar Precodor were chosen to determine the reproductive potential of 13 *M. incognita*, 12 *M. arenaria,* and 10 *M. javanica* populations under greenhouse conditions. Experimental conditions and the assessment of nematode reproduction were similar to those of experiment 1. Data were subjected to analysis of variance.

Results and Discussion.

The availability of preplant soil fumigants such as methyl bromide with herbicidal, fungicidal and nematicidal properties has been a critical factor in the development of high value multiple cropping systems such as the ones studied here. Fumigation with methyl bromide accounted for 63 and 60% of the preplant applications in 1991 and 1994, respectively, (Table 1). Non-fumigants nematicides were applied in 22% of the sites in Maresme county. During the

growing season, root-knot nematodes were easily detected in 6 out of 10 sites fumigated with methyl bromide just before the initial sampling in Baix Llobregat, and in 9 out of 30 sites in Maresme county. All sites treated with non-fumigant nematicides showed population densities of *Meloidogyne* at the end of the crop to which they were applied.

Table 1. Frequency (%) of chemical treatments applied in plastic polytunnel greenhouses before establishing the first crop of the season.

	County	
Chemical	Baix LLobregat*	Maresme**
Methyl bromide	63	60
Metan sodium	13	-
DD + methyl isotiocianate	12	-
Ethoprophos	-	8
Fenamiphos	-	12
Carbofuran	-	2
Untreated	12	18

* Data collected in 1991

** Data collected in 1994

Root-knot nematodes were detected in 33 greenhouses that represented 50% of the 66 sites sampled (Table 2). In most greenhouses, the nematode was found in preplant soil samples. The use of soil fumigants and resistant tomato cultivars probably accounted for the lower nematode detection at harvest. The *Meloidogyne* species identified were *M. incognita, M. arenaria* and *M. javanica*. The esterase phenotypes showed by these populations corresponded to the ones described for each of the nematode species.

Table 2. Distribution and frequency (%) of root-knot nematodes associated to vegetable crops in coastal areas of northeast Spain.

County	Number of sites sampled	Sites infested with *Meloidogyne* spp.	Percentage of infested sites
Baix LLobregat	16	12	75
Maresme	50	21	42
Total	66	33	50

The isolate of *M. javanica* tested against seven resistant and one susceptible tomato cultivar reproduced (Pf/Pi > 1) on the resistant cultivars, whereas the isolates of *M. incognita* and *M. arenaria* did not reproduce (Pf/Pi < 1). Nonetheless *M. javanica* reproduction was lower on the resitant than on the susceptible cultivar (Table 3).

When the reproductive potential of the nematode was examined across species, *M. javanica* showed a Pf/Pi relationship higher (P = 0.05) than that of *M. incognita* or *M. arenaria* on both resistant tomato cultivars (Fig.1). The majority of the *M. incognita* and *M. arenaria* populations reproduced poorly on the resistant cultivars and these reactions can be considered highly resistant, whereas the *M. javanica* populations reacted as moderately resistant.

Table 3. Reproductive potential (final population / initial population) (Pf/Pi) of *Meloidogyne incognita*, *M. arenaria* and *M. javanica* on tomato cultivars carrying the *M. incognita*-resistant gene.

Tomato cultivar	*M. incognita*	*M. arenaria*	*M.javanica*
Resistant			
Carmelo	0.1	0.01	3.8
Carpy	0.1	0.0	4.8
Rambo	0.6	0.2	17.8
Mina	0.2	0.2	5.6
Bermuda	2.0	0.5	na *
Luxor	3.0	0.1	3.0
Empire	na	0.03	na
Susceptible			
Precodor	25	19	85

* Not available

Therefore the existence of Spanish populations of *M. javanica* that show moderates to high reproduction on resistant tomato cultivars emphasizes the need for testing resistant cultivars against local population of the nematode before use in the management of root-knot nematode infested fields. Populations of *M. javanica* able to break resistance on tomato cultivars carrying the M. incognita-resistant gene have been reported recently from Greece (Tzortzakakis and Gowen 1996) and Morocco (Eddaoudi *et al.*, 1997).

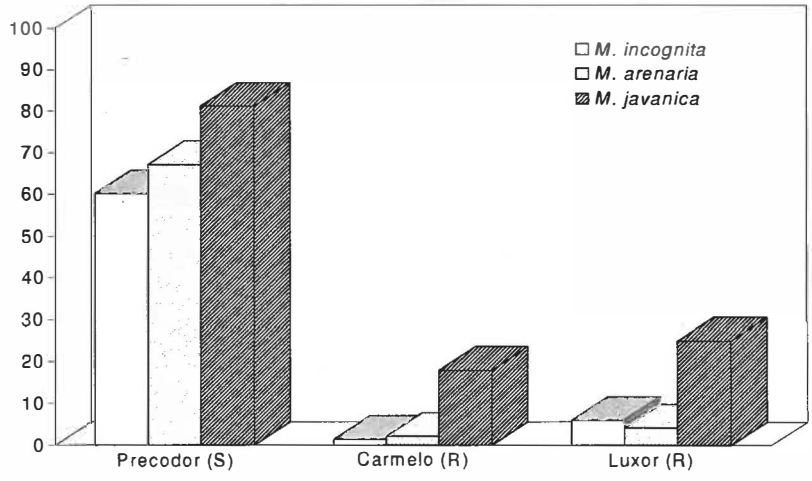


Fig. 1. Reproductive potential (final population / initial population) of the three most common species of *Meloidogyne* present in Spain on two resistant and one susceptible tomato cultivar. Each value is mean of 13 populations of *M. incognita*, 12 populations of *M. arenaria* and 10 populations of *M. javanica*.

References

ANDREU LOPEZ, M., SALMERON PARRA, T., MARTINEZ SIERRA, V.& TOBAR JIMENEZ, A. 1986. Nematodos fitoparásitos de los cultivos horticolas de Trigueros (Huelva). Bol. San.Veg. Plagas. 12: 319-322.

DAVIES, K. G. & BEADLE, J. I. 1995. Use of a cellulase acetate electrophoresis system for the simultaneous characterization of individual root-knot (*Meloidogyne* spp) females using three isozymes. Fundam. appl. Nematol. 18: 459-551.

EDDAOUDI, M., AMMANTI, M., RAMMAH, A. 1997. Identification of the resistance breaking populations of *Meloidogyne* on tomatoes in Morocco and their effect on new sources of resistance. Fundam. appl. Nematol., 20:285-289.

EISENBACK, J. D. 1985. Detailed morphology and anatomy of second-stage juveniles, males and females of the genus *Meloidogyne* (root-knot nematodes). En:. SASSER, J. N., CARTER, C. C. (Eds). An advanced treatise on *Meloidogyne* Volume I: Biology and control. Raleigh: North Carolina State Univ. Graphics, North Carolina, USA, pp.47-77.

HUSSEY, R. S. & BARKER K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp, including a new technique. Plant Dis. Rep. 57: 1025-1028.

JENKINS, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Rep. 48: 692.

RODRIGUEZ RODRIGUEZ, R. 1984. El género *Meloidogyne* en Canarias. 1. Rastreo geográfico preliminar y especies encontradas. Xoba. 4: 41-51.

RODRIGUEZ RODRIGUEZ, R. 1989. Los nematodos del género *Meloidogyne* en hortícolas, biología, daños y control. Cuadernos Fitopat. 20: 95-101.

SORRIBAS, F. J. & VERDEJO-LUCAS, S. 1994. Survey of *Meloidogyne* spp. in tomato production fields of Baix Llobregat county, Spain. J. Nematol. 26: 731-736.

TZORTZAKAKIS, E. A. & GOWEN, S. R. 1996. Occurrence of a resistance breaking pathotype of *Meloidogyne javanica* on tomatoes in Crete, Greece. Fundam. appl. Nematol. 19: 283-288.

Aknowledgement

This work has been partially financed by the Instituto Nacional de Investigaciones Agrarias grant no. SC95-049.

IV. Whiteflies
Aleurodes

CURRENT STATUS OF *BEMISIA TABACI* (GENNADIUS) IN SPAIN: THE PRESENCE OF BIOTYPES OF THIS SPECIES.

F. BEITIA[1], I. MAYO[2], E.M. ROBLES-CHILLIDA[2] , P. GUIRAO[3] & J.L. CENIS[3].

[1] CIT-INIA, Area Protección Vegetal. Ctra. La Coruña, Km.7, 28040 Madrid -SPAIN-
[2] CSIC, Centro de Investigaciones Biológicas. c/ Velazquez, 144, 28006 Madrid -SPAIN-
[3] CIDA, Dpto. Protección Vegetal. Estación Sericícola, 30150 La Alberca, Murcia -SPAIN-

Abstract

The appearance and characterisation of a new biotype of *Bemisia tabaci*, the "B" type, which is now considered as a new species (*Bemisia argentifolii*) for some authors, has led us to investigate the situation of the sweetpotato whitefly in Spain.

Firstly, two methods were carried out: the ability of Spanish populations to induce a phytotoxic disorder in squash plants, the "silvering of leaves", which has only been imputed to the "B" type; and the analysis of Spanish populations by the RAPD-PCR technique.

Results obtained with these two methods have allowed us to identify two different biotypes of *Bemisia tabaci* in Spain: the world-wide well known "B" type and a new one named "non-B" type.

The analysis of differences in the ultrastructural morphology of whitefly pupal cases by using the Scanning Electron Microscopy, and laboratory studies on the comparative biology were added in to confirm remarkable differences between the two biotypes.

These results are very important due to the possible differential incidence on crops (damages, virus transmission, ...) of these biotypes and their influence in the application of biological control of this pest.

1. Introduction

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) 1889, is well known in Spain since 1943, when it was reported for the first time (Gómez-Menor, 1943), but its importance as a pest had not been too remarkable.

However, in the last 10 years, this whitefly has reached the status of a pest, producing significant losses on several vegetable and ornamental crops in the Canary Islands (Carnero et al., 1990) and the southeastern coast of the Iberian Peninsula (Rodríguez et al., 1994).

The current importance of *B. tabaci* around the world have been attributed to a new biotype of this species, named the "B" type (Costa & Brown, 1991). More recently, this biotype has been described as a new species, *Bemisia argentifolii* Bellows & Perring, n. sp. (Bellows et al., 1994), but this description has produced a great controversy (Brown et al., 1995a).

In the present work, we use different tools in order to characterize biotypes of *B. tabaci* present in Spain. Four procedures have been carried out: Firstly, the induction of the silverleaf disorder on squash plants by whiteflies and the analysis of amplified DNA bands using the RAPD-PCR technique, were used to detect the presence of several biotypes of this species. After that, the analysis of biotypes using Scanning Electron Microscopy and their comparative biology were examined.

The importance of the presence of several biotypes of *B.* tabaci in our country is remarkable for the application of integrated control to this insect pest.

2. Material and methods

2.1. Insect populations.

Populations of *B. tabaci* analysed by the induction of silvering on squash plants were collected from different areas of Spain, between 1993 and 1996. Laboratory rearing of these populations was made on several host plants to provide individuals for experiments.
In addition to the Spanish populations, several foreign populations (adult samples fixed in 70% ethanol and supplied by various colleagues) were used in the RAPD-PCR experiment to compare their pattern of amplified DNA bands. All populations are shown in Table 1.

Table 1. Populations of *Bemisia tabaci* used in this work.

Code	Origin	Host Plant	Silvering	Type
1	Almeria (Sp)	Tomato	–	non B
2	Almeria (Sp)	Squash	+	B+non B
3	Barcelona (Sp)	Cabbage	+	B
4	Madrid (Sp)	Poinsettia	+	B
5	Malaga (Sp)	Tomato	+	B
6	Mallorca (Sp)	Tomato	–	non B
7	Murcia (Sp)	Melon	–	non B
8	Murcia (Sp)	Tomato	–	non B
10	Sevilla (Sp)	Cotton	–	non B
11	Tenerife (Sp)	Poinsettia	+	B
12	Valencia (Sp)	Pepper	–	non B
13	Algarve (P) **(1)**	Green Bean		non B
14	Antibes (F) **(2)**	Lantana		B
15	Cesena (I) **(3)**	Tobacco		B
16	Netherlands **(4)**	Gerbera		B
17	Denmark **(5)**	Poinsettia		B
18	Tel Aviv (Is) **(6)**	Tobacco		B
19	India **(7)**	Watermelon		H
20	Pakistan **(7)**	Cotton		K
21	Turkey **(7)**	Cotton		M
22	Japan **(8)**	Cabbage		I
23	Arizona (USA) **(9)**	¿ ?		A
24	Arizona (USA) **(9)**	¿ ?		B
25	California (USA) **(7)**	Cotton		B

Foreign insects supplied by:
(1) Dr. J.Guimaraes. **(2)** Dr. J.C.Onillon. **(3)** Dr. M.Bennuzi. **(4)** Dr. J. Fransen. **(5)** Dr. A.Enkegaard. **(6)** Dr. M.Berlinger.**(7)** I.Bedford. Population 25 characterized as "B" type. **(8)** Dr. M.Matsui. **(9)** Dr. J.K. Brown. Population 23 characterized as "A" type and population 24 as "B" type.

2.2. Induction of silvering.

It is known by the bibliography that the "B" type has the ability to cause phytotoxic disorders on plants, such as squash siverleaf symptoms, only induced by feeding of immatures of this "B" type (Yokomi et al., 1990; Costa & Brown, 1991; Costa et al., 1993). To verify the

presence of the "B" type in Spain, adults of Spanish populations of *B. tabaci* were tested for their ability to induce symptoms of silverleaf in squash plants, *Cucurbita pepo* L., "Nize" cultivar (VILMORIN, La Verpilliere, France).

Three four-leaved squash plants (replicates) were exposed to 50 adults of *B. tabaci* each one, for every whitefly population, and three replicates not exposed to whiteflies were used as controls. Plants with insects were individually placed into ventilated plastic cylinder cages, at 22-27 °C and 60-80 % relative humidity, under a 16 h. fluorescent daylength. Plants were assessed daily for induction of silverleaf symptoms until the appearance of F_1 adults.

2.3. RAPD-PCR reactions.

The application of the RAPD-PCR technique to the biotype determination in *B. tabaci* has been well developed in last years (Gawel & Bartlett, 1993; Perring et al., 1993; Guirao et al., 1994; Brown et al., 1995a).

DNA of insects was extracted as described by Cenis et al. (1993). The PCR reactions were made according to a protocol described by Cenis et al. (1993) and Guirao et al.(1996). Primers used were ten-mer oligonucleotides of arbitrary sequence (Operon technologies): OPB-05, -07, -10, -11, -13, -14, -18, -20, OPC-03, -04, -07, -10, -12, -13, -14, -15, and -16.

The PCR products were loaded into a 1.4 % agarose gel in 1X TBE buffer, and electrophoresis was run at 5 V/cm for 2 hours. Amplified DNA bands were visualized and photographed under UV light after staining the gel with a solution of 0.5 µl/ml of ethidium bromide. Two replications of the reactions were made. The F estimator of similarity of Nei & Li (1979) between pairs of populations was calculated.

2.4. Scanning Electron Microscopy.

Aleyroid pupal cases (exuvium of the fourth nymphal instar) provide many characters which are used to assist in the identification of species (Martin, 1987).; but it is well known that physical characteristics of the host plant can affect the appearence of the puparia of some species, as it is the case in *Bemisia tabaci* (David & Ananthakrishnan, 1976; Mohanty & Basu, 1986 ; Bellows et al., 1994; Rosell et al., 1996).

One representative population of each biotype present in Spain was selected to the SEM experiment: the population from Mallorca ("non B" type) and the population from Tenerife ("B" type). Both populations were reared on gherkin plants, *Cucumis sativus* L. "Petit de Paris" cultivar (VILMORIN, La Verpilliere, France), in the laboratory for approximately 20 generations before their use, in order to avoid the effect of the host plant on the morphology of the puparia.

A total of 20 "B" and "non B" type specimens were processed for the SEM. Pupae were fixed in 4% phosphate-buffered glutaraldehyde for 48 hours, at 0,25 M and pH 7,3 and dehydrated in a graded series of acetone. Specimens were then attached to aluminium stubs and dried by means of the critical point method, before coating with a gold alloy in a vacuum evaporator. They were examined at 20 Kv, using a Philips Edax DX4 Scanning Electron Microscope.

2.5. Comparative biology of biotypes.

Some authors have studied the comparative biology and development of different populations of *Bemisia tabaci*, and this is one character used to distinguish different biotypes of this species (Bethke et al., 1991; Costa & Brown, 1991; Perring, 1996).

In order to continue the characterization of Spanish biotypes, an experiment was developed using two populations of the whitefly: one of the "B" type (Tenerife) and another one of the "non B" type (Mallorca); both of them had been reared in laboratory on gherkin as host plant

for several generations. One-leaved bean plants, *Phaseolus vulgaris* L. "Contender" cultivar (VILMORIN, La Verpilliere, France), maintained in a nutrient solution (Beitia & Garrido, 1991) were used as test plants. Experimental conditions were: 25 ± 1 °C, 60-80 % relative humidity and photoperiod of 16:8 (L:D).
Two groups of experiments have been carried out: **1.** Development and mortality of immatures; **2.** Adult longevity and reproductive capacity.

1.- About 50 adults (males and females) were put into a ventilated cylinder cage (30cm x 11,5cm diameter) that contained a bean plant, with a total of 5 cages (replicates) for each biotype. At the end of the 6-h oviposition period, the adults were removed and 25-30 eggs on the under side of the leaf were selected randomly.
Following eclosion and after crawlers had been fixed on the leaf surface, a sketch of the leaf with the position of each 1st-instar nymph was made. Nymphs were obseved twice in a day to detect moults. When red eye spots appeared on 4th nymphal instar, it was considered as the pupal stage.
Percentages of mortality were analyzed by the construction of 2X2 contingency tables; developmental times were analyzed using the **t** test for comparison of means between two samples and data were transformed by **ln (y+1)** transformation, and means were compared using the **t** test.

2.-. In the pupal stage, many individuals from the stock colonies were selected and individually placed into transparent plastic capsules until adult emergence. For each biotype, a total of 30 couples were formed and placed separatedly into a ventilated cylinder cage (30 cm X 11.5 cm diameter) that contained a bean plant. Eggs layed per female were counted daily and removed from the leaf in order to use the same plants for all the experiment. Observations were made until the death of all couples.
Differences in daily and total oviposition between biotypes were analyzed by the **t** test for comparison of means between two samples and data were transformed by **arcsine square-root** transformation. Differences in adult longevity were analyzed by analysis of variance (ANOVA) with two factors (sex and biotype), and data were previously transformed by **ln (y)** transformation.

3. Results and discussion

3.1. Induction of silvering.

Typical squash "silverleaf" symptoms were observed in six of the populations tested (Table 1). Newly emerged leaves were specially affected by silvering despite the absence of whitefly nymphs, which is in agreement with Yokomi et al. (1990). Initially, silvering was only detected in main veins and after a time the upper surface of the leaf was completely affected. According to many authors (Yokomi et al., 1990; Costa & Brown, 1991; Costa et al., 1993), these populations could be considered as the "B" type of *Bemisia tabaci*.

3.2. RAPD-PCR reactions.

All Spanish populations tested could be grouped in two genetic types according to their pattern of amplified DNA bands (Table 1). Type I includes all populations inducing silvering symptoms on squash plants and for that could be considered as "B" type; this consideration is consistent with the fact that foreign populations tested from California and Arizona

(previously characterized as "B" type and sent to us by american colleagues), were also included in the Type I.
The remaining Spanish populations, just as the Portugese one, were all considered as a Type II ("non B" type), and showed RAPD patterns different from the other foreign populations. Four distinct RAPD patterns were obtained for populations from Arizona (characterized and received as "A" type), India ("H" type), Pakistan ("K" type) and Turkey ("M" type).
The genetic similarity was calculated for all populations. The "A" type from Arizona is very different from the rest (a genetic similarity of 35%). The other populations could be grouped in three clusters: One of this includes populations of the Middle East (India, Pakistan and Turkey), although each of them are distinct; a second cluster includes all populations of the "B" type from all the world; and a third cluster, closer to the last one, assemble the "non B" type populations (Type II).

3.3. Scanning Electron Microscopy.
In the "non B" type, the common pupal shape is oval with slight polar asymmetry. The average size of specimens was 470μm long by 330μm wide. On each lateral anterior margin occur two remarkable and robust wax fringes and a third one is on the caudal margin extending lateral of caudal setae, which is similar to data reported on *B. tabaci* by Bellows et al. (1994). Those fringes correspond with thoracic and caudal tracheal openings (Martin, 1987).
On the other hand, the pupal shape of the "B" type is lanceolated with a pronounced polar asymmetry, and its average size was 383μm long by 258 μm wide. Thoracic and caudal wax fringes are scarce, and narrower and less robust than in the "non B" type. According to Bellows et al. (1994), the character of wax fringes is consistent among specimens from a variety of host plant species, and therefore it could be used to differentiate between biotypes.
In the "non B" type, it was found the presence of seven pairs of short dorsal setae (DS), associated with the head (DS1), the prothorax (DS2), the mesothorax (DS3) and the 1st (DS5), 3rd (DS?), 5th (DS?) and 8th (DS6) abdominal segments, as it has been numbered by Bellows et al. (1994), who pointed out the presence of 6 DS; however, seven pairs of dorsal setae in the pupal case of *B.* tabaci had already been noted by David & Ananthakrishnan (1976). In contrast with those findings, in the pupal case of the "B" type we only found the presence of four pairs of enlarged dorsal setae (DS1, DS2, DS3 and DS5) and one pair of short setae (DS6).
Finally, in the anterior peripherical area, one pair of anterior marginal setae (AMS) was found in pupal cases of the "B" and the "non B" types. And the presence of four pairs of anterior submarginal setae (ASMS) was revealed on the "non B" type, in contrast to three pairs of ASMS in the "B" type.
In spite of the variation in morphological characters of the *Bemisia* pupal case, in response to differences in the leaf surface topology and environmental and physical factors (Rosell et al., 1996), which could affect their use to distinguish between biotypes of *Bemisia tabaci*, our results can be useful taking into account the homogeneus conditions in the rearing of specimens of the two biotypes used for the SEM.

3.4. Comparative biology of biotypes.

3.4.1. Development and survivorship of immatures.
Developmental time for the immature stages of the two biotypes is presented in Table 2. There were significant differences in developmental periods of egg and 1st, 2nd and 3rd nymphal instars. For the 4th nymphal instar and the pupal stage data comparison was not

possible due to the small number of individuals of the "B" type that reached those stages (data in Table 2 correspond with means of 6 individuals for the 4th nymphal instar and 2 individuals for the pupal stage). In general, developmental time of immatures (from egg to adult emergence) is higher in the "B" type (42,39 days) than in the "non B" type (28,75 days). There also were significant differences in percentage of mortality from egg to adult stage for all immature stages, between the two biotypes (Table 3).

Table 2. Developmental periods (days) of immature stages in biotypes of *B. tabaci* (mean ± SE).

Biotype	n	Egg	1st instar	2nd instar	3rd instar	4th instar	Pupal stage
"B"	131	7,76±0,08	9,59±0,42	4,82±0,32	5,39±0,44	*10,33±2,09*	*4,50±2,50*
"non B"	127	8,18±0,06	4,89±0,22	3,37±0,21	3,85±0,16	5,05±0,08	3,41±0,05

Table 3. Mortality percentages of immature stages (and cumulative mortality) in biotypes of *Bemisia tabaci*.

Biotype	Egg	1st instar	2nd instar	3rd instar	4th instar	Pupal stage	From egg to adult
"B"	24,43 **(24,43)**	33,33 **(49,61)**	40,91 **(70,22)**	41,03 **(82,44)**	73,91 **(95,41)**	66,67 **(98,47)**	**98,47**
"non B"	7,09 **(7,09)**	11,02 **(17,32)**	9,52 **(25,19)**	7,37 **(30,70)**	1,14 **(31,49)**	0,00 **(31,49)**	**31,49**

3.4.2. Adult longevity and reproductive capacity.

There were no significant differences in adult longevity considering sex, biotypes and the interaction of both factors (Table 4). The longest individual female and male longevities were respectively recorded at 46 and 49 days for the "B" type, and at 62 and 51 days for the "non B" type.

There also were no significant differences in the total number of eggs per female between the two biotypes (Table 4).

Table 4. Adult longevity and Oviposition in biotypes of *Bemisia tabaci* (mean ±SE).

Biotype	Longevity		Oviposition	
	Female	Male	Eggs/F	Eggs/F/Day
"B"	25,83±1,78	26,73±1,47	143,43±14,04	5,55±0,11
"non B"	31,03±2,61	26,47±1,96	152,67±14,80	4,92±0,11

4. Conclusions

These results suggest the presence in Spain of two different genetic types. According to results of silvering induction and RAPD patterns, we can assign one of the genetic types to the "B" type of *B. tabaci*, which is in agreement with previous information about the presence of biotype "B" in Murcia (Arnó & Gabarra, 1994), Barcelona (R. Gabarra, pers. comm.) and Almeria (I. Bedford, pers. comm.). All "B" type populations studied have a similarity higher than 90%, and this is consistent with the assumption that "B" type has spread throughtout the world and has recently been introduced in Spain. By contrast, the genetic type II, a "non B" type, is genetically closer to the B type than to the other non B populations tested.

If we put together all results obtained until now, we can consider the presence of two different biotypes. And this assumption has also been shown by differences in esterase patterns (Byrne & Devonshire, 1996; Guirao et al., 1997). When considering all differences found between the two types, it would be proposed that the "non B" genetic type could constitute a distinct biotype of *B. tabaci*.

In other studies, some authors have proposed a letter code to designate esterase electromophs corresponding to every biotype of *Bemisia tabaci* (Brown et al., 1995b). The letter to name this "non B" biotype depends on the ending of studies carried out by other authors (J. Brown and I. Bedford, pers. comm.).

These conclusions are very significant since, from now on, all studies about *Bemisia tabaci* populations in Spain (resistance to pesticides, virus transmission, damages on crops, the use of parasitoids as biological control agents,...) should take the presence of the two biotypes into account.

5. Acknowledgments

We hereby like to thank all colleagues that have sent us samples of Spanish and foreign populations of *Bemisia tabaci*. This research has partially been supporrted by a National Research Project (INIA, SC93-045-C2-1).

6. References

ARNO, J. & GABARRA, R., 1994. Whitefly species composition in winter tomato greenhouses. IOBC/WPRS Bull., **17(5)**: 104-109.

BEITIA, F. & GARRIDO, A., 1991. Influence of relative humidity on development and egg-laying in *Panonychus citri* under controlled conditions. EPPO Bull., **21**:719-722.

BELLOWS, T. S. J., PERRING, T. M., GILL, R. J. & HEADRICK, D. H., 1994. Description of a Species of *Bemisia* (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Am., **87(2)**: 195-206.

BETHKE, J. A., PAINE, T. D. & NORESSLY, G. S., 1991. Comparative biology, morphometrics, and development of two populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) on cotton and poinsettia. Ann. Entomol. Soc. Am., **84**: 407-411.

BROWN, J. K., FROHLICH, D. R. & ROSSELL, R. C., 1995a. The sweetpotato or silverleaf whiteflies: Biotypes of *Bemisia tabaci* or a species complex?. Ann.Rev.Entomol., **40**:511-534.

BROWN, J.K., COATS, S.A., BEDFORD, I.D., MARKHAM, P.G., BIRD, J. & FROHLICH, D.R., 1995b. Characterization and distribution of esterase electromorphs in the whitefly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). Biochemical Genetics, **33(7/8)**: 205-214.

BYRNE, F.J. & DEVONSHIRE, A.L., 1996. Biochemical evidence of haplodiploidy in the whitefly *Bemisia tabaci*. Biochemical Genetics, **34(3/4)**: 93-107.

CARNERO, A., MONTESDEOCA, M., PÉREZ, F., SIVERIO, A. & RODRIGUEZ, P., 1990. Presencia de *Bemisia tabaci* (Genn,) en cultivos comerciales de hortícolas y ornamentales en la isla de Tenerife (Islas Canarias). Cuadernos de Fitopatologia, 4° Trimestre, **25**: 176-180

CENIS, J. L., PEREZ, P. & FERERES, A., 1993. Identification of various Aphids (Homoptera: Aphididae) species and clones by Random Amplified Polymorphic DNA (RAPD-PCR). Ann. Entomol. Soc. Am., **86(5)**: 545-550.

COSTA, H. S. & BROWN, J. K., 1991. Variation in biological characteristics and esterase pattems among populations of *Bemisia tabaci* Genn. and the association of one population with silverleaf symptom induction. Entomol. Exp. Appl., **61**: 211-219.

COSTA, H.S., ULLMAN, D.E., JOHNSON, M.W. & TABASHNIK, B.E., 1993. Squash silverleaf symptoms induced by immature, but not adult, *Bemisia tabaci*. Phytopathology, **83(7)**: 763-766.

DAVID, B.V. & ANANTHAKRISHNAN, T.N., 1976. Host correlated variation in *Trialeurodes rara* Singh and *Bemisia tabaci* (Gennadius) (Aleyrodidae: Homoptera: Insecta). Current Science, **45(6)**: 223-225.

GAWEL, N. J. & BARLETT, A. C., 1993. Characterization of differences between whiteflies using RAPD-PCR. Insect Molecular Biology, **2(1)**: 33-38.

GÓMEZ-MENOR, J., 1943. Contribución al conocimiento de los Aleyródidos de España (Hem., Homoptera), 1ª nota. Eos, **19**: 173-209.

GUIRAO, P., BEITIA, F. & CENIS, J. L., 1994. Aplicación de la técnica RAPD-PCR a la taxonomía de moscas blancas (Homoptera:Aleyrodidae). Bol.San.Veg.Plagas, **20(3)**: 757-764.

GUIRAO, P., BEITIA, F. & CENIS, J. L., 1996. Biotipos de *Bemisia tabaci* presentes en España, pp: 47-54. *In*: Consejería de Medio Ambiente, Agricultura y Agua. Región de Murcia (eds.), El virus del rizado amarillo (hoja en cuchara) del tomate (TYLCV) y su vector *Bemisia tabaci*.

GUIRAO, P., BEITIA, F. & CENIS, J.L., 1997. Biotype determination in Spanish populations of *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). Bull. Entomol. Res. (in press).

MARTIN, J. H., 1987. An identification guide to common whitefly pest species of the world (Homoptera, Aleyrodidae). Tropical Pest Management, **33(4)**:298-322.

MOHANTY, A. K. & BASU, A. N., 1986. Effect of host plants and seasonal factors on intraspecific variations in pupal morphology of the whitefly vector, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). J. Entomol. Res., **10**: 19-26.

NEI, M. & LI, W. S., 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA, **76**:5269-5273.

PERRING, T. M., 1996. Biological differences of two species of *Bemisia* that contribute to adaptive advantage, pp: 3-16. *In*: D. Gerling & R. T. Mayer (eds.), Bemisia: 1995. Taxonomy, Biology, Damage, Control and Management. Intercept Ltd., Andover, UK.

PERRING, T. M., COOPER, A. D., RODRÍGUEZ, R. J., FARRAR, C. A. & BELLOWS, T.S. Jr., 1993. Identification of a whitefly species by genomic and behavioral studies. Science, **259**: 74-77.

RODRÍGUEZ, M. D., MORENO, R., TÉLLEZ, M. M., RODRÍGUEZ, M. P. & FERNÁNDEZ, R., 1994. *Eretmocerus mundus (Mercet), Encarsia lutea* (Masi) y *Encarsia transvena* (Timberlake) (Hym., Aphelinidae) parasitoides de *Bemisia tabaci* (Homoptera: Aleyrodidae) en los cultivos hortícolas protegidos almerienses. Bol San. Veg. Plagas, **20**: 695-702.

ROSELL, R. C., BEDFORD, I. D., MARKHAM, P. G., FROHLICH, D. R. & BROWN, J.K., 1996. Morphological variation in *Bemisia* populations, pp: 147-149. *In*: D. Gerling & R. T. Mayer (eds.), Bemisia: 1995. Taxonomy, Biology, Damage, Control and Management. Intercept Ltd., Andover, UK.

YOKOMI, R. K., HOELMER, K. A. & OSBORNE, L. S., 1990. Relationships between the sweetpotato whitefly and the squash silverleaf disorder. Phytopathology, **80**: 895-900.

SUR LA PRESENCE EN TUNISIE DES BIOTYPES "B" ET "NON B" DE *Bemisia tabaci* (HOMOPTERA, ALEYRODIDAE) ET DE LEURS PARASITOIDES ASSOCIES.

Chermiti B., [1] Braham M., [1] Cenis J.L.,[2] Alonso C.,[3] & Beitia F.[3]

[1] École Supérieure d'Horticulture de chott-Mariem. Laboratoire de Zoologie Agricole, 4042 Sousse, TUNISIE.
[2] CIDA, Dept. Protección Vegetal, Estación Sericícola, 30150 La Alberca, Murcia-SPAIN.
[3] CIT INIA, Area Protección Vegetal. Ctra. De la Coruña, Km 7. 28040 Madrid- SPAIN.

Résumé

Des analyses utilisant la technique de l'amplification enzymatique des fragments de l'ADN (RAPD-PCR) pour déterminer les biotypes de *Bemisia tabaci* (Homoptera, Aleyrodidae) existant en Tunisie ont été réalisées sur 19 populations d'adultes collectées sur diverses plantes hôtes dans différents biotopes. Les résultats indiquent que les deux souches "B" et "non B" sont présentes et ce indépendamment de la plante hôte et de la localisation géographique.

Deux espèces de parasitoïdes ont été identifiées par observation des larves parasitées du dernier stade de l'aleurode et par montage des adultes, il s'agit d'*Eretmocerus mundus* Mercet et d' *Encarsia transvena* Timberlake (Hymenoptera, Aphelinidae). Seule *E. mundus* est présente dans tous les biotopes.

1. Introduction

L'aleurode du cotonnier, du tabac ou de la patate douce, *Bemisia tabaci* (Homoptera, Aleyrodidae) est décrit pour la première fois en Grèce en 1889 (Gennadius, 1889; Mound & Halsey, 1978). C'est un insecte des zones tropicales et subtropicales du globe, son importance actuelle dans le monde comme ravageur des cultures maraîchères, florales et ornementales est attribuée à l'introduction et à la dispersion d'un nouveau biotype de l'espèce caractérisé par Costa & Brown (1991) comme le biotype "B". Par la suite certains chercheurs Américains ont décidé de faire la description de ce nouveau biotype comme une nouvelle espèce et lui ont donné le nom de *Bemisia argentifolii* Bellows & Perring n.sp. (Bellows *et al.,* 1994). Il reste cependant une forte controverse sur cette nouvelle espèce (Bartlett & Gawel, 1993; Campbell *et al.*, 1993; Perring *et al.*, 1993; Brown *et al.*, 1995a).

Plusieurs recherches pour identifier et caractériser les biotypes de *B. tabaci* se sont développées durant les cinq dernières années (Bartlett & Gawel, 1993; Bedford *et al.*, 1993; Brown *et al.*, 1995a et b; Guirao *et al.*, 1996; Guirao *et al.,* 1997a). Des récentes recherches entreprises en Espagne ont montré la présence de deux biotypes de l'aleurode: le biotype "B" et un autre biotype "non B" (Guirao *et al.*, 1996; Guirao *et al.,* 1997b).

Par ailleurs, plusieurs auteurs se sont intéressés à l'identification des parasitoïdes associés à *B. tabaci* dont Kirk *et al.*, (1993) et Onillon *et al.*, (1994).

Ce travail préliminaire a pour objectif la connaissance du ou des biotypes tunisiens de *B. tabaci* ainsi que l'inventaire des parasitoïdes se développant sur cet aleurode.

2. Materiels et méthode

2.1. Collections: des prospections ont été réalisées sur différentes plantes hôtes dans diverses zones géographiques aussi bien sous abri qu'en plein champ et ce pendant les mois d'avril, de mai et de novembre 1996.

Les adultes de *Bemisia* sont collectés à l'aide d'un aspirateur à bouche, mis immédiatement dans l'alcool 70° et envoyés au Dr Beitia (CIT INIA, Madrid, Espagne) pour identification.

2.2. Analyse utilisant la technique RAPD-PCR

- L'extraction de l'ADN a été réalisée d'après une méthodologie définie par Cenis & Beitia (1994) pour l'utilisation sur des insectes de très petites taille. Pour chaque population, le ou les adultes de *B. tabaci* sont broyés dans un tube Eppendorf contenant 50 µl d'une solution d'extraction. Après une bonne homogénéisation, on ajoute 15 µl d'huile minérale au mélange et on met les tubes à 60°C pendant 60 minutes puis à 95°C pendant 10 minutes. Ainsi, l'extrait de l'ADN est prêt à l'utilisation ou peut être conservé à - 20°C.

La composition de la solution d'extraction est la suivante (pour 50 µl):
5 µl de tampon PCR (Perkin Elmer Stoffel Fragment, 10X),
0,5 µl de Tween 20 (concentration 20%),
0,3 µl de proteinase K (Sigma, 10 mg/ml), et
44,2 µl d'eau stérilisée (Milli-Qplus 185, Millipore)

Deux populations d'Espagne appartenant aux biotypes "B" et "non B" en élevage au laboratoire et bien caractérisées (Guirao *et al.,* 1997b) ont servi de témoins pour les comparer avec les populations tunisiennes.

- Les réactions PCR ont été réalisées d'après le protocole de Guirao *et al.,* (1997b) avec certaines modifications, dans un thermocycleur (*DNA Thermal Cycler, Perkin Elmer Hispania S.A.*) utilisant la programmation suivante: dénaturation de l'ADN à 94°C pendant 1 minute, recombinaison à 34 °C pendant 1 minute, et extension à 72 °C pendant 2 minutes (pour un total de 40 cycles).

La composition de chaque tube de réaction (pour 10,5 µl de solution) est la suivante:
1X Tampon de l'ADN Polymérase (Amplitaq, Perkin Elmer), 2,5 mM de $MgCl_2$, 0,2 mM de dNTPs, 0,1% de Triton X100, 1 U de l'ADN Polymérase (Amplitaq, Perkin Elmer), 7,5 ng de l'amorce (Operon Technologies), et compléter avec de l'eau stérilisée (Milli-Qplus 185, Millipore) jusqu'à atteindre 10,5 µl.

On ajoute à ce mélange 2 µl de l'extrait de l'ADN qu'on couvre avec 15 µl d'huile minérale.

- Pour visualiser les bandes amplifiées de l'ADN des insectes, les produits de la PCR sont chargés dans un gel d'agarose à 1,4 % en tampon TBE 1X et à l'aide d'un tampon de charge (3µl par produit de réaction). Le gel est coloré avec une solution de 0,8 µg/ ml de bromure d'éthidium. L'électrophorèse a été réalisée à 2,5 v/cm pendant 4 heures. Pour photographier les bandes amplifiées, une caméra Polaroid (Fotodyne) avec un transenlumineur Foto UV21 (Fotodyne) ont été utilisés.

Le marqueur 123 bp Ladder (Sigma) (8 µl au 20%) a été utilisé pour déterminer le nombre de paires de bases.

Les amorces utilisées sont des oligonucléotides de séquence arbitraire d'Operon Technologies (Alameda, CA) de 10 paires de bases. Sur la base d'autres analyses précédentes, les amorces OPC-01, OPC-10, OPC-16, OPB-20 (dont les patrons d'amplification sont considérés des marqueurs génétiques pour les biotypes "B" et "non B" ont été testés avec les 19 populations de *B. tabaci*.

2.3. Emergence et identification des parasitoïdes

En plus, des échantillons des feuilles sont ramenés au laboratoire. Les feuilles sont examinées sous loupe binoculaire. Les pupes parasitées sont mises dans des gélules, deux méthodes sont utilisées pour l'identification des parasitoïdes:

- l'observation de la nymphe vivante et du trou de sortie du parasitoïde à l'intérieur de son hôte, la disposition et la couleur du méconium,

- le montage des adultes dans le baume du Canada, puis l'identification utilisant la clef proposée par Polaszek *et al.*, (1992).

3. Résultats

- Biotypes de *B. tabaci*

Le tableau 1 dresse la liste des plantes hôtes, les lieux de prélèvement et les résultats d'analyse. Les 19 populations ont été testées dans une première analyse à partir de l'extraction de l'ADN de 5 adultes. Les résultats montrent que les populations 1; 2 et 16 présentent le même patron d'amplification que le témoin du biotype "non B". Les patrons des populations 3; 4; 5; 6; 7; 8; 9; 10; 11; 12; 14; 15 et 17 correspondent au témoin du biotype "B". Par contre les populations 13; 18 et 19 ont montré des bandes d'amplification caractéristiques des 2 biotypes témoins.

Une analyse complémentaire par extraction individuelle de l'ADN, pour ces trois populations a confirmé la présence d'individus des deux biotypes dans chaque population, c'est à dire, l'existence d'un mélange de biotypes.

- Parasitoïdes

Indépendamment des biotypes "B" et "non B" de *B. tabaci*, deux espèces de parasitoïdes ont été identifiées; elles appartiennent aux genres *Encarsia* et *Eretmocerus* (Hymenoptera, Aphelinidae) *Eretmocerus mundus* Mercet (pas de méconium) et *Encarsia transvena* Timberlake (corps noir, méconium rouge-orangé situé vers la partie postérieure de la pupe, (tableau 2).

Sur *Lantana* de plein air, un fort pourcentage de parasitisme a été observé sur des populations larvaires importantes de *Bemisia*. Les deux parasitoïdes *Er. mundus* et *E. transvena* ont été recensés. Sur tomate, melon et pastèque sous serre les deux parasitoïdes sont également présents.

Espèce	Plantes hôtes	Localisation géographique	Biotypes
1. *B. tabaci*	*L. camara*[1]	Boucherik (cap Bon)	"Non B"
2. *B. tabaci*	*L. camara*[1]	Parc Ibn Jazzar Sousse	"Non B"
3. *B. tabaci*	Tomate[2]	C. Essaâda Téboulba	"B"
4. *B. tabaci*	Poivron[2]	Téboulba	"B"
5. *B. tabaci*	Tomate[2]	Ras Elain Kébili	"B"
6. *B. tabaci*	Tomate[2]	Ras Elain Kébili	"B"
7. *B. tabaci*	Concombre[2]	Kébili	"B"
8. *B. tabaci*	Pastèque[2]	Oum Elfareth	"B"
9. *B. tabaci*	Concombre[2]	Saidane	"B"
10.*B. tabaci*	Melon[2]	Douz	"B"
11. *B. tabaci*	Melon[2]	Steftimi	"B"
12. *B. tabaci*	Pastèque[2]	Steftimi	"B"
13. *B. tabaci*	Courge[2]	Steftimi	"B" et "non B"
14. *B. tabaci*	Concombre[2]	Elhamma Tozeur	"B"
15. *B. tabaci*	Tomate[2]	Elhamma Tozeur	"B"
16. *B. tabaci*	*Lantana camara*[1]	Elkantaoui Sousse	"Non B"
17. *B. tabaci*	*Cistrum nocturnum*[2]	Sidi Bouali	"B"
18. *B. tabaci*	*Lantana camara*[1]	Mini. Agriculture, Tunis	"B" et "Non B"
19. *B. tabaci*	*Lantana camara*[1]	RETEYA Bizerte	"B" et "Non B"

Tableau 1: Plantes hôtes, localisation géographique et biotypes de *B. tabaci* [1] Plein air [2] Serre

Biotypes de *B. tabaci*	Plantes hôtes	Parasitoïdes
1. "Non B"	*L. camara*	*Er. mundus; E. transvena*
2. "Non B"	*L. camara*	*Er. mundus; E. transvena* + *E. pergandiella* ?
3. "B"	Tomate	*Er. mundus*
5. "B"	Tomate	*Er. mundus; E. transvena* + *E. pergandiella* ?
8. "B"	Pastèque	*Er. mundus, E. transvena*
10. "B"	Melon	*Er. mundus*
16. "Non B"	*L. camara*	*Er. mundus*
17. "B"	*Cistrum nocturnum*	*Er. mundus*
18. "Non B"	*L. camara*	*Er. mundus; E. transvena*
?	*Hibiscus mutabilus*	*Er. mundus; E. transvena*

Tableau 2: Parasitoïdes associés à la plante hôte et aux biotypes de *B. tabaci*.

4. Discussion et conclusions

- Biotypes de *B. tabaci*

Aucun inventaire des biotypes de *B. tabaci* ni de leurs parasitoïdes n'a été entrepris jusqu'à présent en Tunisie malgré l'importance qu'il occupe dans le cadre d'un contrôle biologique de ce ravageur. Plusieurs études utilisant l'analyse du profil enzymatique spécifique ont été conduites pour l'identification des biotypes de *B. tabaci* (Perring *et al.*, 1993; Bergh *et al.*, 1995) ou pour la confirmation des espèces d'aleurodes (Guirao *et al.*, 1994).

On peut dire que la situation de *B. tabaci* en Tunisie pourrait être semblable à celle d'Espagne (Guirao *et al.*, 1996; Guirao *et al.*, 1997b): présence de deux biotypes, le biotype "B" (bien connu dans le monde) et un biotype actuellement dénommé "non B" (bien caractérisé en Espagne). Les deux biotypes peuvent cohabiter dans certains biotopes.

La présence de la souche "B" de *B. tabaci* atteste de sa formidable dispersion et probablement de sa distribution cosmopolite, elle est vraisemblablement d'introduction récente en Tunisie. Elle est reconnue aux Etats-Unis en 1986 puis elle s'est répandue dans toute l'Amérique du Nord, l'Afrique, l'Europe et le bassin méditerranéen (Perring *et al.*, 1993, Bergh *et al.*, 1995; Brown *et al.*, 1995a).

Deux biotypes de *B. tabaci* sont également décrits en Côte d'Ivoire, l'un attaquant le manioc et l'autre polyphage mais évitant cette plante (Burban *et al.*, 1992).

Il faut considérer que ce travail n'est qu'une étude préliminaire et pour l'instant on ne peut pas connaître exactement la distribution géographique des biotypes en Tunisie.

Il est nécessaire de poursuivre les analyses pour connaître davantage la composition des populations de *B. tabaci* présentes en Tunisie.

En Espagne, plusieurs populations de *B. tabaci* sont décrites en 1943 par Gomez-Menor , il a été proposé de correspondre ces populations au biotype "non B" tandis que la présence du biotype "B" serait attribuée à une récente introduction dans le pays (Guirao *et al.*, 1996; Guirao *et al.*, 1997b). Pour proposer une explication de la situation trouvée en Tunisie, il faudrait continuer les analyses des populations, avec des extractions individuelles d'ADN et une étude génétique plus exhaustive. En Espagne; l'étude déjà débutée utilisant la technique RAPD-PCR, sur les différentes populations méditerranéennes de *B. tabaci* permet d'analyser et de proposer l'origine possible de l'aleurode.

- Parasitoïdes

Le nombre de parasitoïdes recensés est faible par rapport à d'autres pays méditerranéens cela se rattacherait à la courte période de prospection d'une part (3 mois) et l'incidence des

traitements chimiques d'autres part. En Crête, Kirk *et al.*, (1993) signalent quatre espèces d'*Encarsia* et une espèce d'*Eretmocerus*. Onillon *et al.*, (1994) dans le sud de la France, recensent trois espèces: *Encarsia pergandiella*, *E. hispida* et *Eretmocerus mundus*.
On a relevé des pupes parasitées par des femelles d'*E. pergandiella* (méconium jaune en position latérale) mais il n y a pas eu d'émergence d'adulte. Cette espèce est vraisemblablement présente en Tunisie mais avec un pourcentage de parasitisme très faible (tableau 2).
Er. mundus est une espèce méditerranéenne, originalement décrite par Mercet (1931) en Espagne et Italie sur *Aleurodes sp* attaquant l'aubergine, signalée par Gameel au Soudan (1969), Hafez *et al.*, en Egypte (1979) et Sharaf en Jordanie (1982). C'est une espèce très intéressante dans le contrôle biologique de *B. tabaci*, pouvant attaquer tous les stades larvaires de l'aleurode à l'exception des nymphes (Braham, 1994); des pourcentages de parasitisme d'environ 80 % sont observés sur tomate (Braham, données non publiées).
Er. mundus a été systématiquement retrouvée dans tous les biotopes; *E. transvena* a été plutôt observée en plein air sur *Lantana* (Tableau 2). Deux souches d'*Er. mundus* sont probablement présentes en Tunisie (l'une colorant la pupe vide marron-clair et l'autre en blanc). Cette variation pourrait être induite par le biotype de l'hôte, par la plante hôte ou autres. Des analyses du profil enzymatique permettraient de donner des éléments de réponse.
E. transvena Timberlake appartient au groupe *E. strenua* est une espèce cosmopolite, elle attaque entre autres *Parabemisia myricae* et *Trialeurodes vaporariorum* (Polaszek *et al.*, 1992).

Références

BARTLETT A.C., & GAWEL N.J., 1993. Determining whiteflies species. *Science*, **261**:1333-1334.

BEDFORD I.D., BRIDDON R.W., MARKHAM P.G., BROWN J.K, & ROSSEL R.C., 1993. A new species of *Bemisia* or biotype *Bemisia tabaci* (Genn.) as a future pest of European agriculture. *Plant health and the European single Market, BCPC Monograph*, **54**:381-386.

BELLOWS T.S., PERRING T.M., GILL R.J., & HEADRICK D.H., 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). *Ann. Entomol. Soc. Am.* **87**: 195-206.

BERGH J.C., PERRING T., M & LE BLANC J.P., 1995. Identification of the Silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae) in Nova Scotia greenhouses. *The Canadian Entomologist* **127**: 141-142.

BRAHAM M., 1994. Modalités du contôle biologique de *Bemisia tabaci* (Gennadius) (Homoptera, Aleyrodidae) à l'aide de deux parasitoïdes *Encarsia hispida* et *Eretmocerus mundus* (Hymenoptera, Aphelinidae). *Mémoire DEA*. Université d'Aix- Marseille III. Faculté des Sciences St Jérôme. 39 p + annexes.

BROWN J.K., FROHLICH D.R., ROSSEL R.C., 1995a. The sweetpotato or silverleaf whiteflies: Biotypes of *Bemisia tabaci* or a species complex ?. *Ann. Rev. Entomol.* **40**: 511-534.

BROWN J.K., COATS S.A., BEDFORD I.D., MARKHAM P.G., BIRD J., ROSSEL R.C., FROHLICH D.R 1995b. Characterization and distribution of esterase electromorphs in the whitefly *Bemisia tabaci* (Genn.), (Homoptera., Aleyrodidae). *Biochemical Genetics* **33 (7/8):** 205-214.

BURBAN C., FISHPOOL L.D.C., FAUQUET C., FARGETTE D., & THOUVENEL J.C., 1992. Host-associated biotypes within west African populations of the Whitefly *Bemisia tabaci* (Genn.), (Hom., Aleyrodidae). *Journal of Applied Entomology* **113**: 416-423.

CAMPBELL B.C., DUFFUS J.E., BAUMANN P., 1993. Determining whitefly species. *Science,* **261**: 1333.

CENIS J.L., & BEITIA F., 1994 Aplicacion de la técnica RAPD-PCR (ADN polimórfico amplificado al azar) a la identificacion de insectos. *Invest. Agri. Prot. prod. Veg.* **9(2)**: 289-297.

COSTA H.S.,& BROWN J.K., 1991. Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci* Genn. and the association of one population with silverleaf symptom induction. *Entomol. Exp. Appl.* **61**: 211-219.

GAMEEL O.I., 1969. Studies on whitefly parasites *Encarsia lutea* Masi and *Eretmocerus mundus* (Hymenoptera, Aphelinidae). *Revue de Zoologie et de Botanique Africaines* **(79) 1& 2**: 55-77.

GENNADIUS P., 1889. Disease of the tobacco plantations in the Trikonia. The aleurodid of tobacco. *Ellenike Ga* **5**:1-3.

GUIRAO P., BEITIA F., & CENIS J.L., 1994. Applicacion de la técnica RAPD-PCR a la taxonomia des moscas blancas (Homoptera, Aleyrodidae). *Bol. San. Veg. Plagas*, **20**: 757-764.

GUIRAO P., CENIS J.L., & BEITIA F., 1996. Determinacion de la presencia en Espana de biotipos de *Bemisia tabaci* (Gennadius). *Phytoma-Espana*, **81**: 30-34.

GUIRAO P., ONILLON J.C., BEITIA F., & CENIS J.L., 1997a. Sur la présence en France du biotype B de *Bemisia tabaci* (Homoptera: Aleyrodidae). *Phytoma* (sous presse)

GUIRAO P., BEITIA F., & CENIS J.L., 1997b. Biotype determination in Spanish populations of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bull. Entomol. Res.* (sous presse).

HAFEZ M., TAWFIK M.FS., AWADALLAH K.T., & SARHAN A. 1979. Studies on *Eretmocerus mundus* Mercet, a parasite of the cotton whitefly, *Bemisia tabaci* (Genn.) in Egypt. *Bull. Soc. Ent. Egypte*, **62**: 15-22.

KIRK A.A., LASEY L.A., RODITAKIS N., & BROWN J.K., 1993. The status of *Bemisia tabaci* **(**Hom. :Aleyrodidae), *Trialeurodes vaporariorum* (Hom. :Aleyrodidae**)** and their natural enemies in Crete. *Entomophaga* **38**: 405-410.

MERCET G.R., 1931. Notas sobre Afelinidos. *Eos. Rev. Esp. Ent.* **7**:395-410.

MOUND L. A., & HALSEY S.H., 1978. Whitefly of the world. A systematic catalogue of the Aleyrodidae (Homoptera) with host plant and natural enemy data. London; British Museum (Natural History), London, *John Wiley & Sons.* 340 p.

ONILLON J.C., POLASZEK A., COCQUEMPOT C., & MAIGNET P., 1994. Premières observations sur l'entomofaune parasitaire inféodée à *Bemisia tabaci* (Homopt. :Aleyrodidae) dans le Sud-Est de la France**.** *OILB/SROP Bulletin.* Integrated Control in Protected Crops. Mediterranean Climate **Vol 17 (5)**: 67-69.

PERRING T.M., COOPER A.D., RODRIGUEZ R.J., FARRAR C.A., & BELLOWS T.S. Jr., 1993. Identification of a whitefly species by genomic and behavioral studies. *Science* **259**: 74-77.

POLASZEK A., EVANS G.A., & BENETT F.D., 1992. *Encarsia* parasitoids of *Bemisia tabaci* (Hymenoptera: Aphelinidae, Homoptera: Aleyrodidae): a preliminary guide to identification. *Bull. Entomol. Res.* **82**: 375-392.

SHARAF N. S., 1982. Parazitisation of the tobacco whitefly *Bemisia tabaci* Genn., (Hom., Aleyrodidae) on *Lantana camara* L in the Jordan Valley. *Z. ang. Ent.* **94**: 263-271.

TOXICITY OF DIFFERENT PESTICIDES ON PUPAE OF *ERETMOCERUS MUNDUS* MERCET (HYMENOPTERA: APHELINIDAE) PARASITIZING *BEMISIA TABACI* (GENN.)(HOMOPTERA: ALEYRODIDAE)

J. E. González-Zamora, J. M. Gallardo, Mª. M. García
Centro de Investigación y Formación Agraria "La Mojonera-La Cañada"
Apartado de Correos 91
04700 El Ejido (Almería, Spain)

Abstract
We present the effect of 19 pesticides of common use in the area of Almería (SE of Spain) over pupal stage of *Ertemocerus mundus* (Mercet), an important and widespread parasitoid of *Bemisia tabaci* (Gennadius). The products against whiteflies and thrips were the most harmful to this parasitoid.

Key words: *Eretmocerus mundus*, *Bemisia tabaci*, pesticides toxicity.

Introduction.

The use of pesticides in modern agriculture is practically a necessity imposed by the losses that insects, acari, fungi or bacteria can made in the production. But their use, many times indiscriminate and without criterion, can produce serious problems: selection of arthropods, fungi or bacteria strains resistant to pesticides, decrease in the number of natural enemies, contamination of the environment, residual effects.

The area of Almería (SE of Spain) is one of the most important producers of vegetables in Europe, concentrating the highest acreage of horticultural protected crops in the Mediterranean basin, with most of its production exported to other european countries. In this area the farmers are used to applied many pesticides, and with the present concern with environmental conditions and health foods there is an increasing interest in the consumers in the way these vegetables are grown. The Integrated Pest Management (IPM) techniques become a necessity, and one of its basis is the use of pesticides compatible with natural enemies, both introduced and indigenous. For this purpose there are many works carryed out in order to know the effect of pesticides in the natural enemies: Waddill, 1978; Garrido *et al.*, 1982; Beitia and Garrido, 1985; Gerling and Sinai, 1994; Biddinger and Hull, 1995; Vandenberg, 1996.

In this work we have selected as target to test some pesticides the hymenopter aphelinid *Eretmocerus mundus* Mercet, a primary parasitoid of the sweetpotato or cotton whitefly *Bemisia tabaci* (Genn.), an important pest of protected crops in the area of Almería. From its first report as pest in 1988 (Rodriguez-Rodriguez *et al.*, 1994) *B. tabaci* has spread to most of the crops in the area, being a serious threat in tomato (*Lycopersicum esculentum* Mill.) crop due to its ability to transmit the Tomato Yellow Leaf Curl Virus (TYLCV), or in cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.) for transmiting some viruses which produce yellowings (Cucumber Yellows Virus, CYV; Cucumber Vein Yellowing Virus, CVYV) (Cuadrado Gomez, 1994). In other crops, as sweet pepper (*Capsicum annuum* L.) or eggplant (*Solanum melongena* L.), the damage is produced when the whitefly reach high populations, weaking and covering plants with honeydew and appearing afterwards the sooty mould (*Fumagina* sp.).

After some years of studying, *E. mundus* has appeared as the most important parasitoid of *B. tabaci* in the conditions of the protected crops in Almería, being able to control *B. tabaci* populations (Rodriguez-Rodriguez *et al.*, 1994; Gonzalez-Zamora *et al.*, 1996; Gonzalez-Zamora, 1996).

In this work we present the effect of different, commonly used, pesticides on *E. mundus* parasiting *B. tabaci*, as help to the technicals who want to apply an IPM schedule.

Material and Methods.

The realization of the assays have followed the general norms recommended by the working group of the IOBC "Pesticides and Beneficial Organism" (Hassan, 1992).

E. mundus was reared on sweet pepper (cv. Espartaco) plants infested with sweetpotato whitefly using cages covered with gauze and illuminated (16:8 L:D), in a room at 25±1 ºC and 60±10 % of relative humidity. Pot plants of sweet pepper with 6-8 leaves were infested with sweetpotato whitefly adults from a stock maintened in a plastic house in the C.I.F.A. (Centro de Investigación y Formación Agraria) "La Mojonera-La Cañada". Adults of *E. mundus* were introduced (from the same plastic house) when whitefly larvae of 2nd and 3rd instar appeared. The plants were regularly observed in order to obtain the parasitoid at the correct stage. In the selection of the stage of the parasitoid has been applied the criterion used by Garrido *et al.* (1982) in their work on *Cales noacki*: the pupae of *E. mundus* parasiting *B. tabaci* were selected when the parasitoid had the eyes formed, beginning these to acquire a reddish coloration, appreciating also the head and extremities. These pupae were left in the own leaves, and pieces containing the pupae were cut and placed in Petri dishes over humidified cotton.

In total four assays were made with five treatments each (except in the assay 3, that consisted of four treatments) and a control in each assay. Six repetitions (three+ three) were made in each treatment, with a minimum of twenty pupae in each repetition, which make at least 120 pupae per treatment. The control was treated with tap water.

The different products were selected according to their more or less extended use in the intensive agriculture of Almería (SE of Spain). It is not an exhaustive list, although it includes insecticides (11), acaricides (3) and fungicides (5), having some of them combined action.

The application of the products were made with a Potter tower (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, England), spraying the Petri dishes. The pressure used was 660-670 mm Hg (13 lb/inch^2), leaving an amount of 1.70 mgr of spray fluid per cm^2. The dose used in each product was the maximum-intermediate of the recommended by the commercial product.

The sprayed Petri dishes, after dry, were left in a chamber at 20±1 ºC, 50±10% of relative humidity and photoperiod 16:8 (L:D). They were counted every seven days until reach twenty-one days, noting the number of empty pupal cases from which the adult of *E. mundus* had emerged completely, considering these as alive, and dead those that remained inside the pupal case or with half body out. The mortality in each repetition was obtained by the expression

$$M = \frac{P_p}{P_e + P_p} \times 100\ (\%)$$

in which P_e is the number of pupae that are empty and P_p the number of pupae that still have the parasitoid.

For the statistical analysis of the results, the transformation $y = arc\ sen\ \sqrt{p}$ was applied, being p the mortality expressed per 1. In each assay an analysis of the variance with two factors, *product* and *day*, was carried out. When the analysis of the variance was significative, the Duncan's multiple range test was applied to separate the mortality means, with an error level of 5%.

For the comparison between all the products used in this work, the corrected mortality (M_c) was calculated according to the expression of Abbot (1925):

$$M_c = \frac{M_p - M_t}{100 - M_t}$$

being M_p the mortality in the product tested and M_t the mortality in the control (expressed as percentage).

Results and Discussion.

The Table 1 shows the results of the four assays, with their statistical significance and the Duncan's multiple range test for the separation of means. Although the assay two gives a low significance (F= 1,746; g.l.: 5, 24; P= 0,1624), the Duncan test was applied, giving a difference between methamidophos and the control. The results refer to one of the factors, the one that we have denominated *product*, being the other factor (*day*) less important to obtain conclusions of the work; anyway there have been no interactions between both factors.

The Table 2 shows the mortality produced by each product, corrected according to the Abbot's formula, with the classification recommended by the IOBC. In this table there are three active ingredients as moderately harmful for the pupae of *E. mundus*: imidacloprid, acrinathrin, and methiocarb. The first is used especially to control whiteflies, and the other two to control thrips, although acrinathrin is also acaricide. It is necessary to consider that the assays have been carried out on pupae of *E. mundus*, which is a developmental stage very protected inside the pupal case of *B. tabaci*. This indicates that the effect of some pesticides tested in this work, which appear as harmless or slightly harmful, tried on the adults of *E. mundus* (a phase much more exposed) could behave as more harmful.

In the case of the methamidophos, the mortality obtained is something superior to that of the control (Table 1, assay 2), but is not excessive, due surely to the protection the parasitoid has inside the pupal case. Both methamidophos and teflubenzuron have a high standard error, but their mean mortality is not exccesive, being classified in the group 1 (harmless), according to the normative of the IOBC.

With the acaricides tested the mortality do not reach high values, while in the fungicides this is practically null.

Conclusions.

Most of the tested products are not especially toxic for the pupae of *E. mundus*, main parasitoid of *B. tabaci* in the conditions of the protected crops of Almeria. This is due to the protection the parasitoid has inside the whitefly pupal case. The most toxic products have turned out to be the used in order to control thrips and whiteflies, two important groups of insect pests in protected crops. A practical conclusion of this work is that many pesticides could be utilized, provided that they were used when most of the parasitoid population were as pupa inside the whitefly pupal case.

The work could be enlarged to other products, and it should be also necessary to carry out assays on adults of this parasitoid, in order to complete the effect on a more sensitive phase to the pesticides, adding studies of the effect of pesticides on the survival and reproduction.

References.

ABBOT, W.S., 1925. A method of computing the efectiveness of an insecticide. J. Econ. Entomol., **18:** 265-267.

BEITIA, F. & GARRIDO, A., 1985. Estudio de la esterilazación de *Cales noacki* How. ((Hym.; Aphelinidae) por el empleo de plaguicidas. An. INIA/Ser. Agric./ vol. **28(3)**: 147-155.

BIDDINGER, D.J. & HULL, L.A., 1995. Effects of several types of insecticides on the mite predator, *Stethorus punctum* (Coleoptera: Coccinellidae), including insect growth regulators and abamectin. J. Econ. Entomol. **88(2)**: 358-366.

CUADRADO GOMEZ, I.M., 1994. Las virosis de las hortalizas en los cultivos de invernadero de Almería. Junta de Andalucia. Consejeria de Agricultura y Pesca. Comunicación I+D Agroalimentaria **5/94. 60 pp**.

GARRIDO, A., TARANCON, J. & DEL BUSTO, T., 1982. Incidencia de algunos plaguicidas sobre estados ninfales de *Cales noacki* How. parasito de *Aleurothrixus floccosus* Mask. An. INIA/Ser. Agric./N. **18:** 73-96.

GERLING, D. & SINAI, P., 1994. Buprofezin effects on two parasitoid species of whitefly (Homoptera, Aleyrodidae). J. Econ. Entomol. **87(4)**: 842-846.

GONZALEZ-ZAMORA, J.E., 1996. Experiencia de liberación de *Macrolophus caliginosus* en tomate bajo plastico en Almeria. Phytoma-España, **84 (Diciembre)**: 20-25.

GONZALEZ-ZAMORA, J.E., MORENO-VAZQUEZ, R., RODRIGUEZ-RODRIGUEZ, Mª.D., RODRIGUEZ-RODRIGUEZ, Mª. P., MIRASOL-CARMONA, E., LASTRES GARCIA-TESTON, J. & MANZANARES-RUIZ, C., 1996. Evolución del parasitismo en *Bemisia tabaci* (Genn.) y *Trialeurodes vaporariorum* (West.) (Homoptera: Aleyrodidae) en invernaderos de Almeria. Bol. San. Veg. Plagas, **22**: 373-389.

HASSAN, S.A., 1992. Meeting of the working group "Pesticides and Beneficial Organisms". In *Guidelines for testing the effects of pesticides on beneficial organisms: description of test methods.* Edited by S.A. Hassan. IOBC/WPRS Bull., **15(3)**: 1-3.

RODRIGUEZ-RODRIGUEZ, Mª.D., MORENO, R; TELLEZ, Mª.M., RODRIGUEZ-RODRIGUEZ, Mª.P. & FERNANDEZ-FERNANDEZ, R., 1994. *Eretmocerus mundus* Mercet, *Encarsia lutea* (Masi) y *Encarsia transvena* (Timberlake) (Hym.: Aphelinidae) parasitoides de *Bemisia tabaci* (Homoptera, Aleyrodidae) en los cultivos horticolas protegidos almerienses. Bol. San. Veg. Plagas, **20**: 695-702.

VANDENBERG, J.D. 1996. Standardized bioassay and screening of *Beauveria bassiana* and *Paecilomyces fumosoroseus* against the russian wheat aphid (Homoptera: Aphididae). J. Econ. Entomol. **89(6)**: 1418-1423.

WADDILL, V.H., 1978. Contact toxicity of four synthetic pyretroids and methomyl to some adult insect parasites. The Florida Entomologist, **61(1)**: 27-30.

Assay	Active ingredient	Mean Mortality (%) + s.e.	Transformation[a]	[b]
Assay 1	F= 23.259 ; g.l.= 5, 24 ; P<0.00001			
	imidacloprid	83.33±4.41	1.19	a
	formetanate	33.56±5.19	0.61	b
	Bacillus thuringiensis	10.83±3.52	0.28	c
	abamectin	12.18±5.73	0.28	c
	myclobutanil	8.66±3.19	0.24	c
	Control	4.90±1.30	0.20	c
Assay 2	F= 1.746 ; g.l.= 5, 24 ; P=0.1624			
	methamidophos	25.56±8.76	0.50	a
	buprofezin	15.18±3.64	0.38	ab
	cymoxanil+zineb	14.63±3.65	0.36	ab
	dichlofuanid+ tebuconazol	12.80±3.37	0.33	ab
	lufenuron	12.22±3.94	0.34	ab
	Control	8.06±3.22	0.23	b
Assay 3	F= 68.968 ; g.l.= 4, 20 ; P<0.00001			
	acrinathrin	91.61±2.38	1.31	a
	methiocarb	86.50±2.23	1.20	a
	teflubenzuron	19.98±9.91	0.39	b
	dinocap	7.32±2.21	0.24	bc
	Control	3.18±3.18	0.08	c
Assay 4	F= 8.724 ; g.l.=5, 24 ; P=0.0001			
	bifenthrin	46.07±2.95	0.75	a
	cyromazine	22.36±4.93	0.48	b
	wetable sulphur	16.72±3.73	0.41	b
	endosulfan	13.07±4.55	0.33	b
	bupirimato	10.95±0.79	0.34	b
	Control	14.52±1.57	0.39	b

(a) $y = arc\ sen \sqrt{p}$, being p the mortality expresed by 1.
(b) Mean mortalities within an assay not followed by a common letter are significantly different ($P \leq 0.05$) by Duncan's multiple range test.

Table 1: Active ingredients used in each assay with the mean mortality (±standard error) produced, statistical significance, transformations and Duncan's test.

Commercial Product	Dose of commercial product (%)	Active ingredient	Dose of active ingredient (ppm)	Corrected Mortality ± s.e. (%)	I.O.B.C. Classification [(a)]
Confidor 20 LS	0.076	imidacloprid	152	82.4 ± 4.6	3
Applaud	0.06	buprofezin	150	7.7 ± 3.0	1
Dicarzol	0.10	formetanate	500	30.1 ± 5.5	2
Rufast	0.04	acrinathrin	60	91.5 ± 2.3	3
Mesurol 50 PM	0.15	methiocarb	750	86.0 ± 2.3	3
Monitor 60	0.10	methamidophos	600	18.7 ± 9.4	1
Nomolt	0.06	teflubenzuron	90	17.8 ± 9.6	1
Match	0.04	lufenuron	20	4.5 ± 3.5	1
Delfin	0.076	*Bacillus thuringiensis* (Var.kurstaki) (clon SA-11)	243	6.3 ± 3.1	1
Trigard 75 WP	0.035	cyromazine	263	9.2 ± 5.6	1
Vertimec	0.06	abamectin	11	7.6 ± 6.0	1
EN-35	0.25	endosulfan	875	-1.7 ± 5.3	1
Talstar 10 LE	0.07	bifenthrin	70	37.0 ± 3.2	2
Karathane LC I	0.05	dinocap	175	4.3 ± 1.9	1
Systhane 12 E	0.076	myclobutanil	91	4.0 ± 3.2	1
Folicur-Combi	0.20	dichlofuanid(40%) + tebuconazol(10%)	800 200	5.2 ± 1.2	1
Milzan	0.30	cymoxanil(4%) + zineb(40%)	120 1200	7.2 ± 2.5	1
Kumulus S	0.25	wetable sulphur	2000	2.6 ± 4.1	1
Nimrod-EC	0.20	bupirimate	500	-4.2 ± 1.1	1

(a) Mortality . <30% . . . . . 1 (Harmless)
" . . 30-79% 2 (Slightly harmful)
" . . 80-98% 3 (Moderatily harmful)
" . . >98% . . . . . 4 (Harmful)

Table 2: Commercial products used, active ingredients and their respective dose, with the corrected mortality (using Abbot's expression) on pupae of *Eretmocerus mundus* parasiting *Bemisia tabaci*, and the IOBC classification.

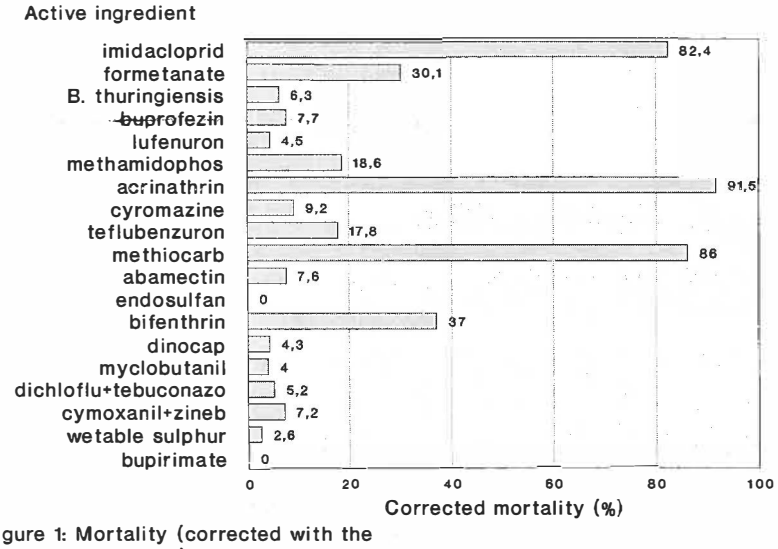


Figure 1: Mortality (corrected with the Abbot's expression) of different pesticides on *Eretmocerus mundus* pupae

PREMIERES DONNEES SUR LE POTENTIEL BIOTIQUE D'*Encarsia hispida* DE SANTIS (HYMENOPT., APHELINIDAE), ENDOPARASITOÏDE DU BIOTYPE « B » DE *Bemisia tabaci* (GENNADIUS) ET DE *Trialeurodes vaporariorum* WEST. (HOMOPT., ALEYRODIDAE)

P. Maignet et J.C. Onillon

Laboratoire de Biologie des Invertébrés
U.R. sur les parasitoïdes d'aleurodes
37, Boulevard du Cap 06606 ANTIBES CEDEX

Résumé

Dans l'inventaire des parasitoïdes indigènes susceptibles, de s'attaquer à la fois au biotype « B » de *Bemisia tabaci* et à *Trialeurodes vaporariorum*, et de ne pas présenter de phénomène d'hyperparasitisme, *Encarsia hispida* a été testée pour définir les caractéristiques majeures de son potentiel biotique. A une température de 22°C constant, 70% d'HR et 16 heures d'éclairement, les femelles d'*E. hispida* montrent, sur larves de *T. vaporariorum*, une fécondité élevée, de l'ordre de 151 œufs par femelle (n=17), une longévité de 54 jours et une prédation moyenne de 77 larves par femelle.

L'examen de l'évolution journalière des paramètres de la ponte (périodes de préoviposition, oviposition et postoviposition) montre une très forte variabilité individuelle chez les femelles. Une ébauche de séparation des individus peut être apportée en fonction de la discontinuité ou de la continuité de la période d'oviposition, le groupe de femelles n'ayant montré aucun arrêt au cours de la période de ponte présentant alors des paramètres du potentiel biotique plus élevés (fécondité de 201 œufs, longévité de 59 jours et prédation de 91 larves).

Mots clés : *Encarsia hispida*, *Bemisia tabaci*, biotype »B », *Trialeurodes vaporariorum*, potentiel biotique, conditions contrôlées.

1. Introduction.

En 1988, une nouvelle espèce d'aleurode, *Bemisia tabaci* (Gennadius) était signalée en France (Della Giustina *et al.*, 1989). Récemment une analyse plus poussée permettait d'affirmer (Guirao *et al.*, 1997) que l'on se trouvait, sans aucune équivoque, en présence du biotype « B » de *B. tabaci* dans les différents biotopes de la Côte d'Azur et en Provence. Entre temps des prospections soutenues dans différentes localités du Sud-Est (Onillon *et al.*, 1994a) avaient mis en évidence l'existence et le maintien d'une entomofaune parasitaire indigène riche et très diversifiée composée en grande majorité d'espèces appartenant aux genres *Encarsia* et *Eretmocerus*. Si *Eretmocerus mundus* Mercet est la seule espèce appartenant au genre *Eretmocerus* qui s'attaque à *B. tabaci*, plusieurs espèces d'*Encarsia* sont susceptibles de s'attaquer avec plus ou moins de réussite aux larves de *B. tabaci* et de *T. vaporariorum*. Parmi celles-ci, deux espèces *Encarsia hispida* De Santis et *Encarsia pergandiella* How. ont fait l'objet d'une analyse poussée des conditions de leur efficacité en cultures de tomate sous serre (Onillon *et al.*, 1994b). Le présent travail vise à apporter les premiers éléments de réponse sur le potentiel biotique d'*E. hispida*, parasitoïde susceptible de s'attaquer aussi bien aux larves de *B. tabaci* qu'à celles de *T. vaporariorum*.

2. Matériel et Méthodes.

2.1. Matériel. Le pondoir : le pondoir type, permettant d'optimiser l'expression du potentiel biotype des femelles de parasitoïde d'aleurode appartenant au genre *Encarsia*, tout au long de la vie de celles-ci, est tronconique avec une base de 3 cm de diamètre et une hauteur de 7 cm. Les deux faces du pondoir sont constituées de grillage très fin. Les pondoirs individuels sont disposé dans une boite de plastique rectangulaire d'une capacité de 2 litres dans laquelle est maintenu un double fond grillagé. Sous ce double fond, un demi-centimètre de coton humide permet de garder une humidité ambiante suffisante, de l'ordre de 70%.

L'aleurode : l'hôte d'élevage est *B. tabaci*. L'espèce d'aleurode sur laquelle a été testé le potentiel du parasitoïde est *T. vaporariorum* pour des problèmes de contamination suffisante sur la plante-hôte utilisée qui est *Ageratum conyzoïdes*.

Le parasitoïde : les femelles d'*E. hispida* testées sont issues des élevages en température contrôlée de 22° C constant, 70% d'humidité relative et une photopériode de 16 heures de jour. La température de test est de 22° C constant, donc identique à la température d'élevage.

2.2. Méthodologie.

Tous les jours des fragments de feuilles portant des larves de *T. vaporariorum* sont découpés à l'aide d'un emporte-pièce ; la zone centrale est nettoyée au pinceau pour ne laisser qu'une vingtaine de larves appartenant aux trois derniers stades larvaires. Le fragment de feuille comportant des larves de l'aleurode est mis en présence de chacune des femelles d'*E. hispida* pendant 24 heures.

Tous les jours les larves de *T. vaporariorum* sont disséquées pour connaître le nombre de larves parasitées, le nombre d'œufs pondus et le nombre de larves sur lesquelles les femelles d'*E. hispida* ont exercé la prédation. La composition de la population larvaire de l'hôte est notée.

Deux répétitions ont été suivies et une analyse de variance a été faite sur les deux répétitions et sur chacun des caractères retenus dans le potentiel biotique d'*E. hispida* : longévité, fécondité, durée de pré et de postoviposition, prédation.

3. Résultats.

3.1. Longévité (tableau n°1).

Sur l'ensemble des 19 femelles d'*E. hispida* testées, 2 femelles n'ont pas pondu. La longévité moyenne des femelles du parasitoïde est de 52,7 jours (σ = 20,3). Les longévités minimale et maximale observées sont respectivement de 10 et de 82 jours.

3.2. Fécondité totale (tableau n°1).

Sur les 17 femelles qui ont effectivement pondu, la fécondité moyenne est de 151,3 œufs (σ = 75,7). La fécondité la plus faible enregistrée est de 31 œufs alors que la valeur maximale notée est de 271 œufs.

3.3. Prédation totale (tableau n°1).

Si l'on considère la valeur de ce paramètre pour l'ensemble de la population testée, soit 19 femelles, l'on observe une valeur moyenne de 77,1 larves prédatées (σ = 31,9) intéressant la totalité des stades larvaires présentés (larves des trois derniers stades). Si maintenant l'on ne considère que la population de femelles ayant pondu, la valeur moyenne du nombre de larves prédatées par femelle est de 79,8 larves (σ = 32,3). Cette faible différence laisse à penser que le nombre de larves prédatées par les deux femelles qui n'ont jamais déposé d'œufs est sensiblement voisin de celui des femelles ayant pondu, les 2 femelles qui n'ont pu pondre

sachant parfaitement utiliser leur ovipositeur pour perforer la cuticule de la larve de *T. vaporariorum* et exercer la prédation.

3.4. Fécondité journalière (tableau n°1).

La fécondité journalière moyenne, calculée sur les 17 femelles ayant déposé des œufs, est de 2,9 œufs (σ = 1,3). Les valeurs minimale et maximale de ce paramètre sont respectivement de 2,1 et 5,7 œufs.

3.5. Prédation journalière (tableau n°1).

La prédation journalière moyenne est de 1,45 larves (σ = 0,2) sur les 19 femelles. Si l'on considère uniquement la valeur de la prédation journalière moyenne sur les 17 femelles qui ont pondu, l'on observe une valeur identique de 1,46 larves prédatées en moyenne par jour. Les 2 femelles qui n'ont pas déposé d'œufs présentent une prédation journalière très proche de la moyenne notée pour l'ensemble des femelles. Les valeurs minimale et maximale de ce paramètre sont respectivement de 1,2 et 1,8 larves tuées par femelle et par jour.

3.6. Distribution temporelle de la ponte des femelles d'*E. hispida* (figures 1 et 2).

Le suivi journalier du dépôt des œufs par les femelles d'*E. hispida* a permis de voir qu'une forte hétérogénéité existait dans le rythme de ponte des femelles du parasitoïde. L'analyse de variance ne montre aucun effet significatif de la répétition au risque de première espèce de 5% sur les différentes variables étudiées (tab.2), permettant de considérer l'ensemble des femelles des deux répétitions pour la formation des groupes sans se soucier de leur origine.

Sur la totalité des femelles qui ont pondu, deux groupes se dégagent en considérant la continuité ou la discontinuité de la période de ponte. Le modèle mixte d'analyse de variance utilisé permet de voir (tab.2) que la durée d'oviposition est significativement différente entre les deux groupes de femelles.

Pour 10 de ces femelles, constituant le groupe X (tab.2), l'on constate une continuité de la période d'oviposition (fig.1) et les quelques arrêts dans la ponte se situent à la fin de la vie de la femelle, époque où des pontes sporadiques, de l'ordre de 1 œuf par jour, sont toujours observées. La durée moyenne d'oviposition est de 48,4 jours pour une longévité moyenne de 59 jours soit un pourcentage de 82%. Les durées moyennes de préoviposition et de postoviposition sont très courtes et ont respectivement comme valeur 0,5 et 2,1 jours. Une très grande ressemblance, entre les différentes femelles, peut être observée dans les courbes de la ponte journalière cumulée (fig.1), notamment au niveau de la branche ascendante de la courbe.

Pour les 6 femelles restantes, constituant le groupe Y (tab.2), les résultats sont différents. L'on constate, pour une longévité de 54,3 jours, donc non significativement différente de la longévité du lot précédent, une période d'oviposition beaucoup plus réduite. Les 27,7 jours d'oviposition ne représentent plus que 51% de la vie des femelles. La durée de postoviposition est notablement plus élevée mais le principal caractère discriminant entre les deux groupes de femelles se situe au niveau d'un arrêt de ponte important intervenant quelques jours après le début de la ponte (fig.2).

A partir de l'existence d'un caractère discriminant qui est la présence d'un arrêt de ponte important intervenant quelques jours après le début de la ponte, il a donc été possible de revoir le potentiel biotique des femelles d'*Encarsia hispida* appartenant à chacun des deux groupes (tab.3). Les femelles du groupe X, présentant une oviposition continue, ont une fécondité moyenne de 201 œufs pour une longévité voisine de 2 mois. Les femelles du groupe

Y, qui ont une oviposition discontinue, ont une fécondité beaucoup plus réduite, de l'ordre de 88 œufs avec une longévité de 54 jours.

4. Discussion et conclusion.

Peu de travaux sont relatifs à une approche, en conditions contrôlées, des potentialités présentées par *Encarsia hispida*, espèce jusqu'alors peu remarquée dans les inventaires des biocénoses d'aleurodes. La synonymie récente établie par Polaszek *et al.*(1992) entre *Encarsia hispida* De Santis et *Encarsia meritoria* Gahan permet d'avoir cependant un élément de comparaison dans la mesure où cette dernière espèce a été étudiée par Avilla *et al.* (1991). Par contre les travaux présentés par Vet et Van Lenteren (1981) ne peuvent être utilisés comme un étalon sûr du fait du statut, non encore assuré, de l'espèce *Encarsia sp.* proche de *meritoria*.

Si l'on observe la fécondité des femelles du parasitoïde, l'on note une parfaite concordance entre les résultats obtenus par Avilla *et al.* (1991) sur *E. meritoria* (n = 7) et le groupe de 10 femelles d'*E. hispida* ayant montré une activité de ponte régulière. A une température constante de 24°C, Avilla *et al.* (1991) observent une fécondité moyenne de 198 œufs, chiffre très voisin des 201 œufs dénombrés en moyenne pour *E. hispida* sous une température constante de 22°C. La longévité est, par contre, très différente, les 34 jours en moyenne notés chez *E. meritoria* étant singulièrement plus faibles que les 59 jours observés chez *E. hispida*. Il est très vraisemblable que la longue période suivant la période de ponte principale, et qui se caractérise par des pontes sporadiques de 1 à 2 œufs entrecoupés de jours sans dépôt d'œufs, est responsable de cette longévité supérieure.

Si l'on compare le potentiel d'*E. hispida* et celui d'*Encarsia formosa*, toutes les deux espèces testées sur les larves de *T. vaporariorum*, l'on constate que pour les mêmes caractéristiques techniques (pondoirs et identité stricte entre les températures d'élevage et de test) et abiotiques (température, hygrométrie et photopériode), la fécondité est supérieure chez *E. formosa* avec 264 œufs en moyenne (Maignet, 1995). Le raccourcissement de la longévité observée chez *E. formosa* avec une longévité moyenne de 39 jours est responsable de la valeur plus élevée (7,3 œufs/jour) de la fécondité journalière.

La mise en évidence de la présence de femelles d'*E. hispida* présentant une évolution différente dans le processus de distribution temporelle des œufs est intéressante. Si ce phénomène était confirmé ultérieurement, en premier lieu sur un plus grand nombre de femelles et ensuite dans une gamme de températures variées, cela pourrait apporter un élément positif pour la confirmation de l'hypothèse émise par Polaszek *et al.* (1992) concernant l'existence d'un complexe d'espèces identifiées pour le moment sous le vocable d'*E. hispida*.

D'ores et déjà, il semble que cette espèce, largement distribuée au niveau du Bassin méditerranéen, justifie de recherches approfondies, tant dans l'expression de son potentiel biotique que dans la diversité de son spectre parasitaire.

5. Remerciements.

Ces recherches ont bénéficié du concours et de l'aide de la C.C.E, que nous remercions, sous la forme du contrat N° AGRE-CT91-0062, dans le cadre du programme ECLAIR.

6.Références.

AVILLA J., VIGGIANI G., DIAZ X., and SARASUA M.J. 1991 - Morphological and Biological notes on *Encarsia meritoria* GAHAN (Hymenopt., *Aphelinidae*), a Parasitoid of

Trialeurodes vaporariorum (WESTWOOD) New in Europe. *Biocontrol science and Technology*, **1**, 289-295.

DELLA GIUSTINA W., MARTINEZ M. et BERTAUX F., 1989 - *Bemisia tabaci* : le nouvel ennemi des cultures sous serres en Europe. *Phytoma*, **406**, 48-52.

GUIRAO P., ONILLON J.C., BEITIA F. et CENIS J.L. 1997 - Sur la présence en France du biotype « B » de *Bemisia tabaci* (Homopt., *Aleyrodidae*). *Phytoma* (sous presse).

MAIGNET P. 1995 - Modalités du contrôle biologique de *Bemisia tabaci* (GENNADIUS, 1889) (Homopt., *Aleyrodidae*) à l'aide de parasitoïdes (Hyménopt., *Aphelinidae*). *Thèse en Sciences de l'Université PARIS-SUD.*, 232 pp.

ONILLON J.C., POLASCEK A., COCQUEMPOT C. et MAIGNET P. 1994a - Premières observations sur l'entomofaune parasitaire inféodée à *Bemisia tabaci* (Homopt., *Aleyrodidae*) dans le Sud-Est de la France. *O.I.L.B./S.R.O.P. Bull. Vol **17 (5)**,* 67-69.

ONILLON J.C., MAIGNET P. et COCQUEMPOT C. 1994b - Premiers résultats sur l'efficacité d'*Encarsia pergandiella* (Hyménopt., *Aphelinidae*) dans le contrôle de *Bemisia tabaci* (Homopt., *Aleyrodidae*) en serre de tomate. . *O.I.L.B./S.R.O.P. Bull. Vol **17 (5)**,* 71-80.

POLASZEK A., EVANS G. A., and BENNETT F.D., 1992 - *Encarsia* parasitoids of *Bemisia tabaci* (*Hymenoptera* : *Aphelinidae*, *Homoptera* : *Aleyrodidae*) : a preliminary guide to identification. *Bull. Entomol. Res.*, **82**, 375-392.

VET L.E.M., and LENTEREN J.C. VAN, 1981 - The parasite host-relationship between *Encarsia formosa* GAHAN (Hyménopt., *Aphelinidae*) and *Trialeurodes vaporariorum* (Homopt., *Aleyrodidae*). X. A comparaison of three *Encarsia* spp. and one *Eretmocerus* sp. to estimate their potentialities in controlling whiteflies on tomatoes in greenhouses with a low temperature regime. *Z. Ang. Ent.*, **91**, 327-348.

a)

	Longévité totale *(jours)*	**Fécondité totale** *(œufs)*	**Prédation totale** *(larves)*	**Fécondité journalière** *(œufs/jour)*	**Prédation journalière** *(larves/jour)*
Moyenne	**52,7**	**135,4**	**77,1**	**2,6**	**1,45**
Ecart-type	20,3	85,9	31,9	1,55	0,2
Minimum	10	0	12	0	1,15
Maximum	82	271	141	5,67	1,78
N	19	19	19	19	19

b)

	Longévité totale *(jours)*	**Fécondité totale** *(œufs)*	**Prédation totale** *(larves)*	**Fécondité journalière** *(œufs/jour)*	**Prédation journalière** *(larves/jour)*
Moyenne	**54,5**	**151,35**	**79,8**	**2,9**	**1,46**
Ecart-type	19,3	75,7	32,3	1,32	0,2
Minimum	10	31	12	2,14	1,2
Maximum	82	271	141	5,67	1,78
N	17	17	17	17	17

Tableau 1 : Caractéristiques biologiques des femelles d'*Encarsia hispida* :a) calculées sur l'ensemble des femelles, b) calculées uniquement sur les femelles ayant pondu.

Source de variation	Durée de préoviposition	Durée d'oviposition	Durée de postoviposition	Longévité	Fécondité totale	Prédation totale
Répétition	0,0881	0,2295	0,2035	0,0796	0,5718	0,7309
Groupe	0,1217	**0,0343**	0,8726	0,2716	0,1618	0,5125
Répét.*Groupe	0,7145	0,7894	0,2692	0,9209	0,2491	0,1937

Tableau 2 : Modèle mixte d'analyse de variance (GLM) relative aux caractéristiques des femelles d'*E. hispida* en fonction des deux répétitions et des groupes X et Y constitués (*les valeurs du risque α sont portées et mises en gras lorsqu'elles sont inférieures au seuil de 0,05 que nous nous sommes fixés*).

		Longévité *(jours)*	**Durée de préoviposition** *(jours)*	**Durée d'oviposition** *(jours)*	**Durée de postoviposition** *(jours)*	**Groupe**
	Moyenne	**59**	**0,5**	**48,4**	**2,1**	
A (2,3,5,7,8,9)	Ecart-type	17,18	0,7	14,2	2,4	**X**
et	Minimum	37	0	35	0	
B (1,6,7,8)	Maximum	82	2	75	7	
	Moyenne	**54,3**	**1,3**	**27,7**	**8,6**	
A (4,6)	Ecart-type	20,1	0,8	8,8	11,2	**Y**
et	Minimum	31	0	18	0	
B (2,3,4,5)	Maximum	80	2	39	25	

Tableau 3 : Caractéristiques biologiques des femelles d'*Encarsia hispida* en fonction de la répartition en 2 groupes X et Y, réalisé à partir des données d'oviposition (température d'élevage et de test : 22°C constant).

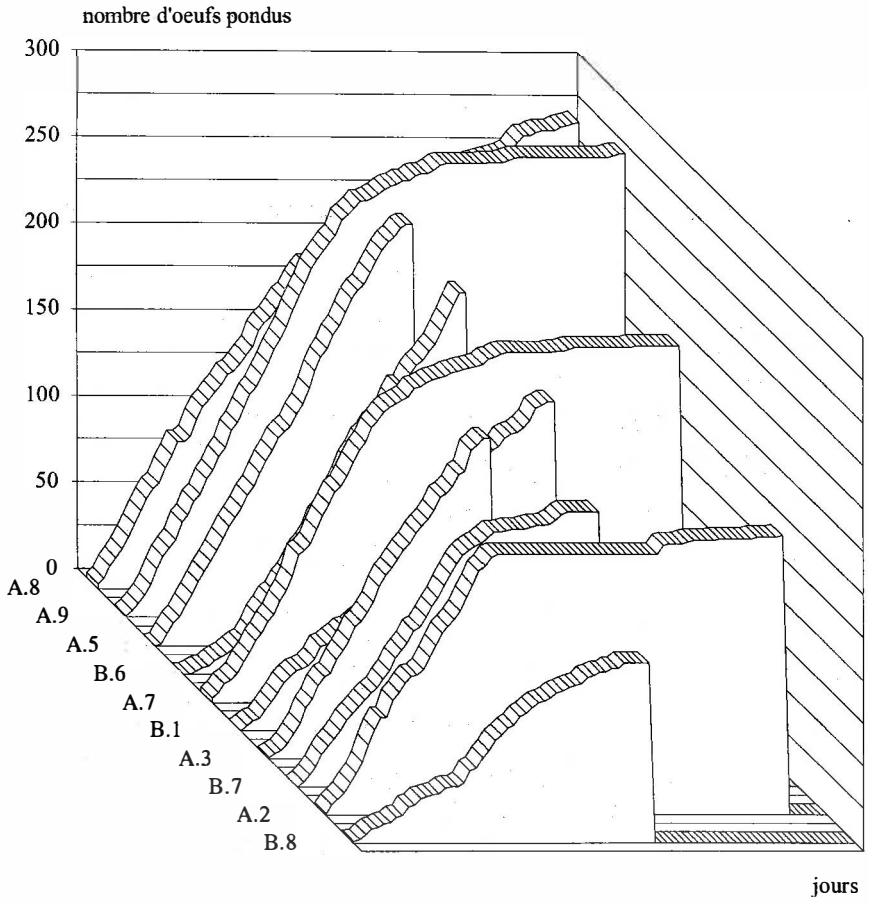


Figure 1. Ponte journalière cumulée des femelles d'**Encarsia hispida** présentant une ponte continue (10 femelles).

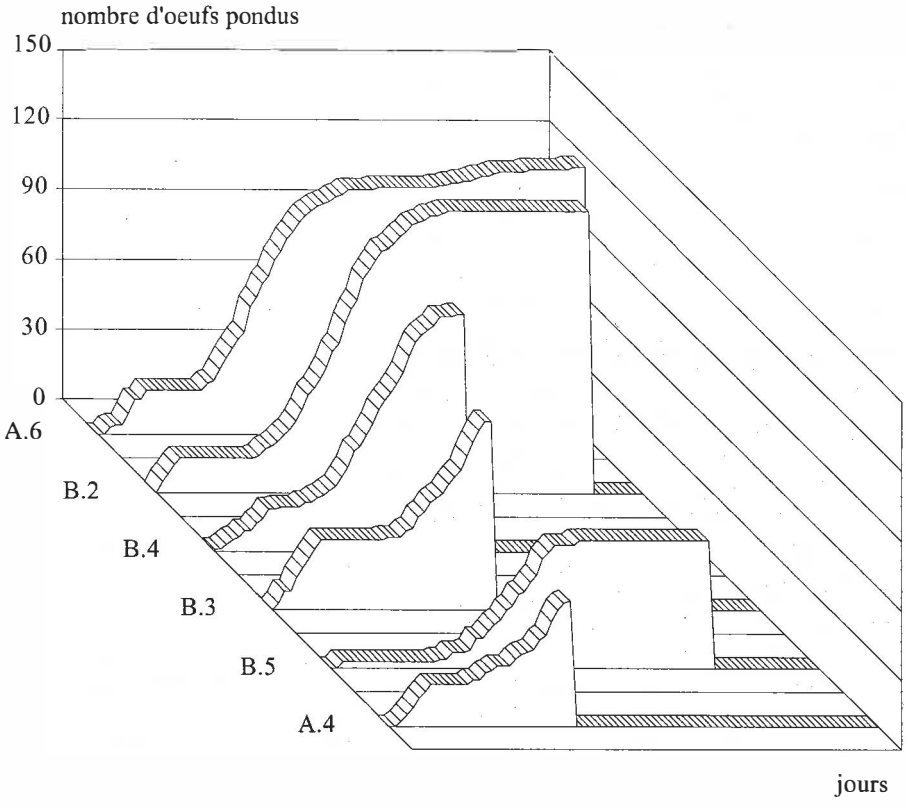


Figure 2. Ponte journalière cumulée des femelles d' **Encarsia hispida** présentant un arrêt de ponte pendant la période d'oviposition (6 femelles).

BIOLOGICAL CONTROL OF WHITEFLY ON POINSETTIA IN ITALY

G. Manzaroli, M.G. Tommasini, M. Mosti & D. Dradi.
BIOLAB - via Masiera I, 1191- Cesena (FO) - Italy.

Summary
An improvement in the control of whiteflies on Poinsettia through the development and the application of the parasitoid *Eretmocerus mundus* (Mercet) is described. The activity was carried out at different levels. Laboratory experiments on fecundity, female longevity and emergence rate of *E. mundus* are reported. Field trials in commercial greenhouses were carried out in a three year period (1994-96) to check the population trend of the pest and the parasitization of *E. mundus*. In some plots an introduction programme with *Encarsia formosa* Gahan alone was used as control. Also a combination of both parasitoids was evaluated in other plots. A small scale commercial application programme in different Italian regions during the 1996 growing season is also described. Thirty growers with a total surface of ca. 2.6 hectares were involved in the application. The combination of both parasitoids was used as standard in association with a sound IPM pest and disease control practices.

Introduction
In the last 6-7 years *Bemisia tabaci* (Genn.) (SPWF) and recently *B. argentifolii* (Bellows & Perring) (SLW), have become world-wide the main pests of Poinsettia, confining *Trialeurodes vaporariorum* (Westw.) to the status of a less common and a very easy problem to solve.
The role of the whitefly complex in the pest control of Poinsettia is still predominant also in Italy since no other pests reach the same importance and their occurrence is rather occasional. Among them spider mites, thrips and fungus-gnats could be found more frequently.
The distribution of the whitefly species is not always clear; in several circumstances the samples collected in different regions and also locally in our field trials were identified as *B. tabaci*, as reported in our previous works (Mosti & Benuzzi, 1992). However, especially in the last seasons, some apparent changes of infestation characteristics and direct damage observations occurred. In fact some whiteflies samples collected during these trials were used for artificial infestations in laboratory or semi-field tests and determined the evident silvering damage pattern on squash typical of SLW (Hoddle *et al.,* 1996). This kind of observations could provide evidence to support that the presence of suspected not widespread population of *B. argentifolii* is very likely to have grown recently. The aspect regarding the direct damage on plants is still under evaluation but in several cases a consistent whiteness of the primary stem of Poinsettia was associated with a high population of *B. tabaci*. In the area of northern Italy (Emilia-Romagna) where the experiments were conducted, the occurrence of *B. tabaci* on other cultivated plants prior to the Poinsettia growing season is very scarce. Sometimes it is possible to find infested spots on different ornamental crops and these pest populations could stay active all year on different hosts when heating systems are used throughout the cold season. The work on Poinsettia at Biolab started in 1988, both as R&D activity and commercial application, (Benuzzi *et al.*, 1990) and made it possible to follow the progressive change of the whitefly pest status and of the pest control techniques on this very important cash crop.
IPM commercial programmes have been set off because of a strong demand of the growers for a "gentler" pest control technique for this crop. The results have been positive also thanks to the awareness of pesticides harmful for the plant and the greenhouse environment and the quick development of resistance or at least reduction of efficacy of the most widespread

pesticides. However, chemical control is still predominant as new products are constantly available on the market. Moreover, too many growers rely on it entirely even if sometimes a very high number of treatments, high doses and increased costs are required to reach a good control.
The general results obtained in the past years in which *E. formosa* was the only natural enemy commercially available were not always satisfactory even if sometimes a very good parasitization level was observed in the fields on *B. tabaci* populations, in agreement with different authors (Boisclar *et al.,* 1990; Enkegaard, 1992).
An integration with other natural enemies and the development of new selective tools was a general request of the growers and also of the technical support and extension people. In our condition however, the occurrence of mixed population of different whitefly species could be considered a standard and needs to be considered in an IPM strategy.
The distribution and the occurrence of the two species of the *Bemisia* "group" are not definite and *B. tabaci* and *T. vaporariorum* could be found together, the latter being reduced rapidly both by chemical and standard biological control with *E. formosa*.
In the present work we focused on different aspects.
In the framework of a study on biology and behaviour of *E. mundus* in the developing process of a mass-rearing technique, some simple experiments were conducted to check emergence ratio, sex ratio, female longevity and fecundity.
In the field experiments we attempt to better evaluate the performance of both parasitoids through a simple comparison between them, applied separately or combined.
Another aim of the study was to determine the performance and to check the adaptability of the native species *E. mundus* that is frequently found naturally in south and central Italy associated with *B. tabaci* infestations.

Materials and methods

Laboratory experiments
All the experiments were carried out in climatic chamber at 25±1°C, 65±5% RH and 16L:8D photoperiod.

1) The emergence ratio and the sex ratio were observed on a sample of 500 pupae. This trial was carried out with pupae both isolated (a) and glued on commercial card (b).
500 pupae close to emergence (when at least red eyes where evident) of *E. mundus* were collected from tobacco leaves. Two different procedures were followed: in the first the pupae were isolated in the holes of an ELISA petri dish then the holes were closed on the top with glued paper; in the second, the pupae were glued on commercial cards (ca. 60 pupae per card). After isolation, newly emerged adults were counted daily. The sex-ratio was recorded at the end of the experiment on the total amount of the emerged adults obtained with the two procedures.
2) Longevity was observed on 25 newly (within 8 hours) emerged females; they were isolated in small Plexiglas cylinders (40 mm diameter and 40 mm height) containing a leaf disc of tobacco on agar. On the leaf a population of 2nd instar *B. tabaci* larvae (>100) was present. The survival rate of females was recorded daily. Unfortunately we could not assess the parasitoid fecundity because the pieces of leaf on agar dried rapidly causing a high mortality of *B. tabaci* and *E. mundus* cultures. For this reason a new experiment was carried out using a leaf attached to the plant to allow a continuous development of the parasitoids.
3) The fecundity test during the whole adult life of the females was carried out taking 25 mated

females, emerged within 24 hours and confined inside small clip-on cages. Two small round plastic 'petri dish type' trays of 45 mm diameter and 10 mm height with a thin gauze tightly closed to the leaf by a clip were used (adapted from Foltyn & Gerling, 1985 and Horowitz *et al.*, 1984). Few tobacco leaves of the same age infested by young stages (mostly 2nd and 3rd instars) of *B. tabaci* were used. The mean number of hosts supplied to each parasitoid female was 140. In order to prevent accidental parasitism after the death of all the females, clip-on cages were opened and the tobacco plant was moved to a climatic chamber where no parasitoids were present. Parasitization was checked only when at least 80% of the parasitoids showed red eyes. All the developing instars of *E. mundus* were then counted including the parasitoids already emerged (from the circular emergence holes).

Field Trials

During the fall-winter season of 1994, 1995 and 1996 in a total of 11 commercial greenhouses an experimental release program was conducted to control the whitefly on Poinsettia. The objective of the experimental activity was not only to compare or assess the best method or the best parasitoid but also to detect information on the behaviour of both pest and natural enemies in commercial greenhouses.

All the plots were located around Cesena in northern Italy and the growers followed the current practices both for disease control and for fertilisation as suggested by cutting producers.

We did not observe whitefly presence in the surrounding area of each plot before transplant. According to the Biolab guidelines for IPM and biological control in this area, no preventive insecticides were used for whiteflies and minor pests, and soon after the cutting transplant we checked the situation weekly before starting with the regular sampling. All the parasitoid introductions were carried out on a weekly basis following an inundative approach (Parrella, 1990). After selecting the plots each year we tried to follow a similar pattern to monitor the population trend and the parasitization level. The characteristics of each plot are summarised in Table 1. In some plots there were different varieties together but in all cases "Sup Ji-Bi" was predominant (more that 60 %) and the sampling plants were always taken on this variety.

Both parasitoids, coming from a small rearing unit at Biolab, were released in parasitized host pupae (*T. vaporariorum* and *B. tabaci*) glued on cards with a procedure comparable with the standard system for *E. formosa* commercialisation. Only in 1994 in the treatment with *E. mundus* the parasitoid was applied as adult.

Sampling

A. Adults whitefly population trend: the infestation was estimated by counting every week the number of adults with the naked eye on 100 plants randomly selected. A simple procedure was used taking the pot out of the bench and observing the underside of every leaf.
B. Young stages population and parasitization trend: On one "middle aged" leaf chosen at random on each plant selected for adult sampling, the number of whitefly pupae (also including the last instar larvae) was estimated with the help of a x10 lens. Evident parasitization was also recorded in this way (the parasitization trend was not recorded in 1994).
C. Parasitization activity: On a weekly or fortnightly basis, according to the availability of suitable specimens, a variable number of apparently non-parasitized young instars were individually tagged and observed every week to monitor their fate (as described by Benuzzi *et al.*, 1990).

   A x30 field lens was used for this purpose to allow the recognition (colour, emergence hole

and residues of faecal material were examined) of the difference between *E. formosa* and *E. mundus* activity.

The fate of each sample was summarised according to the following categories:

- Parasitized i.e. when a complete parasitoid development or rounded emergence holes were evident.
- Non parasitized i.e. whitefly emergence.
- Dead; this category contains all the samples in which there was no pest or parasitoid development including natural mortality, host feeding, non-complete parasitization (i.e. arrested development of parasitoid larva or pupa), predation by other naturally occurring species.

Periodically some samples were taken from the fields to check the general situation and the emergence of both pest and parasitoids more in detail.

Table 1. General condition of the experimental plots

Plot	Treatment	Tot. Release (Parasit. pupae/plant)	Release period (Weeks)	Pots N.
94A	*E formosa*	18,35	33 -46	2500
94B	*E.mundus* (adults)	7,03	33 - 45	1800
95A	*E.mundus*	29,90	32 - 46	2300
95B	*E.mundus*	18,90	32 - 47	4300
95C	*E.mundus*	26,88	32 - 46	775
95D	*E formosa*	9,12	34 - 43	1800
96A	*E.mundus + E. formosa*	17,60	32 - 42	4300
96B	*E.mundus + E. formosa*	17,50	32 - 42	600
96C	*E formosa*	16,50	32 - 42	2150
96D	*E.mundus*	16,70	32 - 42	740
96E	*E.mundus*	16,60	32 - 42	1150

Results and discussion

Laboratory experiments

In figure 1 the daily emergence rate of *E. mundus* is reported. The 76.5% of the total emergence occurs in a close interval and within the first five days from the stage of red eyes. The total percentages of adult emergence and the sex ratio recorded in the two ways are described in table 2. A difference between the two procedures followed to define the percentage of adult emergence was found (χ^2 test, $P<0.05$). However, the lowest result obtained with the isolated pupae could be due to an higher manipulation required. Sex ratio was close to that recorded by Sharaf and Batta (1985).

In the second experiment, female longevity ranged from 2 to 5 days (Tab. 3), with a mean time rather short compared to that recorded by other authors: 9.1 days at the same temperature on tomato (Sharaf & Batta, 1985) and 10.5 days at 30°C on sweet potato (Tawfik *et al.*, 1978-79).

In the third experiment parasitization was checked after 17 days from female isolation, according to the data of Sharaf & Batta (1985) on the development time of *E. mundus*. The mean number of offspring per female was lower than the fecundity recorded by other authors

(Tawfik *et al.*, 1978-79; Sharaf & Batta, 1985) who tested *E. mundus* at different temperatures and on different host plants.

Fig. 1. Daily emergence rate of *E. mundus* at 25°C

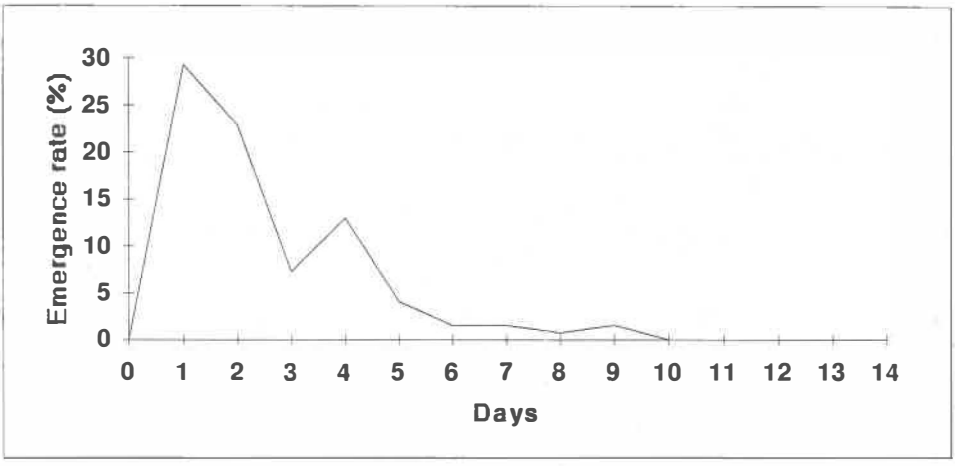


Table 2. Emergence rate and sex ratio of *E. mundus* reared at 25°C on tobacco leaf.

	Emergence rate (%)	**Sex-ratio** (% females)
Isolated pupae (a)	78.3	48.8
Pupae glued on cards (b)	89.5	48.8

They both checked the number of eggs laid by the females while in this experiment only the offspring was recorded. This could justify the different values we found because the data on progeny include the mortality occurring during the parasitoid development. Furthermore we cannot determine if the availability of hosts could influence the fecundity of the females, although no correlation was found between the number of *B. tabaci* offered to each female and the mean progeny per female. Sharaf & Batta (1985) found that the higher parasitization level is reached on tomato with a host/female ratio of 20/1.

Further studies are necessary to determine the relationship between the number of hosts and female performance. Few data on the biology of *E. mundus* are available in literature and the results are often in disagreement mainly because they are obtained in different environmental conditions and on different host plants. Many aspects need to be investigated to assess the main biological parameters of *E. mundus* in relation to the temperature and the host plant on which the pest develops.

Field Trials

As a general trend a very high variability of infestation levels and pest development was

registered in all years and during the trials no significant presence of wild natural enemies was observed.
In the graphics (Fig.2-6) the population trends of some plots are shown. The control was acceptable almost in all cases; even in some plots (94B-Fig.3, 95A-Fig.4-and especially in 95B-Fig.5), where the final level of the pest was beyond what is generally considered a good standard, no damage occurred. On the other hand the parasitization activity (Table 4 and Fig. 7) was very interesting. While in the plots with only one parasitoid species the number of parasitized plus dead pupae did not reach a very high level, an important synergetic effect apparently occurred in plots with both parasitoids (96A, 96B).

Table 3. Female longevity, progeny per female (counted on all the popolation tested) and egg-laying females of *Eretmocerus mundus* reared at 25°C on tobacco leaf.

Longevity females (days) **(means ± SD)**	**Progeny/female** (emerged and not emerged) **(means ± SD)**	**egg-laying females** **(%)**
3.5 ± 0.7	16.3 ± 23.7	80

In 1994 *E. mundus* was released in the adult stage (94B) but the results were not so satisfying also because of the difficult manipulation and distribution. Moreover, even when a low infestation occurred the achievement was not adequate if compared with *E. formosa*, especially in the first weeks after beginning the releases. Subsequently there was an improvement probably due to the following generation born in the greenhouse and to an accidental penetration of *E. formosa* from neighbouring greenhouses that determined a significant parasitization activity (Table B - 94B). Even if not completely satisfactory the remarkable overall control due to the combined action of the two parasitoids gave us the idea of using the two wasps together.
In 1995 due to a very scarce development of the whitefly in the control plot with *E. formosa* we did not have the possibility of making comparisons. In the other plots *E. mundus* was released on cards, that in addition to being very easy to apply ensured in our opinion a better and prolonged action. In the plots with a very high pest population (95A, 95B) the performance of the wasp was excellent not enough, however, to stop the pest population growth at the beginning of the season and to reach a complete control. In 95A a chemical treatment (Buprofezin mixed with Natural Pyrethrum) was necessary in week 40 while in 95B no treatment was applied but the whitefly population trend sharply increased in the last cropping period.
The results of 1996 give a very interesting picture of the possibility for the two species to "work" together as the control level and the overall parasitization (Table 4 -Fig. 7) were very good and no interference between the two species was observed. However, a more detailed experiment could be necessary to study the parasitoids behaviour and interspecific relationship in field conditions.

Commercial programme

In the early summer of 1996, after having informed several growers about the opportunity to set up a release program including *E. formosa* and the "new product" (*E. mundus*), we

proposed this idea to thirty growers in different areas in Italy who accepted to be involved and to cover the costs of the biological agents.

Table 4. Fate of the young instars individually tagged during the cropping seasons.

Quantity				
B. tabaci dead	182	140	336	209
E. mundus emerged	-	128	197	96
E. formosa emerged	191	75	-	114
Total pupae	500	400	734	469

The average of each grower's unit was around 900m^2 with a total area of ca. 2.7 hectares and 180.000 plants. The commercial application was defined with the routine contract of selling on a per plant basis that Biolab had already introduced for some customers in 1991; in this case we offered a more frequent extension service in terms of time and support that we normally do for the standard natural enemies sold.

The combination of parasitoids (50% *E. formosa* and 50% *E. mundus* both in cards) named "Enerpak" was packaged in a single box and distributed as a routine product. The release quantities and the number of introductions were decided by our technical support officers after visiting the grower's site. A scheduled weekly release programme was established on an inundative technique basis according mainly to the following parameters:

-Greenhouse surface;
-Number of plants;
-Diameter of pots and distribution of different plant sizes;
-Date of arrival of cuttings;
-General infestation of *B. tabaci* in the area during the previous seasons.

The growers received a copy of the release sheet and all the instructions of the general approach needed for the success of the programme. In the following visits a change of the established release pattern was sometimes necessary due to changes occurred during the growing season.

The growers selected for this commercial application were already experienced in dealing with parasitoids, so they could easily explain also by phone if anything went wrong. According to the conditions of the contract, the growers had to obtain approval from the Biolab area managers before using any chemical spray.

To check the success of the application we briefly recorded the general trend of the situation, all the specific procedures of the grower and we did a rapid estimation of the parasitization level.

Labels were put on some leafs to follow the development of pest young stages and to give an idea of the parasitoid performance.

To summarise the final response and success of the operation we collected the few data available (see Tab. 5) in 4 categories as follows:

A. Full Control: meeting the growers needs completely;
B. Good Control: with the final level of infestation slightly higher than the standard expected (the acceptance of the grower was however excellent and no chemical insecticides were used;
C. Partial Control: only one treatment with a selective insecticide (Buprofezin mixed with Natural Pyrethrum) was applied;

D. Insufficient Control: one or more chemical treatments were needed and the release programme had to be interrupted to keep a suitable plant selling quality (in any case the general judgement was not very negative because the number of treatments was very low compared with standard chemical control).

Table 5. Number of growers per level of control and area of application (total 30 plots).

	LOCATION		
CATEGORY	North	Central	South
A – Full	12	2	2
B – Good	5	2	-
C – Partial	1	1	1
D - Insufficient	2	1	1

Conclusions

This work gave us the opportunity to assess the possibility for a new species of parasitoid to provide an acceptable control of *B. tabaci* on poinsettia also in commercial conditions.
An easy to use packaging similar to the standard one for *E. formosa* allowed to combine the use of the two wasps.
In order to evaluate more precisely the advantages in applying the two species together, other work is needed and an improvement of the rearing system has to be achieved through more detailed laboratory tests.
This seems to be a possible solution in Mediterranean climate in which *E. mundus* is very common in association with *B. tabaci*.

Acknowledgement

The regional IPM program of Emilia-Romagna supported the research.
Thanks are due to the growers involved in the trials and in the commercial programme for their help and patience.

References

BENUZZI M., NICOLI, G. & MANZAROLI, G., 1990. IOBC/WPRS Bull.**XIII/5**, 27-31.
BOISCLAIR, J., BRUEREN, G.J. & VAN LENTEREN, J.C., 1990. IOBC/WPRS Bull.**XIII/5**, 32-35.
ENKEGAARD, A., 1992. Bionomics and interactions between the cotton whitefly *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae) and its parasitoid, *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) on poinsettia in relation to biological control. -Ph.D. Thesis -University of Copenaghen.
FOLTYN, S. & GERLING, D., 1985. The parasitoids of the aleyrodid *Bemisia tabaci* in Israel: development, host preference and discrimination of the aphelinid wasp *Eretmocerus mundus*. Entomol. exp. appl. **38**: 255-260.
HODDLE, M.S., VAN DRIESCHE, R., ROY, S., SMITH, T., MAZZOLA, M., LOPES, P. & SANDERSON, J., 1996. - Floral facts - UMass Extension Publications, June 1996.
HOROWITZ, A., PODOLER, H. & GERLING, D., 1984. Life table analysis of the tobacco whitefly *Bemisia tabaci* (Gennadius) in cotton fields in Israel. Oecol. Applic. **5 (3):** 221-233.
MOSTI, M., & BENUZZI, M., 1992. *Encarsia formosa* - Inf. fitopatologico **9**:25-31
PARRELLA, M.P., 1990. IOBC/WPRS Bull.**XIII/5**, 161-168.

SHARAF, N, & BATTA, Y., 1985. Effect of some factors on the relationship between the whitefly *Bemisia tabaci* Genn. (Homopt., Aleyrodidea) and the parasitoid *Eretmocerus mundus* Mercet (Hymenopt., Aphelinidae). Z. ang. Ent. **99**: 267-276.

TAWFIK, M.F.S.; AWADALLAH, K.T., HAFEZ, H. & SARHAN, K.T., 1978-79. Biology of the Aphelinid parasite *Eretmocerus mundus* Mercet. Bull. Soc. ent. Egypte, **62**: 33-48.

Fig. 2 - Pest population trend - Plot 94A

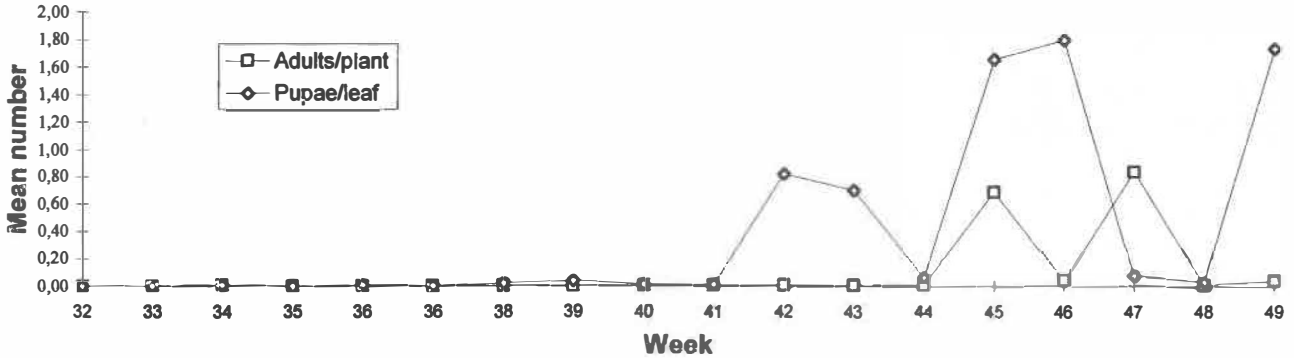


Fig.3 - Pest population trend - Plot 94B

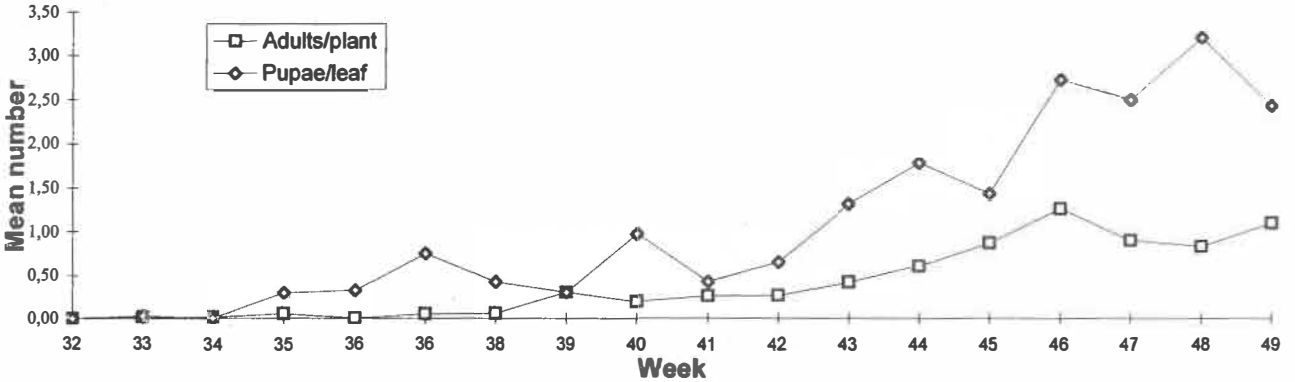


Fig. 4 - Pest population and parasitization trend
Plot 95A

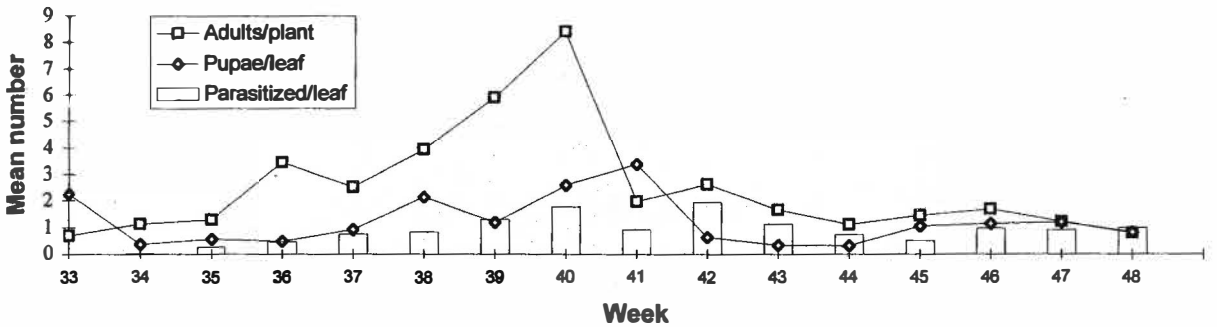


Fig. 5 - Pest population and parasitization trend
Plot 95B

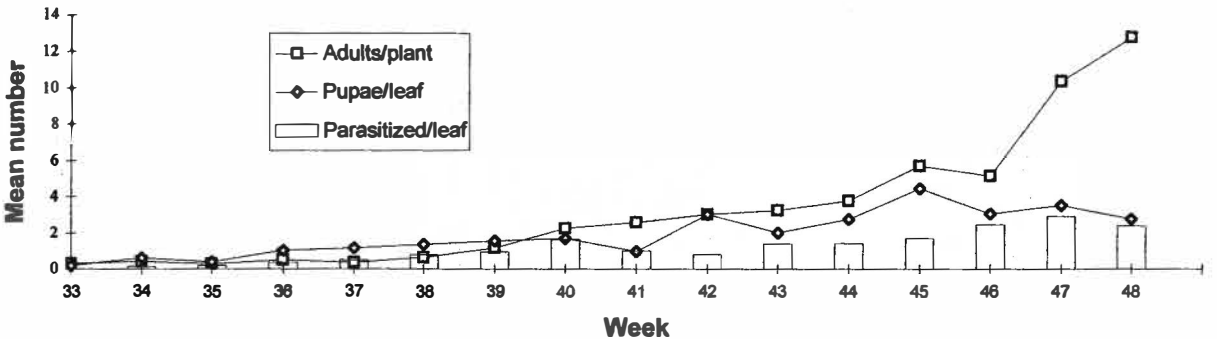


Fig.6 - Pest population and parasitization trend - Plot 96A

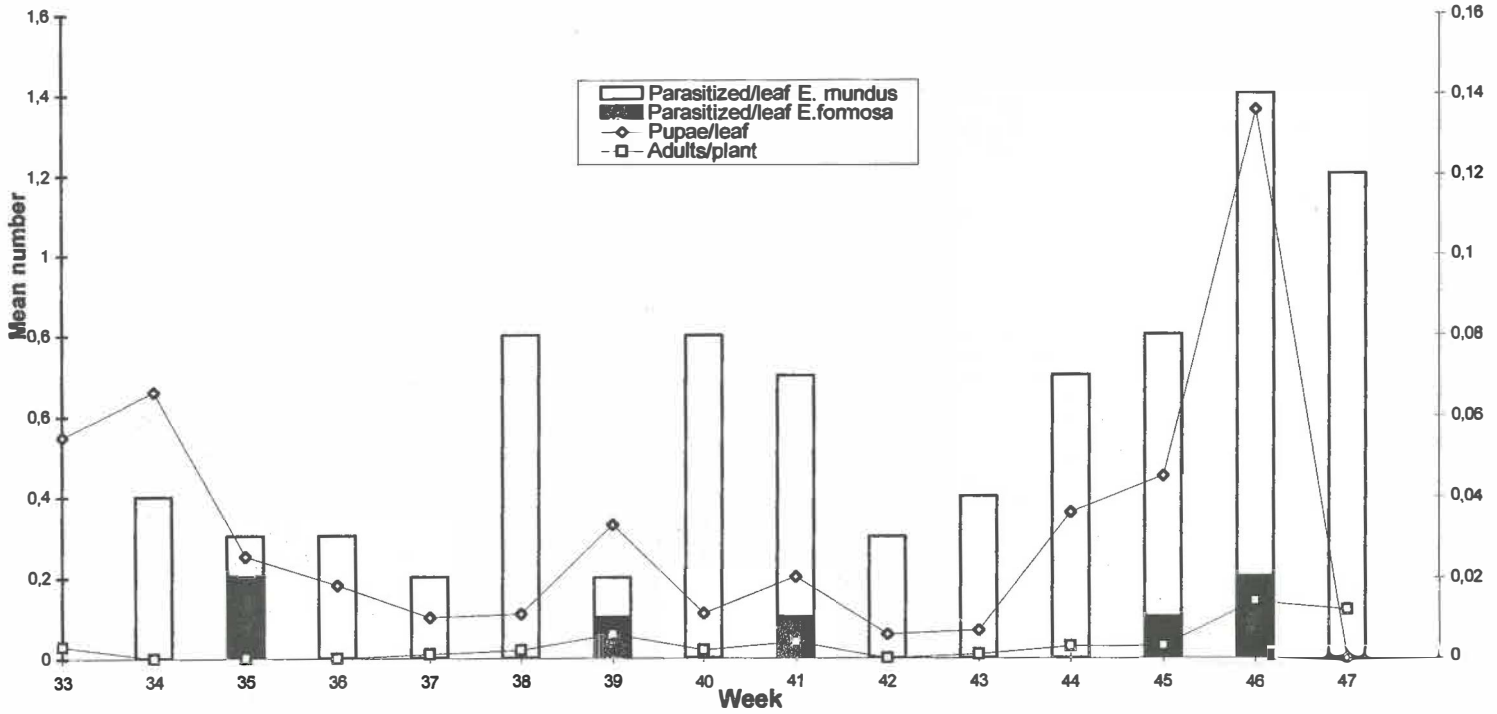
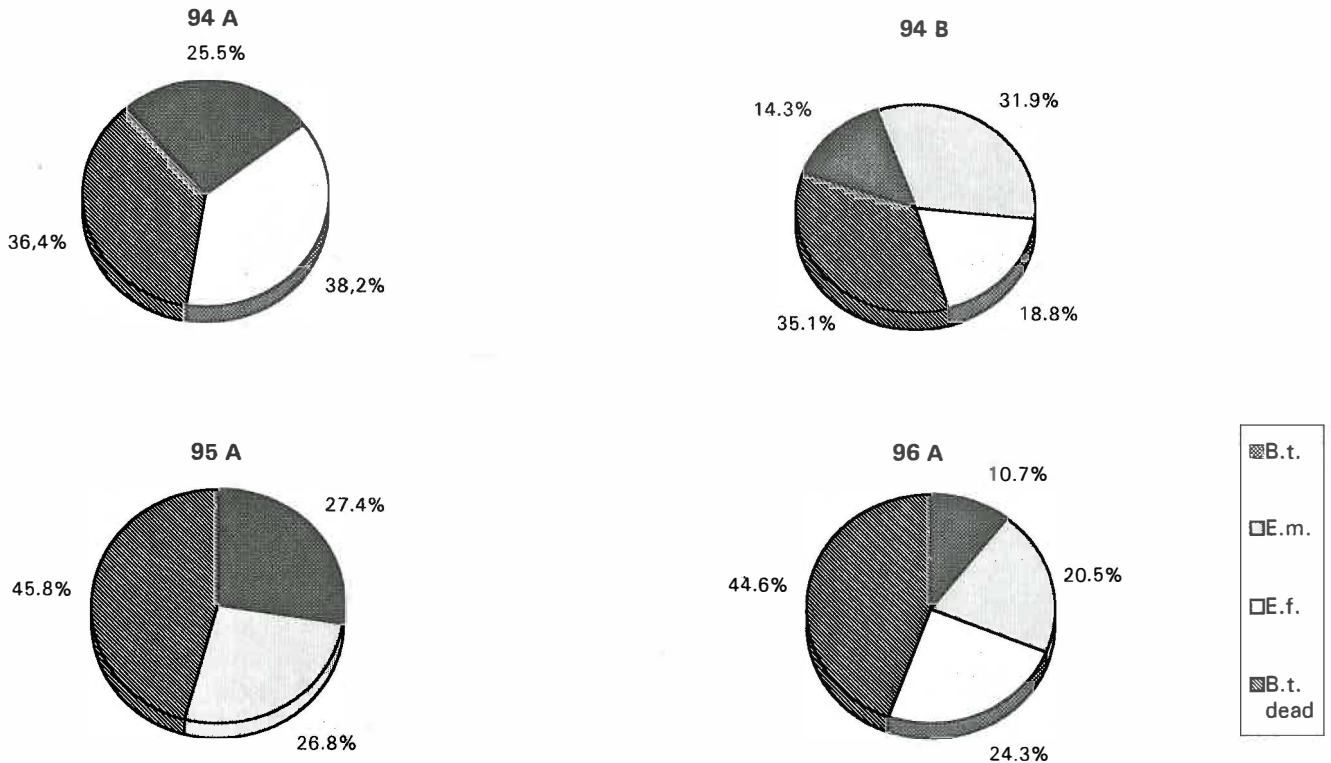


Fig. 7. Overall percentage of parasitization activity and fate following the selected categories.



DEVELOPMENT, OVIPOSITION AND FEMALE LONGEVITY OF TWO BIOTYPES OF *Bemisia tabaci* (HOMOPTERA: ALEYRODIDAE) ON THREE VARIETIES OF *Capsicum annuum* L.

M. MUÑIZ & G. NOMBELA
Departamento de Protección Vegetal. Centro de Ciencias Medioambientales (CSIC)
c/ Serrano 115 Dpdo. 28006 Madrid (Spain)

Abstract

Development periods of immature stages, fecundity and longevity of females of two biotypes ("B" and "non B") of *Bemisia tabaci* (Gennadius) on three varieties of *Capsicum annuum* L. at constant temperature (25 ± 1ºC) were studied. The mean (± SE) development time (egg-adult; in days) of "B" biotype on Morrón (16 ± 0.37) was significantly ($p < 0.05$) shorter than was on Yolo-wonder (22.83 ± 0.48) and Piquillo (19.02 ± 0.52). In contrast, the "non B" biotype developed faster on Yolo-wonder (16.91 ± 0.42) than on Morrón (19.70 ± 0.37) and Piquillo (18.89 ± 0.38). The average oviposition (eggs per female) of "B" biotype was significantly greater on Piquillo (214.00 ± 6.77) than was on Morrón (194.13 ± 5.71) and Yolo-wonder (66.53 ± 4.56). In contrast, "non B" females laid more eggs on Morrón (217.20 ± 7.71) and Yolo-wonder (226.20 ± 6.14) than on Piquillo (134.40 ± 3.72). With respect to adult longevity (in days), "B" females lived longer on Piquillo plants (24.00 ± 0.62) than on Morrón (18.27 ± 0.43) and Yolo-wonder (15.33 ± 0.65). In contrast, the average longevity of "non B" females was shorter on Piquillo (18.73 ± 0.64) than was on Morrón (24.47 ± 0.74) and Yolo-wonder (24.87 ± 0.70).
From these results we conclude that there was evidence of different reproductive capacity between "B" and "non B" biotypes of *B. tabaci* on some *C. annuum* varieties: Whereas Piquillo was more suitable host for "B" biotype, Morrón and Yolo-wonder were better hosts for "non B".

KEY WORDS: Whitefly, Pepper, Fecundity, Fertility, Longevity.

Introduction

Bemisia tabaci (Gennadius) has become a major agricultural pest of protected horticultural crops (eg. tomato, pepper, melon, lettuce, etc.) in tropical and subtropical areas causing direct damage through feeding and honeydew deposition, as well as geminiviruses transmission. In addition, populations of *B. tabaci* have developed pesticide resistance (Prabhaker et al., 1985) contributing to make more difficult their control. In addition to protected crops, weed hosts, like *Malva parviflora* L., *Brassica kaber* (DC.) and *Capsella bursa-pastoris* (L.) could also serve as suitable overwintering reservoirs for whiteflies (Gerling, 1984; Muñiz & Zalom, 1997). Because of the wide host range of *B. tabaci* and the possibility of continuous development through the winter in many areas, regional management of this insect is necessary to decrease its overall impact (Watson *et al.*, 1992). Several authors have obtained differences in reproductive capacity of *B. tabaci* depending on the host (Coudriet *et al.*, 1985, 1986; Butler *et al.*, 1989; Van Lenteren *et al.*, 1990; Zalom *et al.*, 1995). However, almost no references on the insect development and reproduction on pepper plants are available. Wagner (1995) emphasized the need for understanding the fundamental relationships between biotypes of *B. tabaci* (Wang & Tsai, 1996). This insect has recently been considered a pest in horticultural and ornamental crops in Tenerife, Spain (Carnero et al., 1990). Two well genetically different biotypes, "B" (probably *B. argentifolii* Bellows and Perring, n. sp.) and "non

B" (probably a Spanish native type) have been reported (Guirao et al., 1996). In a previous work, host preference, pupal production and sex ratio of both, "B" and "non B" biotypes of *B. tabaci* on three varieties of *C. annuum* (Morrón, Piquillo and Yolo-wonder) were determined (Muñiz & Nombela, 1997). In order to obtain a better understanding of the reproductive biology of this insect on this crop for IPM programs, development time, fecundity and female longevity of these *B. tabaci* biotypes on the same three varieties of *C. annuum* have been studied in this research.

Material and methods

Seeds of three varieties of C*apsicum annuum* L. (Morrón, Piquillo and Yolo-wonder) were germinated in a climatic chamber at 26:18ºC, 16:8 (L:D) h. and 75-80% R.H. 30 plants of each variety were used. When plants were 60 day-old adult whiteflies were collected with an aspirator from a large stock colony reared on the yolo-wonder variety in a climatic chamber at 22 ± 1ºC and 75-80% R.H. One male and one female of "B" and "non B" biotypes of *B. tabaci* were introduced into a 30 cm^3 plastic truncated cone clip-cage (3.6 cm maximum diameter; 2.6 cm minimum diameter; 4 cm high). One cage per plant was attached to one leaf of each plant. In order to determine the developmental periods of immature stages of *B. tabaci,* the caged adults were held at 25 ± 1ºC for 24-h oviposition period, after which the adults were removed and 10 eggs on abaxial surface of the leaf were selected arbitrarily. Female oviposition and longevity were obtained with the same method but adults were kept into clip-cages in the climatic chamber until the last female died (Wang & Tsai, 1996). Data were analyzed using 1-way ANOVA and Fisher´ s protected LSD. The experiments were carried out at the Centro de Ciencias Medioambientales (CSIC), Madrid (Spain).

Results and discussion

In this study significant differences in lengths of each stadium between populations of the two before mentioned biotypes of *B. tabaci* on some *C. annuum* varieties were found. Individuals from the "B" biotype developed significantly ($p<0.05$) faster on Morrón than on Yolo-wonder. In contrast, the development times of the "non B" biotype were significantly shorter on Yolo-wonder. The total development times (egg-adult) for the "B" biotype ranged from 16.60 d on Morrón to 22.83 d on Yolo-wonder. For the "non B" biotype they ranged from 16.91 d on Yolo-wonder to 19.89 d on Piquillo (Table 1), which is similar to that obtained by Cabello *et al.* (1995) on pepper.

The rate of oviposition on these plants also varied with the biotype of *B. tabaci*. Female "B" whiteflies laid a significantly ($p<0.05$) shorter number of eggs on Yolo-wonder plants than did on Morrón and Piquillo. However, "non B" females laid significantly more eggs on Yolo-wonder and Morrón than did on Piquillo. The average longevity of "B" females was significantly ($p<0.05$) greater on Piquillo plants. However, "non B" females lived significantly longer on Yolo-wonder and Morrón varieties than on Piquillo (Table 2).

In this study evidence of reproductive differences between both, "B" and "non B" biotypes of B. tabaci (previously reported in Spain) on three varieties of *Capsicum annuum* L. was obtained from development time, oviposition and longevity criteria: Generally, developmental periods of immature stages of "B" biotype were shorter and female oviposition and longevity were greater on Morrón and Piquillo varieties of *C. annuum* than were on Yolo-wonder. In contrast, development, female longevity and reproductive capacity of "non B" biotype were better on Yolo-wonder. According to that, Piquillo was more

suitable host for "B" biotype than was for "non B". However, Yolo-wonder was better host for "non B" biotype. Morrón was a similar host for both, "B" and "non B" biotypes.

Table 1. Mean development periods (days ± SE) of eggs, immature stadia (st.) and pupae of *B. tabaci* at 25±1ºC on three *C. annuum* varieties.

Plant type	*B.tabaci* biotype	Egg	1rst st.	2nd st.	3rd st.	Pupa	Egg-adult
Morrón	B	4.75±0.10 a	2.40±0.09 a	2.39±0.09 a	2.53±0.09 a	4.53±0.10 a	16.60±0.37 a
Piquillo	B	4.85±0.12 ac	3.16±0.16 b	3.17±0.13 b	3.19±0.14 b	4.65±0.12 ac	19.02±0.52 b
Yolo-wonder	B	6.09±0.12 d	3.65±0.13 c	3.60±0.14 c	3.67±0.15 c	5.83±0.12 b	22.83±0.48 c
Morrón	non B	5.15±0.12 ab	3.01±0.10 b	3.21±0.11 b	3.38±0.12 b	4.94±0.14 cd	19.70±0.37 b
Piquillo	non B	5.29±0.16 b	3.10±0.10 b	3.27±0.10 b	3.17±0.10 b	5.07±0.15 d	19.89±0.38 b
Yolo-wonder	non B	4.85±0.11 ac	2.53±0.08 a	2.43±0.11 a	2.43±0.10 a	4.68±0.11 ac	16.91±0.42 a

Means within columns followed by the same letter do not differ significantly ($p<0.05$) by Fisher's Protected LSD

Table 2. Oviposition (eggs per female, mean ± SE) and longevity (in days ± SE) of female *B. tabaci* at 25ºC on three varieties of *C. annuum*.

Plant type	*B.tabaci* biotype	Total eggs/ female	Daily eggs/ female	Female longevity
Morrón	B	194.13±5.71 a	10.66±0.29 a	18.27±0.43 a
Piquillo	B	214.00±6.77 b	8.94±0.25 b	24.00±0.62 b
Yolo-wonder	B	66.53±4.56 c	4.45±0.30 c	15.13±0.65 c
Morrón	non B	217.20±7.17 b	8.89±0.20 b	24 .47±0.74b
Piquillo	non B	134.40±3.72 d	7.25±0.24 d	18.73±0.64 d
Yolo-wonder	non B	226.20±6.14 b	9.14±0.25 b	24.87±0.70 b

Means within columns followed by the same letter do not differ significantly ($p<0.05$) by Fisher's Protected LSD

Acknowledgements

This research was supported by funds from the Ministerio de Educación y Ciencia of Spain. (CICYT). Plan Nacional de I+D. Proyecto AGF 95-0253-C02-01.

References

BUTLER, G.D., Jr., COUDRIET, D. & HENNEBERRY, T.J., 1989. Sweetpotato whitefly: Host plant preference and repellent effect of plnat-derived oils on cotton, squash, lettuce and cantaloupe. Southwest Entomol. **14**: 9-16.

CABELLO, T., CARRICONDO, I., JUSTICIA, L. & BELDA, J. 1995. Biología de las especies de mosca blanca de los invernaderos: *Trialeurodes vaporariorum* y *Bemisia tabaci* (Hom., Aleyrodidae). In: Reúmenes de las V Jornadas científicas de la Sociedad Española de Entomología Aplicada. Sevilla 20-24 de Noviembre de 1995: 104.

CARNERO HERNÁNDEZ, A., MONTESDEOCA MONTESDEOCA, M., PÉREZ PADRÓN, F., SILVERIO NÚÑEZ, A. & RODRÍGUEZ LÓPEZ, P., 1990. Presencia de *Bemisia tabaci* (Genn.) en cultivos hortícolas y ornamentales en la isla de Tenerife (Islas Canarias). Cuadernos de Fitopatología, 4º Trimestre, 176-180.

COUDRIET, D.L., PRABHAKER, N., KISHABA, A.N. & MEYERDIRK, D.E., 1985. Variation in development rate on different hosts and overwintering of the sweetpotato whitefly Bemisia tabaci (Homoptera: Aleyrodidae). Environ. Entomol. **14**: 516-519.

COUDRIET, D.L, MEYERDIRK, D.E., PRABHAKER, N. & KISHABA, A.N. 1986. Bionomics of sweetpotato whitefly (Homoptera: Aleyrodidae) on weed hosts in the Imperial Valley, California. Environ. Entomol. **15**: 1179-1183.

GERLING, D. 1984. The overwintering mode of Bemisia tabaci and its parasitoids in Israel.

GÓMEZ-MENOR ORTEGA, J. 1943. Contribución al conocimiento de los Aleyródidos de España (Hem. Homóptera) 1ª nota. EOS, **19**: 173-209.

GUIRAO, P., BEITIA, F. & CENIS, J.L. 1996. Determination of Biotypes of *Bemisia tabaci* (Gennadius) in Spain. Entomol. Exp. Appl. (In press).

MUÑIZ, M. & NOMBELA, G. 1997. Preference of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) on three varieties of *Capsicum annuum* varieties. In: Abstracts of the Symposium International Production et Protection Intégrées des Cultures. Agadir, 6-9 May 1997: 111.

MUÑIZ, M. & ZALOM, F.G. 1996. Host preference of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on some common winter weeds. J. Agric. Entomol. (in press).

PRABHAKER, N., COUDRIET, D.L. & MEYERDIRK, D.E. 1985. Insecticide resistance in the sweetpotato whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae). J. Econ. Entomol. **78**: 748-752.

VAN LENTEREN, J.C. & NOLDUS, L.P.J.J., 1990. Whitefly-plant relationships: Behavioral and ecological aspects. In: D. Gerling (Ed.): Whiteflies: Their bionomics, pest status and maanagement. Intercept Ltd. Sandover, Hants. U.K.: 47-89.

WAGNER, T.L., 1995. Temperature-dependent development, mortality and adult size of sweet potato whitefly biotype B (Homoptera: Aleyrodidae) on cotton. Environ. Entomol. **24**: 1179-1188.

WANG, K & TSAI, J.H. 1996. Temperature effect on development and reproduction of silverleaf whitefly (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Amer. **89(3):** 375-384.

WATSON, T.F., SILVERTOOTH, J.C., TELLER, A. & LASTRA, L. 1992. Selection of some winter-spring vegetable crop hosts by *Bemisia argentifolii* (Homoptera: Aleyrodidae). J. Econ. Entomol. **88**: 70-76.

ZALOM, F.G., CASTAÑÉ, C. & GABARRA, R. 1995. Selection of some winter-spring vegetable crop hosts by Bemisia tabaci (Homoptera: Aleyrodidae). J. Econ. Entomol. **88**: 70-76.

HOST-FEEDING ACTIVITY OF *Encarsia pergandiella* Howard ON *Bemisia tabaci* (Gennadius)

P. Videllet[1], R. Albajes[1] & R. Gabarra[2]

[1] Universitat de Lleida, Centre UdL-IRTA, Rovira Roure 177, E-25006 Lleida, Spain
[2] IRTA, Centre de Cabrils, Carretera de Cabrils s/n, E-08348 Cabrils (Barcelona), Spain

Abstract

Host-feeding or predation is a common habit of many synovigenic parasitoids that are tested for biological control purposes. The capacity of parasitoids to prey on hosts has been considered as a positive trait of candidates for biological control if the host-feeding behaviour is non-concurrent with ovipositing behaviour. The exotic aphelinid *Encarsia pergandiella* was introduced in Europe in the early 80s to control *Trialeurodes vaporariorum* and has become established in the Mediterranean area. More recently, the parasitoid has been evaluated as a biocontrol agent of *Bemisia tabaci*. The work described in this contribution aimed to evaluate the host-feeding capacity of *E. pergandiella* on *B. tabaci* and to find which the host nymph instar preferred by the parasitoid to prey on in order to determine whether the host-feeding and oviposition are concurrent on the same whitefly nymph instar. Eleven unmated *E. pergandiella* females were allowed to choose among all nymphal instars of *B. tabaci* to prey on in cages clipped on tomato leaflets throughout their life span. Arenas with whitefly nymphs (between 50 and 120 individuals) were changed every 24 hours and the number of nymphs killed after exposure to the parasitoid was counted. *E. pergandiella* preyed on relatively more third instar nymphs (N3) than fixed-second and fourth instars. Since N3 is also the preferred host nymphal instar for oviposition, the significance of host-feeding for biological control of *B. tabaci* by *E. pergandiella* is discussed.

Introduction

Since *Bemisia tabaci* (Gennadius) became a world-wide serious pest of vegetables and ornamentals, many natural enemies, among which the arrhenotokous autoparasitoid *Encarsia pergandiella* Howard (Heinz & Parrella, 1994; Onillon *et al.* 1994), have been investigated for their use in biological control of the whitefly. *E. pergandiella* was imported in the Mediterranean area in the late 70s (Viggiani & Mazzone, 1980) and since then become established there (Onillon *et al.* 1994; author's unpublished results). As a consequence, when *Encarsia formosa* is used for whitefly control in greenhouses, *E. pergandiella* hyperparasites the former and may reduce its efficacy (author's unpublished results).

Among the criteria used to select a parasitoid for biological control, the capacity of parasitoid females to prey on hosts have been largely neglected. Host-feeding may have a strong impact on host-parasitoid dynamics through several mechanisms: by allowing adult parasitoids to obtain nutrients and water and thus to increase their fecundity and foraging capacity, by complementing the host mortality due to parasitoid oviposition or eliminating hosts previously parasitized by competing parasitoid species (Heimpel & Collier, 1996; Ehler 1994).

Whether a host is used for feeding or ovipositing is a crucial decision to be made by the parasitoid female in order to maximize benefits and minimize costs. For biological control purposes, the occurrence of non-concurrent destructive host-feeding behaviour (the use of different host individuals for either destructive host-feeding or oviposition) has been cited as a positive trait of candidates (Jervis & Kidd, 1996; Jervis *et al.* 1996). Concerning the relationship between *B. tabaci/B. argentifolii* and *E. pergandiella*, Liu & Stansly (1996)

reported that the parasitoid prefers the 3rd and 4th instar nymph for oviposition. Results from literature are rather contradictory about which *Bemisia* nymphal instar is preferred to feed on. Whereas Schuster & Price (1996) did not find significant differences in host mortality among host nymph ages when *E. pergandiella* females were not given a choice of whitefly instar, Liu & Stansly (1996) recorded higher mortality on younger instars under host age choice conditions but more older nymphs were killed when females could not choose among differently aged *Bemisia* nymphs.

The work reported here aimed to evaluate the capacity of *E. pergandiella* to prey on *B. tabaci* nymphs and to determine which host nymphal instars are preferred by parasitoid females to feed on.

Materials and Methods

Both *B. tabaci* and *E. pergandiella* individuals used in the experiments came from laboratory rearings and were collected originally in Cabrils area (25 km. North of Barcelona). In the rearing unit, *B. tabaci* had been reared on *Brassica* sp. for several (>30) generations and *E. pergandiella* on the system *Trialeurodes vaporariorum* (Westwood)/tomato. The *B. tabaci* strain used in experiments was identified as B-strain by T. Perring in 1993 and can thus be referred to as *B. argentifolii* according to Perring *et al.* (1993). Tomato potted plants were grown in a parasitoid-free compartment with *B. tabaci*-infested *Brassica* plants. Tomato leaflets with a variable number of *B. tabaci* nymphs (50 to 120) of different ages were chosen for the experiments. From here onwards fixed first-second, third and fourth instar nymphs are referred to in the text as N1-2, N3 and N4 respectively. Some leaflets also contained a few pharate adults (PhA). To obtain unmated females, greenhouse whitefly nymphs parasitized by *E. pergandiella* were detached from tomato leaves and kept individually in transparent gelatine 00 vials until adult emergence. Newly emerged parasitoids (<24 h. old) were sexed and placed for 24 h into ∅ 3 cm clip cages with the tomato leaflets infested with *B. tabaci* nymphs. Before caging, the number of N1-2, N3, N4 and PhA infesting experimental leaflets were counted under binocular in 5 of the 11 females caged. Throughout the experimental period, at least one infested leaflet was caged with no parasitoid for 24 h in order to estimate daily natural mortality of nymphs. Experiments were carried out at 23±2ºC, photoperiod of 16L:8D and relative humidity between 30 and 45 %.

The arena was renewed every 24 h, after which the number of dead nymphs (dry and flattened) was counted under binocular. A total of 11 females were used.

To determine the preference of the parasitoid for host age, a χ^2 test was performed. The influence of female age on host-feeding was analysed by ANOVA and means were compared by Student-Newman-Keuls's test. A Statgraphics computer package was used (Statgraphics, 1987).

Results

Host-feeding capacity. A mean of 9.62±1.63 (mean±s.d, n=181) *B. tabaci* dead nymphs was recorded in cages with parasitoid. In control cages the mean was 4.45±1.51 (mean±s.d, n=42) dead nymphs. Thus, the number of *B. tabaci* nymphs killed per unmated *E. pergandiella* female for feeding may be estimated as 5.17 nymphs/24 h.

Longevity of females averaged 16.45±1.76 (mean±s.d, n=11), so a total of 85.0 nymphs killed per female throughout its life span can be expected.

In Figure 1 the number of dead nymphs recorded in control cages (natural mortality) was subtracted from the number of dead nymphs recorded in parasitoid cages and then the means of the differences at each female's age were calculated. Figure 1 therefore shows the

estimated number of nymphs killed by *E. pergandiella* females. The age of females significantly influenced (F=10.61, d.f.=19,161, P<0.001) their host-feeding capacity. This peaked at an age of 9 and 10 days (15 nymphs/24 h), was lower but still high when females were 8 and 11 days old (about 11 nymphs/day) and decreased for the remaining ages. The youngest and oldest parasitoid females showed the lowest host-feeding capacity.

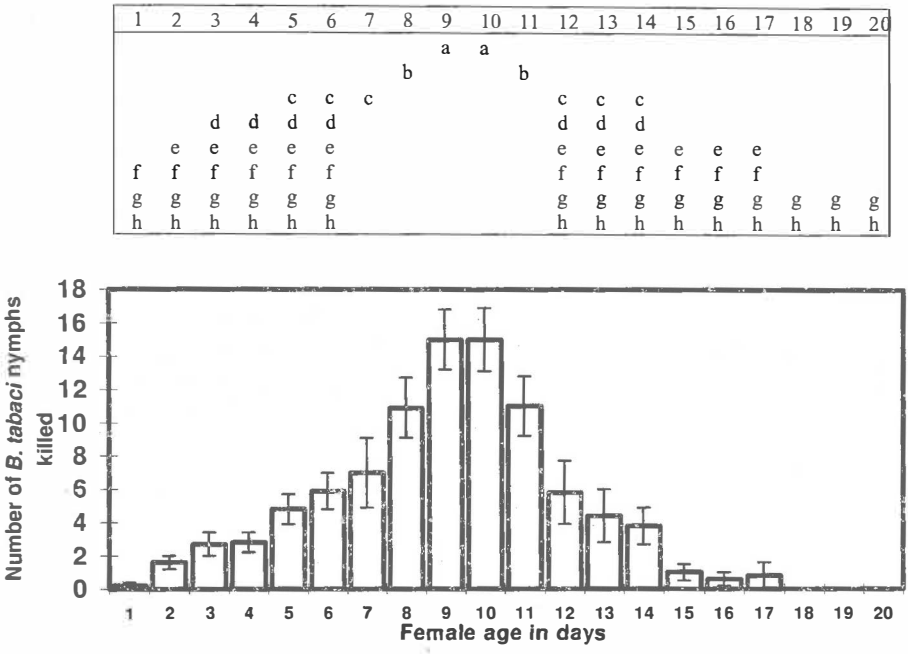


Figure 1. Below. Number (mean±s.d.) of *B. tabaci* nymphs killed by unmated *E. pergandiella* females in 24 hours according to their age. Above. Ages followed by the same letter are not significantly (P<0.01) different accorging to Tukey's test. The number of females used for calculating the mean was 11, except in the last ages (days 15 to 20) in which the number of females was variable (from 9 in the day 15 to 1 in the day 20)

Preference for host instar to feed on. In 5 of the 11 females tested and in control cages the daily number of N1-2, N3, N4 and PhA in the arena was counted. The number and instar of dead nymphs after parasitoid removal was also counted. Expected and observed values for each nymph instar were compared by the χ^2 test (Table 1). Comparison of PhA was not done because of the low numbers of this instar in arenas. The number of young dead nymphs was much lower than expected, whereas the number of third instar dead nymphs was twice the value expected and the number of fourth instar dead nymphs was significantly higher than expected. No significant (P>0.05) differences among nymphal instars were found when observed and expected values in control cages were compared. Thus, differences among nymphal instars found in parasitoid cages were effectively due to the preference of *E. pergandiella* to feed on N3 and N4 to the detriment of N1-2.

Mean number of dead nymphs	N1-2	N3	N4
Observed	1.67	4.22	2,44
Expected	4.58	2.11	1.65
χ^2 (d.f.=80)	189.17 (P<0.001)	281.95 (P<0.001)	151.23 (P<0.001)

Table 1. Comparison of means of expected and observed number of dead nymphs per instar. N1-2: fixed first-second instar, N3: third instar N4: fourth instar. PhA were not included in the analysis

Host nymph instar	Duration	N. of nymphs killed by host-feeding	Experimental conditions	Observations	Ref.
3rd	72 h	5.5-2.4 Unmated females	27.6ºC, 14L:10D Female age: 1-4 d. No instar choice	Range in two poinsettia cultivars Natural mortality subtracted	(1)
3rd	72 h	6.3-3.4 Mated females	as above	as above	(1)
N1 N2 N3 N4^{early}	48 h	28.2 24.7 15.4 14.4 Mated females	26.7ºC 14L:10D HR55±5% Instar choice. Female age not specified	5 females per cage Natural mortality not subtracted Host plant: sweet potato	(2)
1st-4th	24 h	1.3-1.7* Mated females	25.3ºC, 12L:12D No instar choice No signif. differences among host instars	3 females per cage Host plant: tomato Natural mortality estimated and subtracted	(3)

Table 2. Main results of host-feeding activity of E. pergandiella *on* B. argentifolii *and experimental conditions in the following references: (1) Heinz & Parrella, 1994. (2) Liu & Stansly, 1996. (3) Schuster & Price, 1996. *The number of nymphs killed was recalculated from original results shown in the article.*

Discussion

E. pergandiella is an arrhenotoukous autoparasitoid of several whitefly species. Females ara able to kill host nymphs by feeding upon them (Heinz and Parrella, 1994; Liu & Stansly, 1996; Schuster & Price, 1996). Only a few eggs are laid by unmated females (Gerling 1966; Heinz & Parrella, 1994), so mortality caused by female oviposition activites may have been neglected in the present discussion. The main results of the only three references that have been found in the literature concerning host-feeding of *E. pergandiella* upon *B. tabaci/argentifolii* are summarized in Table 2. Many factors influence the number of hosts killed by host-feeding and this makes it difficult to compare our results with those shown in Table 2, because the experimental conditions and set-up used by the authors are quite different from each other. Relative humidity (HR) is not indicated in two of the mentioned references and it probably influences natural mortality of *B. tabaci/argentifolii* nymphs (Sharaf & Batta, 1984). In fact, the low HR under which our experiments were conducted could be the principal cause of the mortality recorded in control cages. Heinz and Parrella (1994) did not find differences between mated and unmated *E. pergandiella* females in the number of *B. argentifolii* nymphs killed by host-feeding. The highest values reported were recorded by Liu & Stansly (1996), but they did not subtract natural mortality from the number of dead nymphs found in parasitoid cages. In this case, values of uncorrected mortality (means per female and per 24 h) were slightly lower than ours before subtracting

natural mortality. Host-feeding could be enhanced in the present work by low relative humidities of the environment, as has been observed in *E. formosa* feeding on *T. vaporariorum* (Liu & Tian 1987).

The killing capacity of hosts by host-feeding recorded in the present work for *E. pergandiella* females throughout their lifespan (85 nymphs, but it could be higher because longevity decreased probably by parasitoid manipulation every 24 h.), is higher than the mortality derived from parasitism that is recorded in the literature on *B. argentifolii* (Heinz & Parrella, 1994) and may not be neglected when the use of this parasitoid for biological control is considered.

It has been frequently observed that older parasitoids reduce their host-feeding activity (Jervis & Kidd, 1986) and this agrees with the results reported here. The influence of parasitoid age on host-feeding has been related to egg load and life expectancy of the foraging parasitoid (Heimpel & Collier, 1996). In unmated females that do not lay eggs, as is mostly the case of *E. pergandiella*, egg load does not probably affect host-feeding activity. In that case, females would tend to extend their life in order to increase the probability of finding a male for mating. Low host-feeding numbers recorded in young females could be explained by their inexperience in finding hosts to feed upon or because they resorb unlaid eggs and and have a far lesser need to obtain proteinaceous nutrients.

About the *Bemisia* nymph instars preferred to prey upon by *E. pergandiella* females, results in the literature are rather contradictory, but preference for older instars, as has been found in the present work, has never been reported (Liu & Stansly, 1996; Schuster & Price, 1996). In these two works, the authors used mated females for their experiments, and according to Liu and Stansly's (1996) results, females could avoid feeding on those instar which are preferred for oviposition. In our case, we tested unmated females and, so they were not forced to choose between ovipositing and preying upon one host nymph. Under these circumstances, females do not need to avoid concurrence of oviposition and feeding on the same hosts, and tend to choose those hosts with higher quality which provide the maximum gains in terms of prolonging their life. Van Lenteren *et al.* (1987) observed that *E. formosa* females allowed to oviposit and feed on large hosts live longer than females just fed on honeydew, a difference that was not found when the hosts available were smaller. In summary, the capacity of *E. pergandiella* females to switch off preference of host instar to feed upon as a function of whether or not they are mated, would complement the behavioural and physiological plasticity observed by Liu & Stansly (1996).

In summary, high values of host-feeding activity of *E. pergandiella* on *B. tabaci* should be taken into account when this parasitoid is considered as a biological control agent of the whitefly. However, in cases where sex ratios of parasitoid populations shift strongly to females (absence or low numbers of secondary hosts in the parental generation to produce males in the progeny) older host instars may be preferred for host-feeding, leading to a decrease in avaibility of preferred host instars for parasitization.

References cited

EHLER, L.E. 1994. Parasitoid communities, parasitoid guilds, and biological control. *In* Hawkins B.A. & Sheeman, W. (eds.) "Parasitoid Community Ecology". Oxford University Press, Oxford, U.K. pp. 418-436.

GERLING, D. 1966. Studies with whitefly parasites of Southern California. I. *Encarsia pergandiella* Howard (Hym.: Aphelinidae). Can.Entomol. **98**:707-724.

HEIMPEL, G.E. & COLLIER, T.R. 1996. The evolution of host-feeding behaviour in insect parasitoids. Biol. Rev. **71**:373-400.

HEINZ, M.H. & PARRELLA M.P., 1994. Poinsettia (*Euphorbia opulcherrima* Willd. ex Koltz.) Cultivar-Mediated Differences in Performance of Five Natural Enemies of *Bemisia argentifolii* Bellows and Perring, n.sp. (Homoptera: Aleyrodidae). Biol.Control **4**: 305-318.

JERVIS, M.A. & KIDD, N.A.C. 1986. Host-feeding strategies in hymenopteran parasitoids. Biol.Rev. **61**:395-434.

JERVIS, M.A., HAWKINS B. A. & KIDD, N.A. 1996. The usefulness of destructive host feeding parasitoids in classical biological control: theory and observation conflict. Ecol. Entomol. **21**:41-46.

KIDD, N.A.C. & JERVIS, M.A. 1996. Population dynamics. *In* Jervis, M.A. & Kidd, N.A.C. (eds.)"Insect Natural Enemies. Practical Approaches to their study and evaluation". Chapman & Hall, London, U.K. pp.293-374.

LIU, J.J. & TIAN, Y.Q. 1987. Influence of environmental factors on the number of host killed by Encarsia formosa Gahan through host feeding and parasitizing. Chinese Journal of Biological Control 3: 152-156. (cited in Noldus, L.P.J.J. & van Lenteren, J.C. 1990. Host aggregation and parasitoid behaviour: biological control in a closed system. In Mackauer, M., Ehler L.E. & Roland, J. (eds.) "Critical Issues in Biological Control". Intercept, Andover, Hants, U.K. pp.229-262.

LIU, T.-X. & STANSLY, P.A. 1996. Oviposition, Development, and Survivorship of *Encarsia pergandiella* (Hymenoptera: Aphelinidae) in Four Instars of *Bemisia argentifolii* (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Am. **89**:96-102.

ONILLON, J.C., MAIGNET, P. & COCQUEMPOT, C. 1994. Premiers résultats sur l'efficacité ***d'Encarsia pergandiella*** (Hymenoptera: Aphelinidae) dans le contrôle de *Bemisia tabaci* (Homoptera: Aleurodidae) en serres de tomate. IOBC/WPRS Bull. **17(5)**: 71-80.

PERRING,T.M., COOPER, A.D., RODRIGUEZ, R.J.,FARRAR, C.A. & BELLOWS, T.S. 1993. Identification of a whitefly species by genomic and behavioural studies. Science **259**: 74-77.

SCHUSTER, D.J. & PRICE, J.F. 1996. Parasitization of *Bemisia argentifolii* (Hom.: Aleyrodidae) by *Encarsia pergandiella* (Hym.: Aphelinidae). Entomophaga **41**: 95-103.

SHARAF, N. & BATTA, Y. 1984. Effect of Sun Factors on the Real Relationship between the Whitefly *Bemisia tabaci* Gennadius (Hom., Aleyrodidae) and the Parasitoid *Eretmocerus mundus* Mercet (Hymen., Aphelinidae). Z. angew. Entomol. **99**:267-276.

STATGRAPHICS 1987. Statistical Graphic System by Statistical Graphic Corporation. Plus Ware, Rockville.

van LENTEREN, J.C., van VIANEN, A., GAST, H. F. & KORTENHOFF A. 1987. The parasite-host relationship between *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* (Westwood)(Homoptera: Aleyrodidae). XVI. Food effects on oogenesis, lifespan and fecundity of *Encarsia formosa* and other Hymenopterous parasites. J. Appl. Entomol. **103**: 69-84.

VIGGIANI, G. & MAZZONE, P. 1980. Sull'introduzione in Italia di Encarsia pergandiella Howard (Hym.: Aphelinidae), parassita di Trialeurodes vaporariorum (Westw.)(Hom.: Aleyrodidae). Boll. Lab. di Ent. Agr."Filippo Silvestri" Port. **XXXVII**: 39-43.

V. Thrips

WESTERN FLOWER THRIPS PHENOLOGY IN ISRAEL

M.J Berlinger[1], Sara Lebiush-Mordechi[1], Dvora Fridja[1], V. Khasdan[1], E. Siti[2], R. Rodman[3]

[1] Entomology Laboratory, ARO, Gilat Regional Experiment Station, Mobile Post Negev 85-280, Israel.
[2] Ministry of Agriculture, Plant Protection, Extension Service, Beer Sheva, Israel.
[3] R. Rodman, Organic Agricultural Adviser, Talme-Yoseph, Israel.

Abstract

Western flower thrips (WFT) were first identified in Israel in 1988. They have since spread throughout the country, attacking vegetable crops, flowers, ornamentals and wild plants. WFT were trapped outdoors throughout the year; their numbers were very low during December-March, with a clear general peak during April-November, sometimes subdivided into two secondary peaks. The total population density varied from year to year. In a screened tomato greenhouse, the pattern of WFT catches was similar indoors and outdoors, although the population was much lower indoors. WFT populations were compared on greenhouse tomatoes and pepper. On leaves- WFT numbers were lowest in tomatoes, and the population density pattern was accurately reflected by the numbers trapped. In flowers- the WFT densities were highest in pepper, where they appeared prior to their being caught in traps.

Introduction

Western flower thrips (WFT), *Frankliniella occidentalis*, which are responsible for severe economic losses in many protected and open-field crops, were described one hundred years ago in California. In 1980 they started to disperse quickly to many countries world-wide (Ben-Dov, 1987). They were discovered in Dutch greenhouses in 1983 (Mantel, 1989), and in Danish greenhouses in 1985 (Brodsgaard, 1993). They were inadvertently introduced to Israel in 1988, most likely on imported ornamentals (Argaman, 1989). They were first found on chrysanthemums and ornamentals in a greenhouse near Tel Aviv (Brosh, 1989).

Since then they have spread throughout the country, attacking flowers, ornamentals, vegetable crops and wild plants in greenhouses and in the open field. Chemical control is hazardous and difficult. Exclusion screens significantly reduce crop invasions but not absolutely. Biocontrol has so far proven insufficient. Nor is plastic mulch an effective solution (Brown and Brown, 1992). Preliminary studies (Berlinger *et al.*, 1996; Berlinger, unpublished) showed that WFT are attracted by blue, green, yellow and white sticky traps, arranged in a vertical position, especially when traps are illuminated by sunshine in the morning hours. Optimal trap specifications were established in accordance with the findings: color (blue), height (40cm) (Berlinger *et al.*, 1996), position (vertical), direction in the open field (south/south-east), and exposure in the greenhouse (full sun) (Berlinger, unpublished) Conclusively, blue sticky traps were used to study WFT phenology during 1993-1996.

The purpose of this summary was to study the seasonal appearance of the WFT as the basis for the preparation of an improved control program.

Materials and Methods

Thrips monitoring by traps. The phenology of WFT was studied in open fields at the Gilat Experiment Station and the Bessor experimental farm; and in screened greenhouses as well as outdoors at Kadesh Barneia and Eshel HaNassi. All sites are in the southern, semi-arid zone of Israel. The traps were made of disposable plastic Petri dishes, 9cm in diameter, fixed by

iron wire on a blue corrugated sheet (10x10cm). The inside of each Petri dish was smeared with insect-glue (Rimifoot®). The traps were exposed vertically, approximately 40cm above the ground, facing south-west to maximize sunshine illumination in the morning hours. Ten traps were set outdoors at 5 meter intervals, and ten traps per 1,000 sq.m indoors.

Monitoring thrips on plant material. Thrips present in flowers or on leaves of pepper and tomato plants were counted on plant samples by a stereoscopic binocular at x10 magnification.

Comparing indoor/outdoor catches. The number of WFT caught on 20 traps in a tomato greenhouse was compared with the number trapped on 20 traps outdoors. The traps were changed weekly. The observations took place at Kadesh-Barneia from September 1995 to June 1996.

Results and Discussion

Phenology. WFT phenology was studied by positioning traps in uncultivated fields, or in proximity to an organically cultivated field. In general, various numbers of WFT are trapped throughout the year. During winter, from December to March-April, very few WFT were trapped (fig.1). During the summer months much higher numbers were trapped, forming a single peak (Gilat, 1993,1994), or two peaks; one in May-June, and the second in September-October (Gilat, 1993, Gilat-Organic field, 1995). This pattern of trapping was similar whether the traps were located in uncultivated fields like in Gilat (Fig 1, 1993-1996) and Bessor (Fig 1, 1993), or at the edge of an organic cultivated field at Gilat (Fig 1, 1994-1995). The same pattern of appearance was also found at Kadesh-Barneia (data not shown).

There were significant differences in WFT numbers trapped at Gilat during different years: the highest numbers of WFT were trapped in 1994, lower numbers in 1993, and the lowest numbers in 1996, the year at which the peaks were the least defined.

Comparing indoor/outdoor catches. In a tomato crop at Kadesh Barneia, a positive correlation (R^2=0.7) was found during the period from September 1995 to June 1996 between thrips trapped indoors and outdoors, although the number of WFT trapped indoors was significantly lower than the number trapped outdoors at the same time. This indicates that indoor traps reliably indicate the immigration rate of WFT into a greenhouse screened by whitefly-proof exclusion screens.

The efficacy of blue sticky traps. The timing of the appearance of the WFT in traps was compared to their appearance on tomato and pepper plants (Fig. 2). When monitoring pepper leaves the thrips appeared first in the traps, then on the leaves. But when monitoring pepper flowers it was found that thrips were present in the flowers prior to their appearance in the traps, and in higher numbers. Therefore, one should not rely on traps as the sole monitoring method in pepper. In the tomato crop, no WFT were found in the flowers. Thrips were detected in traps before they were found on the leaves. Thus, blue sticky traps can be used for monitoring WFT in tomato crops.

In conclusion

* WFT is on the wing year round, with low numbers in winter, and two peaks; one in spring and one in autumn.
* In general, blue sticky traps are a convenient and reliable device for monitoring WFT.
* Traps efficacy is variable, depending upon the crop being monitored.

Bibliography

ARGAMAN, Q., Z. KLEIN, Y. BEN-DOV, AND Z. MENDEL (1989) *Frankliniella occidentalis* (Thysanoptera: Thripidae), an injurious intruder. Hassadeh, **69**:1268-1269 (in Hebrew with English summary).

BEN-DOV, Y. (1987) The western flower thrips *Frankliniella occidentalis* has appeared in Europe; let us prevent its penetration into Israel. Hassadeh, **67**:1701. (in Hebrew).

BERLINGER, M.J.; SARA LEBIUSH-MORDECHI; DVORA FRIDJA, AND NETA MOR (1993) The effect of types of greenhouse screens on the presence of western flower thrips: A preliminary study. IOBC/WPRS Bull. **16(2):**13-16.

BERLINGER, M.J., S. SINGER, S. LEBIUSH-MORDECHI AND B. RACCAH (1996) Monitoring insect vectors with special emphasis on ornamental viruses in protected crops. Acta Hort. November 1996, No. **432**:188-194.

BRODSGAARD, H.F. (1993) Monitoring thrips in glasshouse pot plant crops by means of blue sticky traps. IOBC/WPRS Bull. **16(8):**29-32.

BROSH, S. (1989) [The Western Flower Thrips and its Control] Israel Ministry of Agriculture, Plant Protection, Extension Service, 1p. (in Hebrew).

BROWN, S.L. AND BROWN, J.E. (1992) Effect of plastic mulch color and insecticides on thrips populations and damage to tomatoes. HortTechnology **2(2):**208-211.

MANTEL, W.P. (1989) Bibliography of the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera : Thripidae. IOBC/WPRS Bull. 1989/**XII/3**. pp. 29-66.

Fig. 1. Phenology of the WFT

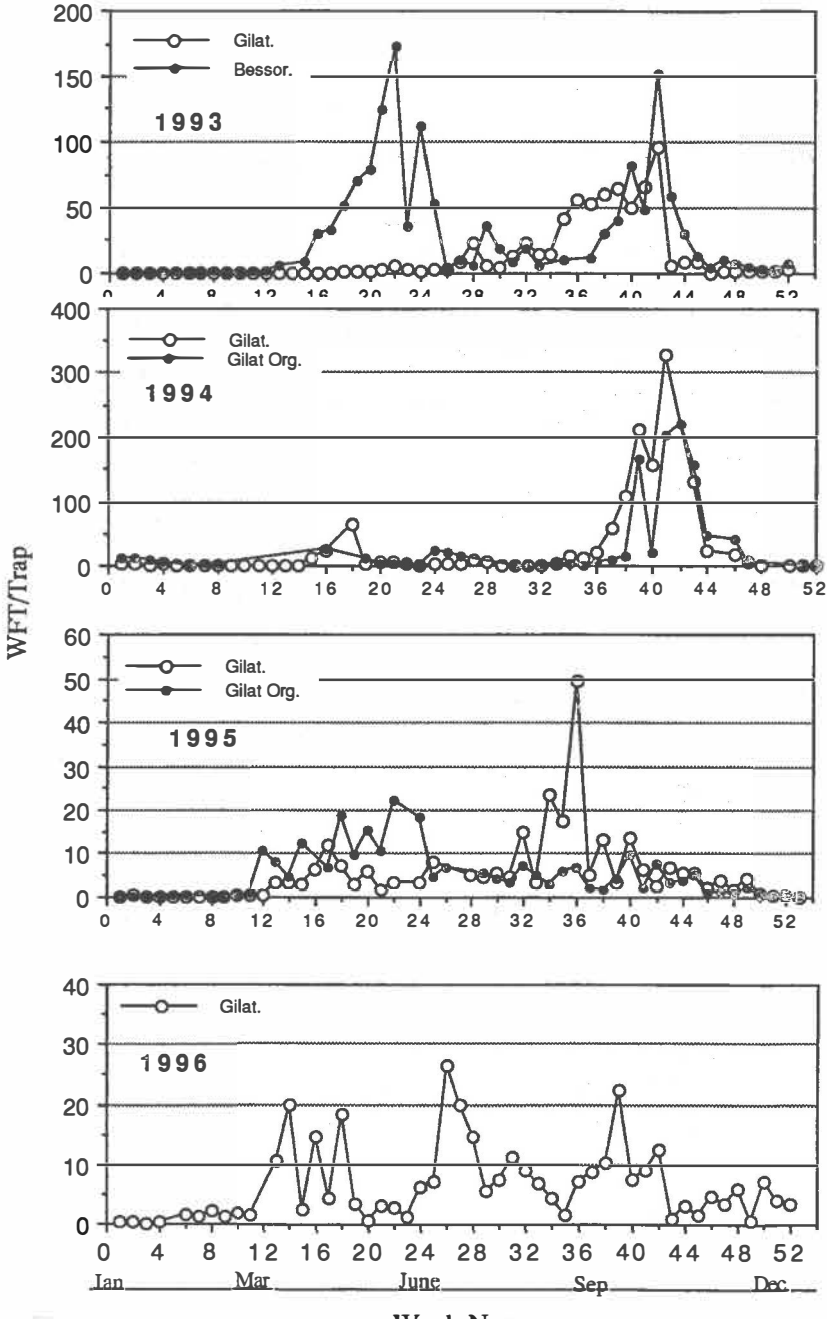
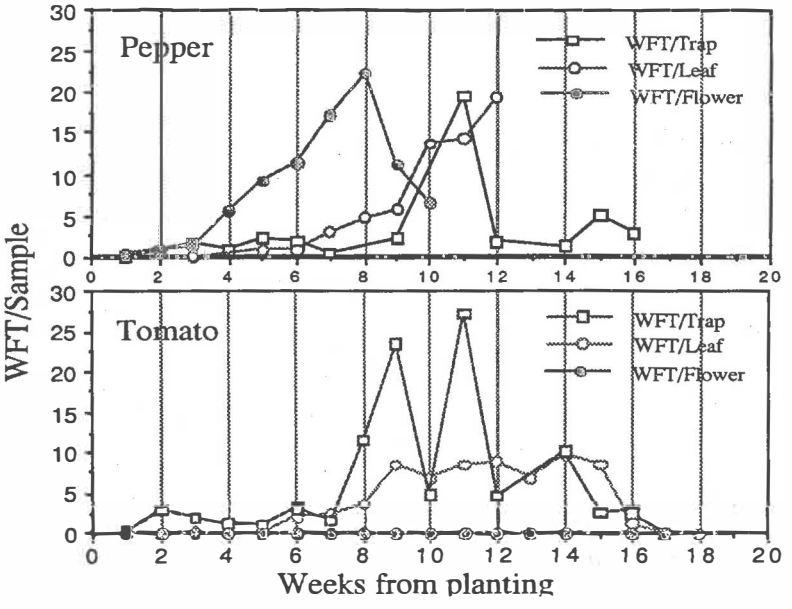


Fig. 2. Appearence of WFT in blue sticky traps, in pepper and in tomato crops



STICKY TRAPS' COLOUR AND *Frankliniella occidentalis'* SEX RATIO IN GREENHOUSE CROPS

Célia Mateus (1), Jorge Araújo (1) & António Mexia (2)

(1) Évora University, Biology Department., Apart. 94, 7001 Évora Codex, Portugal
(2) Agronomical Institute, IPM Section, Tapada da Ajuda, 1399 Lisboa Codex, Portugal.

Abstract

Male and female *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) responses to sticky traps' colours were studied in four greenhouses grown roses, carnations, spray- type carnations and strawberries. A consistent influence of colour in thrips' sex ratio was not detected: in two crops (roses and carnations) an effect (but not equal) was observed and, in the other two crops, there were no significant differences between different traps. A possible seasons and/ or crops effect is suggested and the importance of sex ratio determination in crops is emphasized.

Introduction

Apart from the importance of studying adults sex ratio owing to its ecological and evolutionary significance, there is also the possibility of using it for crops protection: Higgins & Myers (1992) and Reynaud et al. (1993) detected a relation between *F. occidentalis* sex ratio and populations densities, which can be used to predict this pest populations dynamics.

Mateus (1993) and Mateus (not published) data indicate that their model should be modified for situations where pesticide treatments occur: a pesticide effect on this pests sex ratio was observed, with more males being detected after spraying. Houston (1981) also observed this effect for *Anastrepha ludens* (Diptera).

If males and females have, in fact, a different economical impact in crops, analysis of sex ratio evolution will help predicting this impact, and should be taken into account when economic decision levels are calculated and used.

Sex ratio data may also be important in diseases dynamics analysis: Choudhury & Rosenkranz (1983) detected a different vector capacity in males and females of *Graminella nigrifons* (Forbes) (Homoptera: Cicadellidae) in relation to MCD Virus.

F. occidentalis monitoring has been done mainly using coloured sticky traps (Robb, 1989; Shipp & Zarifa, 1990; Higgins, 1992; Mateus 1993; Shipp, 1995; and many others).

For a correct use of sex ratio in crop protection, it is important to determine if sex ratio is affected by traps colours used in monitoring programs.

Materials and Methods

Field experiments. Experiments took place in four plastic greenhouses, each with one of the following crops: roses, carnations, spray-type carnations and strawberries (different cultivars were present). Sticky traps were 3mm thick coloured acrylic Plexiglas plates, 10x15 cm, covered on both sides with Napvis glue. In roses and carnations greenhouses, in summer, we tested three white shades (Plexiglas no. 199, 123 and 815, an opaque, a translucid and a cream white, respectively), three yellow shades (Plexiglas no. 566, 552 and 577, a bright, a dark yellow and an orange, respectively) and three blue shades (Plexiglas no. 326, 353 and

308, a bright, a dark opaque and a translucid dark blue, respectively). In spray-type carnations and strawberries greenhouses, in spring, we tested: the whites 199 and 815, the yellows 566 and 552 and the blues 326 and 353. Traps were hung vertically, just over the plants, in a balanced incomplete block design (Cox 1958): twelve blocks in roses and carnations and ten in spray-type carnations and strawberries, each block with three traps of different colours separated by about 35cm. In the first two experiments, there was a total of four plates of each colour and, in the other two, there were five. One week later, traps were removed and examined.

Data analysis. The proportion of males (number of males in trap x/ total of individuals in trap x) was calculated for each trap. After transformation log (x+0.5), the analysis of variance (ANOVA) was performed and, when recommended, the adjusted means of each colour were compared by Duncan`s Multiple Range Test (Montgomery 1991) for a 5% level of significance.

Reflectance spectra. Methodology and results are presented in Mateus (1993) and Mateus & Mexia (1995).

Results

The adjusted mean proportions of males for each shade are presented in Table 1. In carnation and rose, significant differences between some colours were detected. This was not so for spray-type carnation and strawberry (ANOVA analysis did not recommended means comparison).

Discussion

Male and female *F.occidentalis* attraction to reflectance spectra, which human eyes see as specific colours/ shades, was studied. Data analysis was performed using the proportion of males in each trap (in relation to the total captures in that trap) and not the sex ratio (males/ females or females/ males), to avoid as much as possible mathematical indeterminations (cases of no males or females being trapped).

Results did not revealed a consistent influence of traps colour in the sex ratio of thrips captured: in the two crops where that effect was observed, it was not working in the same way; on the other two, colour did not affect sex ratio.

F. occidentalis male and female electroretinogram revealed minimal differences between sexes spectral efficiency (Matteson et al., 1992) and fieldwork conducted by Matteson & Terry (1992) indicated those relative preferences for colours were similar between males and females. However, in Vernon & Gillespie's (1990) studies, some differences were seen, in spite of, in most cases, male and female colour preferences had been similar.

Since we are talking about different crops and different seasons, these two factors' influence should *be* explored in order to clarify this question.

F. occidentalis is a pest causing serious problems in many countries of Europe and America. Standardise a trap is a possibility, but it may not be easily available to all growers in different countries, and so they should be given the opportunity of choosing the most economical and practical solution between a range of traps (of different materials and colours).

Table 1. Adjusted mean proportions of males *Frankliniella occidentalis* on sticky traps of different shades of white (w), yellow (y) and blue (b), in greenhouses with carnations, roses, spray-type carnation and strawberries

Crop	Colours								
Carnation	w123	y577	y552	w199	y566	b326	w815	b353	b308
	-0.76 a	-0.73 a	-0.46 ab	-0.45 abc	-0.25 bcd	-0.21 bcd	-0.06 bcd	0.00 cd	0.07 d
Rose	y577	w123	b353	w199	y552	b326	w815	b308	y566
	-0.77	-0.13 a	-0.02 a	0.08 a	0.09 a	0.13 a	0.22 a	0.29 a	0.31 a
Spray carnat.	y566	b353	y552	w199	b326	w815			
	-0.13	0.11	0.17	0.17	0.24	0.31			
Strawberry	b353	w815	w199	Y566	y552	b326			
	-0.09	0.02	0.03	0.09	0.11	0.16			

Adjusted means (transformed data) above the same letter within rows are not significantly different (Duncan's multiple range test, ((= 0,05); standard errors: 0.16 (rose); 0.13 (carnation); 0.06 (strawberry).

Acknowledgements

Junta Nacional de Investigação Científica e Tecnológica (JNICT) financed the research with a grant attributed to the first author.

References

CHOUDHURY & ROSENKRANZ, 1983. (in Hunt et al. 1993. Influence of leafhopper (Homoptera: Cicadelidae) gender and female mating status on plant disease dynamics within a simple habitat. Env. Ent., **22**(1): 109-115)

COX, D.R., 1958. Planning of experiments. John Wiley & Sons Inc. New York.

HIGGINS, C. J., 1992. Western Flower Thrips (Thysanoptera: Thripidae) in greenhouses: population dynamics, distribution on plants and associations with predators. J. Econ. Ent., **85**(5): 1891-1903

HIGGINS, C. J. & MYERS, J. H., 1992. Sex ratio patterns and population dynamics of Western Flower Thrips (Thysanoptera: Thripidae). Env. Ent. **21** (2): 322-330.

HOUSTON, W. W. K., 1981. Fluctuations in numbers and significance of the sex ratio of the mexican fruit fly, Anastrepha ludens caught in Mcphail traps. Ent. Exp. & Appl., **30**: 140-150

MATEUS, C., 1993. A praga *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) e a implementação da protecção integrada no seu combate. M. Sc. Thesis, Instituto Superior de Agronomia, Lisboa.

MATEUS, C. & MEXIA, A., 1995. Western Flower Thrips response to colour. International Conference on Thysanoptera. 28-30 Sept. 1993, Vermont. In: Thrips biology and management. Eds: B. Parker, M. Skinner & T. Lewis. NATO ASI Series, vol. **276**, 636 pp.

MATTESON, N. & TERRY, L. I., 1992. Response to colour by male and female Frankliniella occidentalis during swarming and non- swarming behavior. Ent. Exp. App., **63**:187-201

MATTESON, N., TERRY, L. I., ASCCOLI-CHRISTENSEN A. & GILBERT, C., 1992. Spectral efficiency of the western flower thrips Frankliniella occidentalis. J. Insect Physiol., **38**(6): 453-459

MONTGOMERY, D., 1991. Design and analysis of experiments. John Wiley & Sons Inc., New York.

REYNAUD, B., BOISSOT, N., RENARD, V., PATERNELLE, M. C., NABENEZA, S., 1993. Dynamique des populations de Frankliniella occidentalis et apidemiologie du Tomato Wilt Virus (TSWV) dans une culture florale de plein champ (Callistephus chinensis) a la Reunion. ANPP- III Conf. Int. Ravag. Agric., 7-9 Dec. 1993, Montpellier.

ROBB, K., 1989. Analysis of Frankliniella occidentalis as a pest of a floricultural crops in California greenhouses. PH. D. Thesis, Univ. California, Riverside, USA, 135pp

SHIPP, L. 1995. Monitoring of Western Flower Thrips on glasshouse and vegetable crops. International Conference on Thysanoptera. 28-30 Sept. 1993, Vermont. In: Thrips biology and management. Eds: B. Parker, M. Skinner & T. Lewis. NATO ASI Series, vol. **276**, 636 pp.

SHIPP, L & ZARIFFA, N., 1990. Developing a sampling program for Western Flower Thrips on greenhouse peppers. SROP/ WPRS Bull. **13**(5): 194-197

VERNON, R. S. & GILLESPIE, D. R., 1990. Spectral responsiveness of Frankliniella occidentalis (Thysanoptera: Thripidae) determined by trap catches in greenhouses. Env. Ent., **19**(5): 1229-1241

THRIPS PALMI: A NEXT THRIPS PEST IN LINE TO BE INTODUCED INTO EUROPE?

A.J.M. Loomans & G. Vierbergen.
Plant Protection Service, P.O. Box 9102, 6700 HC Wageningen, The Netherlands

Abstract
Thrips palmi Karny is an important pest throughout large parts of tropical and subtropical vegetable and flower producing areas. After the introduction and establishment of *Frankliniella occidentalis* (Pergande) into Europe about 15 years ago, this could be a next economic thrips pest in line. Exchange of horticultural products all over the world, makes this quarantine pest a serious threat to Europe as well. Interceptions from vegetables and cut flowers imported from the Caribbean and Asia show an increase in numbers over recent years. Except a threat for greenhouse crops in temperate areas, it can particularly be a potential problem for the horticultural industry in the Mediterranean Region. Here we give an account of its taxonomy, geographical distribution and ways of introduction with international trade. We also make an analysis of its risk to various crops, based on the plant host range, biological and ecological characteristics and indicate ways of control.

Introduction

During the past twenty years, the economic significance of the thrips species, *Thrips palmi* Karny and *Frankliniella occidentalis* (Pergande), as pests of vegetables and ornamental crops worldwide, has increased greatly. Started *F. occidentalis* its expansion in Europe, *T. palmi* did so in the Far East and the Pacific. End of the eighties, *F. occidentalis* was recorded first in the Mediterranean Region and established itself rapidly, both in the greenhouse and in the open (Loomans *et al.*, 1995). *T. palmi* has not established itself in Europe yet, but is considered as an EPPO and EU quarantine organism (A1 status: EPPO/CABI, 1997) with a zero tolerance level and a high priority to prevention of entry and establishment. In 1992, infestations in The Netherlands on *Ficus benjamina* urged the Dutch Plant Protection Service to start a drastic eradication programme (Van Halteren, 1995) and since then EU import inspections were intensified. Nevertheless, because of various characteristics of the pest itself as well as the ever increasing pressure by international trade, it is not unlikely that sooner or later *T. palmi* will obtain a foothold here. Below the major pathways of entry for *T. palmi* as well as similarities and differences with *F. occidentalis* and major ways of management are indicated.

Detection and identification

The introduction of *F. occidentalis* took Europe by surprise as did *T. palmi* in the Far East. In both cases a lack of proper taxonomic descriptions, misidentifications, confusion with other species and a lack of adequate quarantine programmes in the newly invaded areas hampered proper actions to be taken and enhanced early establishment (Mantel, 1989; Sakimura *et al.*, 1986). Its major field characteristics: *T. palmi* is about 0.9 mm (male) - 1.2 mm (female) in size, with the first two antennal segments light in colour. The body is uniformely coloured, light yellow in the male and clear yellow without any blotch in the female. Without microscopic examination (for details see Sakimura *et al.* (1986), Zur Strassen (1989)) however, it can easily be confused with other yellow species native to Europe within the genus *Thrips,* as for instance pest species like *Thrips tabaci* Lindeman or *T. nigropilosus* Uzel or plant dwellers like *T.*

Table 1: Interceptions of *Thrips palmi* (adults and/or larvae) at import products in European ports of entry (auctions and airports). Based on Vierbergen (1996), supplemented with notifications of various Plant Protection Services, situation April 1997.

Plant family	plant species	origin	year	country (# interceptions)
ornamentals				
Amaranthaceae	*Amaranthus* sp.	Mauritius	1996	France (1)
	Celosia sp.	Australia	1997	Netherlands (1)
Compositae	*Dendranthema*	Japan	1987	Netherlands (1)
Moraceae	*Ficus benjamina*	USA (Florida)	1996	Netherlands (1)
Orchidaceae	not identified	Singapore	1995	Netherlands (1)
			1996	France (9)
			1997	Italy (1)
		Thailand	1988	Netherlands (2)
			1991	Finland (3), Germany (1)
			1992	Finland (4), Norway (1)
			1993	Finland (3)
			1995	Denmark (3), Finland (1), Netherlands (4), Sweden (1)
			1996	Denmark (1), Finland (1), France (14)
			1997	France (8), Germany (2), Netherlands (5), Spain (3) Sweden (1)
	Dendrobium sp.	Malaysia	1996	France (2), Netherlands (2)
		Singapore	1996	Netherlands (1)
		Thailand	1996	France (10), Netherlands (1)
			1997	Germany (1), Italy (24), Netherlands (1), UK (2)
Rosaceae	*Rosa* sp.	India	1997	Netherlands (4)
Umbelliferae	*Coriandrum* sp.	Thailand	1997	France (4)
vegetables				
Solanaceae	*Basella alba*	Mauritius	1987	UK (1)
	Solanum sp.	Thailand	1997	France (1)
	Solanum macrocarpon	Mauritius	1996	France (1)
	Solanum melongena	Guadeloupe	1988	France (1)
		Mauritius	1996	France (6)
		Dom. Rep.	1996	France (3)
			1997	France (1)
		Thailand	1997	France (1)
	Capsicum sp.	Mauritius	1996	France (1)
Cucurbitaceae	*Cucurbita maxima*	Mauritius	1996	France (3)
	Momordica sp.	Dom. Rep.	1996	France (2)
			1997	France (1)
		Thailand	1997	France (12)
	Momordica charantia	Dom. Rep.	1997	France (3)
		Thailand	1997	France (2)

Table 2: Infestations by *Thrips palmi* of potted plants in greenhouses in The Netherlands

year	plant family	plant species	# infested greenhouses
1988	Cactaceae	not identified	1 x
1992	Moraceae	*Ficus benjamina*	3 x
1994	Moraceae	*Ficus benjamina*	21 x
1995	Moraceae	*Ficus benjamina*	10 x
1996	Moraceae	*Ficus benjamina*	3 x

pillichi Priesner, *T. brevicornis* Priesner, *T. albopilosus* Uzel, *T. urticae* Fabricius, *T. alni* Uzel or the widely distributed *T. flavus* Schrank.

Geographical distribution and international spread.

T. palmi originates from the Oriental region. It was first described from tobacco in Sumatra, Indonesia in 1921, but it is native within the Pakistan-Taiwan-Indonesia triangle. Its economic significance remained obscure for a long time, but at the end of the 1970's it was recorded as a major pest in vegetable crops in The Philippines, Japan and various islands in the Pacific. Except for a few odd records from mainland Africa (Sudan, Nigeria), it is now found throughout Asia and tropical Australia and it has been spreading rapidly in the Caribbean extending its range to Florida-USA, Venezuela, Guyana and recently to Cuba. It settled in Mauritius and Reunion and recently the species invaded Brazil (Vierbergen, 1995; EPPO/CABI, 1997). A single record from The Canary Islands in 1981 (Miyazaki *et al.*, 1984) has not been confirmed.

The international trade of plant material is the most important way of transport and its rapid dispersal to new areas (Vierbergen, 1995). Large quantities of horticultural products are imported daily from all over the world into Europe. Regular inspections by phytosanitary authorities show an increase in interceptions of *T. palmi* and rejections of imported products at ports of entry in recent years. Two main pathways of entry can be identified:

(1) fresh market products such as cut flowers (roses, orchids) imported from Southeast Asia and vegetables from the Caribbean and Mauritius (table 1; Vierbergen, 1996; Reynaud & Mercadier, 1996). Because these directly marketable products have a low shelf-life, and only adults and larvae were detectable, the real infestation level is likely to exceed actual interception numbers.

(2) direct import of propagation material, i.c. plants which have been reared outside Europe and that are grown for a number of months in European greenhouses before they are redistributed (table 2; Reynaud & Mercadier, 1996).

Both patways present a potential, but different risk to the European horticultural industry. Establishment due to entry or contamination by fresh market produce at auctions, repackaging points or at the consumers level, is not very likely. In case of entry with propagation material, population build-up can take place while grown in the greenhouse, on the imported crop or on neighbouring vulnerable host crops, thus presenting a much higher risk of establishment. Since March 1996 the EU therefore requires an international plant passport for *Ficus* plants in order to prevent further spread within its area. Surveys performed in all EU-countries on *Ficus* shipments and on field crops however revealed no other infested areas (Schmidt, 1994; Vierbergen, 1996; Reynaud & Mercadier, 1996).

Host plants and damage

Like *F. occidentalis, T. palmi* is extremely polyphagous: it has been recorded from over 210 species of plants, belonging to 51 families. It is known to reproduce on 33 species of plants, within 14 families, many of which are commercially important crops (table 1), e.g. Cucurbitaceae (cucumber, melons, pumpkin, squash), Solanaceae (eggplant, sweet pepper, potato, but not tomato) and Leguminosae (various types of bean). Larvae and adults feed close to veins and midribs on the under and upper side of the leaves, on stems, growing tips, flowers and fruits, causing direct damage. Severe injuries by defoliation, scarring and deformations of fruits cause large economic losses due to *T. palmi* infestations (Etienne *et al.*, 1990; Kawai, 1990a). In Hawaii, in mixed populations in cucumber, infestations by *F. occidentalis* were responsible for scarring and deformations of fruit, whereas *T. palmi* was primarily found feeding on the foliage (Rosenheim *et al.,* 1990). Until its detection on *Ficus benjamina* in 1992 (Vierbergen, 1996), except for *Ficus carica* (fig), *Ficus* species were unknown as host plants for *T. palmi.* Experiments performed under quarantine conditions however, have shown that though *F. benjamina* is not a primary host plant, *T. palmi* readily oviposits in the soft young leaves and growing tips and can complete its development successfully on various cultivars (Loomans, unpubl.).

Besides direct damage from feeding, indirect damage due to transmission of tospoviruses has been reported. *F. occidentalis* is a well known vector of this group of plantborne viruses. *T. palmi* is also known as a vector, e.g. of Peanut Bud Necrose Virus in India. Its importance as a vector of TSWV however remains yet unclear: populations endemic to Taiwan and Japan have been reported to transmit TSWV infecting watermelon, but attempts to transmit other isolates of TSWV in Florida and elsewhere have failed (Tsai *et al.*, 1995).

Biology and ecology

Most of the information on the biology and ecology of *T. palmi* is based on work in Japan (e.g. Kawai, 1990a) and the Caribbean/Florida (Tsai *et al.*, 1995). Table 3 shows some of its major life-history parameters on cucumber, its preferred host plant. The life-cycle of *T. palmi* is rather similar to related thrips pest species, e.g. *F. occidentalis* (see EPPO/CABI, 1997). In both species the sex-ratio is largely female biased and developmental times are comparable as well, e.g. on cucumber (Kawai, 1990a; Gaum *et al.*, 1994). An important difference between the two is the relative period required for each juvenile life stage (figure 1): *T. palmi* spends about 40% as an egg inside leaf tissue and 25% as pupae in the soil (in total 60-70% in hidden phases) and only 30-35% of the time is spent in the larval stage. In *F. occidentalis,* the egg stage takes about 30%, pupal stages 20-25% (50-55% in hidden phases) and 41-47% of the time is required to complete the larval stage. Although differences in developmental time occur, depending on host plant species and cultivars, this feature is characteristic for *T. palmi*. On cucumber, the pre-oviposition period of *T. palmi* is long compared to that of *F. occidentalis*, resulting in a relative long generation time (egg-to-egg period). Whereas fecundity in *T. palmi* peaks around 25^{0}C (table 3), in *F. occidentalis* it increases up to 30^{0}C (Gaum *et al.*, 1994). Intrinsic growth rate of *F. occidentalis* exceeds that of *T. palmi* in most crops (Kawai, 1990a).

F. occidentalis feeds on pollen as well as on cell-tissue and pollen feeding positively affects fecundity. Although *T. palmi* feeds on pollen too (per.s obs.), it primarily feeds on cell-tissue. Consequently, *T. palmi* adults and larvae are mostly found on leaves (beans, cucurbits), but also on flowers (orchids, roses) and/or fruits (eggplant, pepper) (Kawai, 1990a). In mixed populations on cucumber, *F. occidentalis* largely predominated and aggregated strongly in flowers, whereas *T. palmi* densities were highest on foliage (Rosenheim *et al.*, 1990).

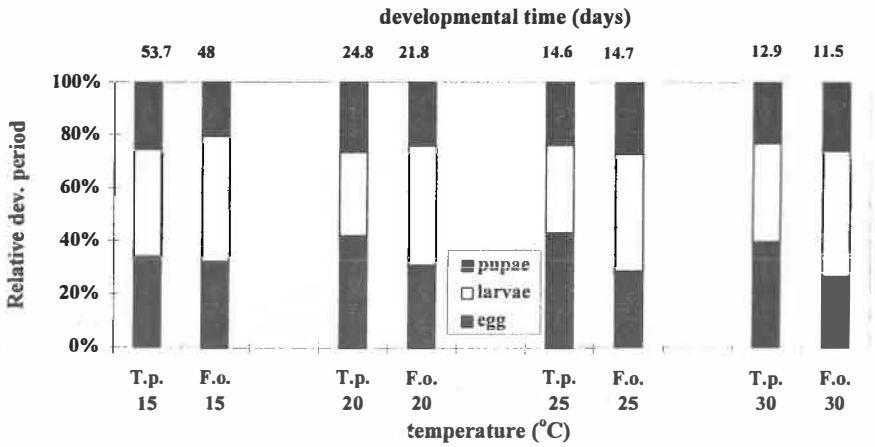


Figure 1: The percentage of developmental time required for each life stage of *Thrips palmi* (Kawai, 1990a) and *Frankliniella occidentalis* (Gaum et al., 1994) on cucumber.

T. palmi by itself has only moderate active dispersal potential. This even decreases when a UV absorbing coverage is used (Vierbergen, 1996; Kawai, 1990b). *F. occidentalis* is very active and spreads actively (EPPO/CABI, 1997).

Pest management strategies

During quarantine actions in The Netherlands in 1992, eradication of *T. palmi* infestations was first based on mechanical destruction of the infested crops, desinfection of the greenhouse and the soil. Later it was based on chemical treatments by a combination of various insecticides. Application of aerosols (dichloorvos), followed by treatment of the crop, based on soil application of the systemic insecticide imidacloprid, in addition with the foliar applications of permethrin and methiocarb and the use of carbofuran in the soil, resulted in elimination of the pest, but put a high pressure on the environment. During the quarantine period (depending on temperature, with a minimum of 1 month), the development of the population was monitored by blue and yellow sticky traps every week (Vierbergen, 1996).

In areas where *T. palmi* established as a pest, chemical control of *T. palmi* often proves to be difficult, because a large part of the juvenile stages escapes treatment (concealed during 60-70% of its developmental time), its resistance to a range of commonly used insecticides (Kawai, 1990b) or control is limited by phytotoxic effects (Seal *et al.*, 1993). Application of organo-phosphoresters even enhanced outbreaks of the pest in the Caribbean, destroying natural enemies and leaving the pest unharmed (Etienne *et al.*, 1990). Although a large variety of predators (anthocorids, mirids, predatory thrips and mites), fungal parasites, nematodes (Walker, 1994) and parasitoids (Loomans *et al.*, 1995) are known, control practices are still largely based on chemical applications (Seal *et al.*, 1993). Monitoring with (white) sticky traps and timely applications of pesticides proved successfull in combination with cultural practices (mulching, UV absorbing screens, mass trapping: Kawai, 1990b). IPM strategies are currently being developed for solanaceous and cucurbit crops, based on the releases of predatory bugs (*Orius sauteri* (Poppius)) in Japan (reviewed by Yano, 1996) or predatory mites (*Amblyseius*

Table 3. Life-history parameters of *Thrips palmi* at different temperatures on cucumber. K = Kawai (1990a; Japan), T = Tsai *et al.* (1995; Florida).

	15°C K	15°C T	20°C K	25°C K	26°C T	30°C K	32°C T
Egg to egg period (T) (days)	80,2	53,8	40,7	24,8	25,1	20,5	17,4
Nr. female offspring (R_o)	16,5	12,0	25,9	28,0	22,9	19,1	11,2
Intrinsic growth rate (r_m) (/day)	.035	.046	.080	.134	.125	.144	.139
Longevity female (days)	35,9	26,6	22,8	15,8	19,8	10,9	13,1
Longevity male (days)	35,7	13,8	20,6	13,2	20,1	11,3	12,4
Fecundity (eggs/female)	32,4	-	48,8	59,6	-	35,4	-

cucumeris (Oudemans) in Florida. Biological control measures, which have proven to be effective to keep populations of *F. occidentalis* and *T. tabaci* down in Europe, using predatory bugs (*Orius*) and predatory mites (*Amblyseius, Hypoaspis*), will likely be able to control *T. palmi* as well once established, and are currently under investigation.

Concluding remarks

A rapid, though acurate detection and identification of economically significant species like *T. palmi* and their pathways of entry, besides an adequate pest risk assessment, are a prerequisite for making proper control decisions and anticipating establishment. Because *T. palmi* prefers young leaf tissue rather than flowers and fruits, the economic injury level of *T. palmi* will depend on the type of marketable product and is expected to be higher (flowers, fruits) than or similar (potted green plants) to that of *F. occidentalis*. However, because of a comparable host plant range, including many weeds, its preference for temperatures above 20°C, together with the ability to survive mild winters and a low sensitivity to insecticides, make *T. palmi* a high potential risk to field crops and greenhouse grown crops in the Mediterranean Region.

Acknowledgements

We greatly appreciate comments on an earlier draft by Berend Aukema and Marie-Josée Jenneskens. The first author was financially supported by the "Produktschap Voor Siergewassen", The Hague, The Netherlands.

References

EPPO/CABI, 1997. Data sheets on quarantine pests. In: Smith, I.M., McNamara, D.G., Scott, P.R. and Holderness, M. (eds.). Quarantine pests for Europe. Datasheets on quarantine pests for the European Union and Mediterranean Plant Protection Organisations. CAB Int., Wallingford, UK, 2nd Ed. : 267-272 (*Frankliniella occidentalis*); 324-328 (*Thrips palmi*).

ETIENNE, J., GUYOT, J. AND VANDERWATERMEULEN, X., 1990. Effects of insecticides, predation, and precipitation on populations of *Thrips palmi* on aubergine (eggplant) in Guadeloupe. Fla. Entomol., 73: 339-342.

GAUM, W.G., GILIOMEE, J.H. and PRINGLE, K.L., 1994. Life-history and life tables of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), on English cucumbers. Bull. Ent. Res., 84: 219-224.

KAWAI, A., 1990a. Life-cycle and population dynamics of *Thrips palmi*. Japan Agric. Res. Quarterly, 23(4): 283-288.

KAWAI, A., 1990b. Control of *Thrips palmi* Karny in Japan. Japan Agric. Res. Quarterly, 24: 43-48.

LOOMANS, A.J.M., VAN LENTEREN, J.C., TOMASSINI, M.G., MAINI, S. and RIUDAVETS, J., 1995. Biological control of thrips pests. Wag. Agric. Univ. Papers 95-1, 201pp.

MANTEL, W., 1989. Bibliography of the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Bull. IOBC/WPRS, 12(3): 29-66.

MIYAZAKI, M., KUDO, I. and IQBAL, A., 1984. Notes on the Thrips (Thysanoptera) occurring on the soybean in Java. Kontyû, 52(4): 482-486.

REYNAUD, P. and MERCADIER D., 1996. Enquête officielle *Thrips palmi* Karny. Résultats France 1996. Rapport LNP Vegetaux - Unité d'Entomologie, 31pp.

ROSENHEIM, J.A., WELTER, S.C., JOHNSON, M.W., MAU, R.F.L. and GUSUKUMA-MINUTO, L.R., 1990. Direct feeding damage on cucumber by mixed species infestations of *Thrips palmi* and *Frankliniella occidentalis* (Thysanoptera: Thripidae). J. Econ. Ent., 83(4): 1519-1525.

SAKIMURA, K., NAKAHARA, L.M. and DENMARK, H.A.,1986. A thrips, *Thrips palmi* Karny. Ent. Circular Fla. Dept. Agric. Consumer Serv., Talahassee, 280:1-4.

SCHMIDT, M., 1994. *Thrips palmi* - ein Proträt: Wissenswertes über den neuen Quarantänenschädling. Der Gartenbau, 23: 23-25.

SEAL, D.R., BARANOWSKI, R.M. and BISHOP, J.D., 1993. Effectiveness of insecticides in controlling *Thrips palmi* Karny (Thysanoptera: Thripidae) on different vegetable crops in south Florida. Proc. Fla. Hort. Soc., 106: 228-233.

TSAI, J.H., YUE, B., WEBB, S.E. , FUNDERBURK, J.E. and H.T. HSU, 1995. Effects of host plant and temperature on growth and reproduction of *Thrips palmi* (Thysanoptera: Thripidae). Env. Entomol., 24(6): 1598-1603.

VAN HALTEREN, P., 1995. A diagnostic network for the EPPO region. Bull. OEPP/EPPO Bull., 25: 1-4.

VIERBERGEN, G. 1995. International movement, detection and quarantine of Thysanoptera pests. In: B.L. Parker, M. Skinner & T. Lewis (eds.), Thrips biology and management. NATO ASI series. Series A, Life sciences 276: 119-132.

VIERBERGEN, G., 1996. After introduction of *Frankliniella occidentalis* in Europe: prevention of establishment of *Thrips palmi* (Thysanoptera: Thripidae). Acta Phytopathol. Ent. Hungarica, 31 (3-4): 267-273.

WALKER, A.K., 1994. A review of the pest status and natural enemies of *Thrips palmi*. Biocontrol News and Information, 15(1): 7N-10N.

YANO, E., 1996. Biology of *Orius sauteri* (Poppius) and its potential as a biocontrol agent for *Thrips palmi* Karny. Bull IOBC/WPRS, 19(1): 203-206).

ZUR STRASSEN, R., 1989. Was ist *Thrips palmi*? Ein neuer Quarantäne-Schädling in Europa. Gesunde Pflanzen, 41(2): 63-68.

INTEGRATED CONTROL OF *Frankliniella occidentalis* IN CRETE-GREECE

S. E. MICHELAKIS[1] and A. AMRI[2]

1. Agricultural Research Centre of Crete and islands
2. Mediterranean Agronomic Institute of Chania

Abstract

The western flower thrips *Frankliniella occidentalis* is widely spread in all the greenhouse crops of Crete, since 1987. With these experiments effort is made to develop the integrated control of this pest based mainly on the use of predators and the blue sticky traps.

The biological control of *F. occidentalis* was carried out with *Orius insidiosus* and proved to be an effective control agent of the pest. The most effective control was obtained when the predator was used in the predator / prey ratio of 6:15.

The effectiveness of blue sticky traps and *O. insidiosus* was also compared with that of the chemical pesticide Vydate (Oxamyl 10%). Vydate showed an immediate effect when it was applied at a dose of 2.0 gr./plant, while predators showed a long time effectiveness.

The combined effect of sticky traps and the predator *O. laevigatus* was also compared with that of other chemicals. (Dichlorvos and Methiocarb). It was found that this combination is very effective against *F. occidentalis*.

Concerning the different colour traps; the blue shades were as good as the white ones without ultraviolet reflectance for catching the pest.

The treatments using predators and traps were all effective in reducing western flower thrips populations to a satisfactory low level and in obtaining healthy plants and good quality produce when compared to the untreated plots.

1. Introduction

Since its introduction in 1983, western flower thrips *Frankliniella occidentalis* has became a nery important incect pest in Europe. It has become a pest of vegatable and ornamental crops especially in the Mediterranean area both in the greenhouse and open field crops (Riudavets et al., 1993). The control of this thrips is generally difficult, since the pest prefers hidden places. The employment of synthetic chemicals, on the other hand, has greatly contributed to the degradation of the environment. During recent years effort have been made for the development of Integrated Pest Management of *F. occidentalis* with the use of *Orius sp (*Fransen 1992, Fransen et al, 1993). In our experiments the efficiency of the predators *Orius insidiosus* and *Orius laevigatus* was tested and was compared with that of the blue sticky traps and some chemicals.

2. Materials and methods

2.1. The efficiency of different predator : prey, ratios

Commercial grasshouses were used for these experiments. Each plant was covered with a white net having a hole of less than 0.18 mm in size to isolate the movements of pests and predators among the plant hosts and exclude and prevent invasion of other insects. The experimental desigh used was the completely randomised with 4 treatments including the control in four replications. Replications were randomly assigned to each of the four treatments because of the homogeneity; each plant was an experimental unit. The plants of the experiment were artificially infested by immature stages of thrips and the insects were allowed to establish

before any treatment was made. The tested treatments were the three predator: prey ratios *O. insidiosus* 2:15, 6:15 and 10:15 and the control without predators. The inscect population was followed by countings every 5 days of all thrips in all the leaves per plant.

2.2. Efficiency of several alternatives of chemical methods of control.

a. The experiment was carried out in the glasshouse of the Institute of Subtropial Plants and Olive Trees in Chania. Cucumber plants (*Cucumis sativis* 1., Variety:Mubis F1 hybrid) were used in the experiment and were grown in a peat-perlite mixture in pots. Each pot was isolated from the others in order to reduce and prevent the movement of pests and predators from one plant to the other. The experimental design used was the completely randomised design with 4 treatments including the control in 4 replications which were randomly assigned to each of the 4 treatments. All the cucumber plants were initially artificially infested by thrips collected from flowers of wild vegetation and were distributed to all leaves at the rate of ten thrips per plant. The insects were allowed to establish before any treatment was made. The treatments tested in addition to control were the following:
i) predatory bugs of *Orius insidiosus* ii) the trapping system with blue sticky traps (Horriver-TR)
iii) the chemical control including Vydate (Oxamyl 10%) granules at a rate of 2g/plant, which was applied to young plants as soil granules and then watered to facilitate immediate absorption by the root systems. The number of *O. insidiosus* was eight predators per plant distributed over the leaves of each treated plant. Blue sticky traps were suspended over the top of the plants, the traps were raised as the plants grew and the trapped adult insects were counted at weekly intervals by using a magnifying glass.The damage caused by western flower thrips was assessed by counting infested leaves as well as contaminated fruit considering the symptoms of thrips. The thrips population was followed in all treatments by countings every 4 days.

b. Several materials were also tested in commercial plastic greenhouse. The cucumber cv Mubis F1 hybrid was sown in March and transplanted in middle April in a plastic greenhouse. The randomized complete block design was used for the experiment, with four blocks each containing four experimental plots. Each plot included nine plants for the application of each treatment. The introduction of California thrips was made artificially from flower of wild vegetation with 15 thrips per plant. The climatic conditions were similar in all sides of the greenhouse. The treatments applied were the following: i) Dichlorvos (Dedevape) with employed dose 2%o. ii) Mesurol (Methiocarb) with the employed dose 2%o. iii) Light blue sticky traps (15 X 8 cm) coated with insect trapping adhesive Tangle Trap in combination with releases of *Orius laevigatus* iv) Control, that is without any treatment. The insect population was followed by weekly counting of the mobile stages of *F. occidentalis* on the undersides of all the leaves from three plants per experimental plot. Insects trapped were also counted weekly.

2.3. Colour trap preference.

In commercial strawberries the experiment of the efficiency of various colours of sticky traps for the detection of *F. occidentalis* was carried out outdoors. The available colour traps tested were white, light-blue, dark-blue, yellow, light green, green and orange. The traps were pieces of painted carboard (14.8X20.8 cm) and were coated with latex paints obtained locally. The sticky traps were mounted on wooden poles at the same height just above the crop canopy. traps were arranged in a completely rondomised design of seven colour treatments with four replications. The number of western flower thrips adults on each trap was counted weekly by

using a magnifying glass and the traps were replaced each time by new ones suspended in the same position.
Statistical analysis, in all experiments, was carried out by the analysis of variance and the means separation by Duncans multiple range test (p= 0.05).

3. Results and discussion

3.1. The efficiency of different predator: prey ratios.

The thrips population was homogenous before treatments. An increase of the thrips population was followed in all treatments but this increase was more pronounced in the control plants (Table 1). This increase lasted up to the middle of May and during this period no difference was observed among the different predator/prey ratios. The only statistical difference observed was the one between each predator treatment and the control. Up to the beginning of May the only existed difference was the one between the radio 6:15 and the control, later and up to the middle of May the increase continued in all treatments without any statistical differences among the different ratios applied .

Counting dates	Treatments			
	Ratio 10:15	Ratio 6:15	Ratio 2:15	Control
25 April	15.50 a	15.00 a	14.75 a	15.75 a
30 April	31.00 ab	16.50 b	31.25 ab	37.00 a
5 May	26.25 ab	18.00 b	29.75 ab	49.25 a
10 May	28.75 b	22.50 b	41.25 ab	61.25 a
15 May	36.50 b	26.25 b	41.25 b	89.25 a
20 May	33.50 b	27.75 b	36.50 b	80.25 a
25 May	7.25 b	9.75 b	19.75 ab	31.50 a
30 May	4.74 b	4.50 b	10.50 b	47.50 a
4 June	5.50 b	10.00 b	15.50 b	47.75 a
9 June	9.75 b	9.00 b	13.50 b	48.50 a
14 June	9.75 b	10.25 b	16.50 ab	51.25 a
19 June	7.50 b	9.25 b	19.00 ab	59.50 a
24 June	6.75 b	8.25 b	13.25 b	56.25 a
29 June	5.00 b	5.25 b	12.25 b	47.25 a

Table 1.- Mean populations of the western flower thrips variation under different release predator:prey ratios before and after the treatments. Means followed by the same letter in the same row are not significantly different at the 5% level of significance according to Duncan's multiple range test.

After the middle of May a dicrease was observed in the thrips population which was more pronounced in the ratios 10:15 and 6:15 but again not statistical difference existed among the different pradator/prey ratios. The thrips population in the control was however much higher with statistical difference than the population in plots treated with the predators.

3.2. Alternatives of chemical methods of control.

a. The thrips population on the plants was homogenous before the application of the treatments as it was expected. After the application of the different treatments an immediate

reduction of thrips population occured in the plants treated by oxamyl while in the other treatments the thrips population continued to increase and this increase was more pronounced in the control (Table 2).

About 10 days after the application of treatments the thrips population started increasing gradually again in the plots treated with oxamyl and one month after the start of the experiment the thrips population was higher on the plants treated by oxamyl than on the plants where predators and traps were used. On the whole and with the exception to two sampling dates no statistical differences were obseved among the plots treated by Oxamyl, predators and traps but the thrips population in all these treatments was different from the thrips population in the control.

b. The numbers of western flowers thrips per plant were homogeneous in all experimental plots, of the plastic greenhouse before applying the treatments.

There was an immediate effect, on the reduction of thrips population, after the application of Methiocarb and Dedevape while the effect of the blue traps and predator was less pronounced.

Counting dates	Treatments			
	Oxamyl	Predators	Traps	Control
26 April	11.00 a	9.75 a	10.75 a	10.50 a
30 April	0.00 b	15.25 a	16.00 a	22.00 a
4 May	0.75 c	16.00 b	15.00 b	29.00 a
8 May	1.00 b	19.75 b	19.00 b	43.00 a
12 May	1.25 c	43.75 b	38.25 b	92.50 a
16 May	2.50 b	19.25 b	22.50 b	71.75 a
20 May	5. 00 b	12.50 b	15.50 b	34.50 a
24 May	7.25 b	7.75 b	12.00 b	33.75 a
28 May	14.25 b	8.25 b	12.25 b	35.50 a
1 June	16.25 b	6.50 b	12.25 b	38.00 a

Table 2.- Mean numbers of western flower thrips per plant over time under predator, trap and Oxamyl treatments. Means followed by the same letter in the same line are not significantly different at the 5% level of significance according to Duncan's multiple range test.

In the control plots the thrips population continued to increase and this lasted up to end of May (Table 3). The thrips population also started increasing in the plots of Methiocarb, Dedevape and Traps but the rate of increase was less important than in the control.

After May and up to the end of the experiment the thrips population in the control started decreasing but it remained in higher level than in the other treatments although a decrease also appeared in their plots. In the plots with traps the predators *O.laevigatus* (5 insects/plant) was added end of May and the thrips population started decreasing continuously. The thrips population in Dichlorvos plots remained in the same level as with traps and predator up to the end of the experiment. In plots with methiocarb the thrips started increasing to the level of the thrips population in the control and for the thrips population reduction Dichlorvos mixed with the faty acid (savona) was applied to all these plots.

Counting dates	Treatments			
	Methiocarb	Dichlorvos	Tr. + Pr.	Control
27 April	8.50 a	10.33 a	9.91 a	8.91 a
3 May	0.00 c	4.50 b	10.25 a	11.50 a
9 May	0.16 d	6.66 c	11.83 b	17.25 a
15 May	3.41 c	18.33 c	41.50 b	67.83 a
21 May	7.58 d	31.41 c	47.25 b	65.83 a
26 May	9.67 d	38.58 c	64.17 b	104.67 a
1 June	10.08 b	23.25 b	21.25 b	58.41 a
7 June	20.16 b	25.25 b	23.58 b	65.58 a
13 June	48.41 b	28.50 c	31.41 c	80.50 a
19 June	58.66 a	22.91 b	24.25 b	70.08 a
26 June	11.83 b	16.50 b	16.08 b	40.66 a
2 July	12.00 b	17.00 b	11.25 b	39.33 a
8 July	12.16 b	15.58 b	9.66 b	45.91 a
14 July	13.25 b	15.00 b	10.33 b	44.66 a

Table 3.- Means of western flower thrips populations per plant over time under Methiocarb, Dichlorvos and trap + predator treatments. Means followed by the same letter in the same line are not significantly different at the 5% level of significance according to Duncan's multiple range test. Tr.: Traps Pr.: Predators

3.3. Colour trap preference

The results showed that under outdoor conditions the greatest preference of *F. occidentalis* was for two shades of blue and for white. (Table 4) On the other hand it was less for yellow and the lowest for light green, green and orange.

There was no difference in colour preference of *F. occidentalis* for white and light-blue which both trapped the highest number of western flower thrips.

C. dates	Treatments						
	Orange	Green	L. green	Yellow	D. blue	L. blue	White
23 May	5.75 b	6.25 b	11.50 b	60.25 b	188.00 a	290.75 a	240.00 a
30 May	7.50 d	12.75 d	23.50 cd	60.50 c	232.25 b	280.50 a	238.5 ab
06 June	4.50 d	7.00 d	13.00 d	75.00 c	257.25 b	330.25 a	326.25 a
13 June	3.00 b	4.00 b	5.75 b	34.00 b	102.50 a	144.25 a	126.50 a
21 June	2.50 c	3.50 c	5.00 c	41.25 b	158.25 a	158.75 a	168.00 a
27 June	3.00 d	3.50 d	5.75 d	50.75 c	133.00 b	156.0 ab	193.25 a
04 July	3.75 c	3.75 c	6.25 c	47.50 b	143.50 a	175.25 a	160.25 a
11 July	3.50 c	5.00 c	6.50 c	41.00 b	127.75 a	160.75 a	154.25 a
18 July	3.00 d	4.50 d	10.00 d	31.00 c	147.50 b	190.00 a	176.50 a

L. green: Light green, D. blue: Dark blue, L. blue: Light blue, C. dates: Counting dates

Table 4.: Population means of western flower thrips adults caught on different colour traps/week. Means followed by the same letter in the same line are not significantly different at the 5% level of significance according to Duncan's multiple range test.

Discussion and Conclusions

During the first five days after releasing the predator, western flower thrips population continue to increase but under the predator prey ratio of 6:15 the increase was slow. The more rapid increase in thrips population was in the control and in the 2:15 and the 10:15 ratios. The 6:15 ratio was the most efficient in keeping the western flower thrips in low population numbers throughout the whole experiment period. Moreover only the 6:15 ratio and the control had significant differences just after treatments and throughout the experiment period. This may be because of the competition between predator in the ratio 10:15 and the small number of predators released in the ratio 2:15. This result is in agreement with Ravensberg et al., (1992) who noted that *O. insidiosus* is able to diminish and clean thrips densities during 3 to 4 weeks when high numbers of predatory bugs (5-10 per plant) are released in cucumber crops. He also showed that in cucumber plants *O. insidiosus* is not able to be established in a complete absence of thrips or on very low thrips densities. The effectiveness of *O. insidiosus* have been demostrated by other workers. At the rate of 0.5 *O. insidiosus* per m^2 could be effective against *F.occidentalis* in peppers (Meiracker and Ramakers, 1991). Fransen, 1992 showed that high rates of *O. insidiosus* reduced flower damage on chrysanthemum from about 90% on untreated plots to 20% where *Orius* was introduced. The continuous increase, however, of the thrips population up to 20th of May in all treatments may be explained by the favourable average temperature during this period and the good stage of the plants with soft lower leaves. After the end of May with the presence of new generation and the increasing population of predators the number of western flower thrips counted was even less than at the beginning particularly in the 6:15 and 10:15 ratios.

Bournier (1990) suggested that oxamyl is among the most active ingredients for the chemical control of *F. occidentalis* in various countries and in our experiments gave satisfactory results. The oxamyl was found to protect cucumber plants for a period of five weeks (Michelakis, 1987) and tomato plants for about three weeks (Kassis and Michelakis, 1993). In our experiment western flower thrips population started increasing about eight days after the application of oxamyl and later the increase became more rapid and more important than in the previous ones.

Plants in which traps were installed showed a population increase in the beginning and then a conciderable reductions in western flower thrips which lasted up to the end of the experiment. Brodsgaard (1989,1993) reported that *F. occidentalis* has a very positive performance for blue sticky traps and that is a very effective tool for early detection of initial attacks and monitoring development of attacks even at very low densities in greenhouses. An important and constant thrips reduction occured in the plots with blue sticky, traps after the introduction of the predator *O.laevigatus* end of May. A considarable multiplication of *O.laevigatus* occured after their introduction and the efficiency of these predatory bugs led to a continuous decrease in western flower thrips population up to the end of the experiment. It is obvious that *O. laevigatus* is very efficient in controlling *F.occidentalis* as it adapts very well to a protected environment. It was found to colonise naturally the infested pepper cultivations without any artificial aids and multiplied successfully when pesticide spaying was lacking (Tavella et al, 1991). The results also confirm Riudavets and Castane (1994) who reported that *O.laevigatus* can be considered as a promising biological control agent for *F. occidentalis* on pepper and cucumber. It was also recorded that populations of this predator established readily on strawberry with high potentials for the control of *F.occidentalis* (Villevielle and Millot, 1991)

The chemicals Dichlorvos (Vapona) and Mesurol (Methiocarb) have been used successfully against western flower thrips (Bournier, 1990; Villevieille and Millot, 1991; Labanowski, 1992).

The results from the colour attractiveness experiment support the findings of Beavers et al. (1971) who found that white traps are highly attractive to *F. occidentalis*. These traps showed a good estination of thrips populations inside the crop habitat. This result is also in agreement with Vernon and Gillespie (1990) who showed the response of *F. occidentalis* to colour in cucumber greenhouse and found that the insect alighted preferably on traps coloured blue, violet, yellow and white whereas green and orange were not attractive.

It can be concluded from our experiments that the *Orius* species tested (*O insidiosus* and *O . laevigatus)* were successful in suppressing western flower thrips populations in cucumber crops. Although the feeding sources consisted mainly of thrips because of the absence of pollen from the parthenocarpic variety of cucumber, the *Orius* population built up successfully. The blue sticky traps caught a considerable number of actively flying adults of western flower thrips and their efficiency can be combined successfully with the efficient of the proper *Orius* species.

References

BEAVERS J.B., SHAW A.C. and HAMPTON R.B. (1971). Colour and height preference of the Citrus thrips in a navel orange grove. J. Econ. Entomol **64**: 1112-1113.

BOURNIER, J.P. (1990). Chemical control of *Frakliniella occidentalis*. Phytoma., **422(35)**: 38-39.

BRODSGAARD, H.F. (1989). Coloured sticky traps for *Frakliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) in glasshouses. J. Appl. Ent., **107**(2): 136 - 140.

BRODSGAARD, H.F. 1993. Monitoring thrips in glasshouse pot plant crops by means of blue sticky traps. Bull OILB/SROP, **16(8)**: 29 - 32.

FRASEN, J.J. (1992). Releases of *Orius insidiosus* against western flower thrips on crysanthemums. Bull. SROP/WPRS, September 1992, Cambridge, England.

FRASEN, J.J., BOOGAARD, H.F., and TOLSMA, J. (1993). The minute pirate bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), as a predator of western flower thrips, *Frakliniella occidentalis* Pergande, in chrysanthemum, rose and saintpaulia. Bull. OILB/SROP, **16(8):** 73-77.

KASSIS, G. and MICHELAKIS, S. (1993). The effectiveness of the biological control of the glasshouse Whitefly *Trialeurodes vaporariorum* west by *Encarsia formosa* (Hym., Aphelinidae) J. Appl. Entomol. 116 (1993), 298-302.

LABANOWSKI, G.S. (1992). *Frakliniella occidentalis* on ornamental cropS in Poland and its control. Bulletin OEPP/EPPO, **22(3):** 367-376.

MEIRACKER, R.A.F. VAN DEN and RAMAKERS, P.M.J. (1991). Biological control of the western flower thrips, *Frakliniella occidentalis*, in sweer pepper with the anthocorid predator *Orius insidiosus*. Med. Fac. landbouww. Rijksuniv. Gent. **56**/2a:241-249.

MICHELAKIS, S. (1987). An integrated control of *Trialeurodes vaporarium* westwood in greenhouses of Crete, Greece. Joint Experts Meeting on Integrated Pest Management in protected vegetable crops. Barcelona, Spain.

RAVENSBERG, W. J., DISSEVELT, M. ALTENA, K. and SIMONSE M.P. (1992). Developments in the integrated control of *Frakliniella occidentalis* in capsicum and cucumber. Bull. OEPP/EPPO, **22**(3): 387-396.

RIUDAVETS, J., GABARRA, R., and CASTANE', C. (1993). *Frakliniella occidentalis* predation by native natural enemies. IOBC/WPRS,**16**(2): 137-140.

RIUDAVETS, J. and CASTANE', C. (1994). Abudance and host plant preferences for oviposition of *Orius spp.* (Heteroptera: Anthocoridae) along the Mediterranean coast of Spain. Bull. OILB/SROP, **17**(5):230-236.

TAVELLA, L., ARZONE, A. and ALMA, A. (1991). Researches on *Orius laevigatus* (Fieb), a Predator of *Frankliniella occidentalis* (Perg.) in greenhouses. A preliminary note.Bull. WPRS/SROP, **XIV**/5: 65-72.

VERNON R.S. and GILLESPIE D.R. (1990). Spectral respectiveness of *Frankliniella occidentalis* (Thysanoptera: Thripidae) determined by trap catehes in greenhouses. Environ. Entomol., **19**(5): 1229-1241.

VILLEVIELLE, M. and MILLOT, P. (1991). Lutte biologique contre *Frankliniella occidentalis* avec *Orius laevigatus* sur Fraisier. Bull. SROP/WPRS, **XIV**/ 5: 57-61.

RESPONSE OF THE ANTHOCORIDS *Orius laevigatus* AND *Orius albidipennis* AND THE PHYTOSEIID *Amblyseius cucumeris* FOR THE CONTROL OF *Frankliniella occidenatalis* IN COMMERCIAL CROPS OF SWEET PEPPERS IN PLASTIC HOUSES IN MURCIA (SPAIN).

J.A. SANCHEZ [1], F. GARCÍA [2], A. LÀCASA [1], L. GUTIERREZ [1], M. ONCINA [1], J. CONTRERAS [3], Y J. GOMEZ [2].

[1] Dept. Protección Vegetal. Centro de Investigación y Desarrollo Agroalimentario. C/ Mayor, s/n. 30150 La Alberca (Murcia-ESPAÑA).

[2] Novartis BCM, Polig. Industrial La Redonda, Sta Mª del Aguila, 04710 El Ejido (Almería-ESPAÑA).

[3] Dept. Producción Vegetal. Escuela Técnica Superior de Ingenieros Agrónomos. C/ Alfonso XII, 34. Cartagena (Murcia-ESPAÑA).

SUMMARY

Since the first appearance of Tomato Spotted Wilt Virus (TSWV) in Southeast Spain in 1990, the complex of this virus and its vector, *Frankliniella occidentalis* constitutes the most important phytopathological problem in the region. In crops of sweet peppers under plastic, the control of *F.occidentalis* is being achieved by chemical methods. Sometimes these treatments are insufficient to maintain the populations under acceptable economic thresholds, considering the damage caused by this species as a vector of TSWV.

During the 1995/96 season IPM trials were carried out in two commercial plastic houses to test the efficacy of the simultaneous use of *Amblyseius cucumeris*, *Orius laevigatus* and *Orius albidipennis* in the control of *F.occidentalis*. Another plastic house using chemical control was used as a comparison. In each of the three plastic houses, populations of the relevant species were monitored by taking samples of leaves and flowers weekly, from the beginning to the end of the crop.

The use of natural enemies for the control of Thrips gave satisfactory results, comparable with the results achieved with chemical control. In one of the plastic houses where integrated pest management was used, *Amblyseius cucumeris* reached a maximum of 3,8 predatory mites/flower in the middle of March; *F.occidentalis* reached its maximum population in the middle of May with 4,7 individuals/flower, and one month later *Orius spp.* reached a peak level of 3,6 individuals/flower. In the second plastic house a peak of 17,5 *Amblyseius cucumeris*/flower was recorded in the middle of February; *F.occidentalis* reached a maximum of 10,7 individuals/flower in early June, and *Orius spp.* reached a peak of 1 individual/flower one month later. In the plastic house where chemical control was used, 6,6 *F.occidentalis* were present in each flower by the middle of May. A maximum of 20 individuals/flower was recorded at the end of the crop, after the final chemical treatments.

INTRODUCTION

In the Murcia region over 1200 Has. of sweet-peppers are grown in plastic-houses. The complex of *Frankliniella occidentalis* and Tomato Spotted Wilt Virus (TSWV) has constituted the most important phytopathological problem, since 1990, when the presence of TSWV was detected (LACASA, *et al.* 1994). The control of Thrips populations is absolutely vital to reduce or limit the virus incidence. The control of *F.occidentalis* as vector is mainly carried out by chemical methods. When the conditions are optimum for the development, multiplication and movements of thrips, chemical control is insufficient to maintain the level

of virus incidence under the economic thresholds (LACASA Y CONTRERAS, 1993). Also, the frequent treatments during spring and summer cause problems of resistance or are incompatibe with programmes of integrated pest management.

Since 1991, different alternative and complementary methods of control have been tested, to reduce the number of chemical interventions: use of dense mesh in the openings of the plastic-houses (LACASA, *et al.* 1994), elimination of infested plants and the use of natural enemies (SANCHEZ, *et al.* 1995). In the sweet-pepper growing areas , the thrips are being multiplied in outside crops, where they remain active throughout the year and produce maximun populations in spring, summer and autumn. A proportion of the alternative host crops are susceptible to TSWV.

The activity and efficacy of some phytoseiids from the *Amblyseius* genus and *Anthocorids* of the genus *Orius* in the control of thrips in the sweet-pepper crops, has been stated by RAMAKERS (1990; 1993), TEILLER and STEINER (1990) and TAVELLA *et al.* (1994). Some of them consider that the most effective control is given by the asocciation of the two types of predators.

The objective of this work was to confirm the response of the predators *A.cucumeris, O. laevigatus* and *O.albidipennis* in commercial sweet-pepper crops for the control of *F.occidentalis*, following the results obtained by SANCHEZ *et al.* (1995) in preliminary trials.

MATERIALS AND METHODS

Three commercial plastic-houses "Tipo capilla" have been used , with drip irrigation and mesh of 14x10 threads/cm in the ventilation opening. The plastic-houses A and C were planted with "Lamuyo Type" peppers while Californian peppers were planted in plastic-house B, with a density of 1x0'4 m. In the three plastic-houses the cultural practices common in the area were used.

Plastic-house A was treated with Lufenuron on 18th December and later with the following natural enemies were released for thrips control: *A.cucumeris* (1 sachet/plant on 13rd Jan and 0'5 sachets/plant on 1st March and 3rd May); *O.laevigatus* (2 adults/m^2 on 2nd Feb. and 8th Feb.) and *O.albidipennis* (1 adults/m^2 the 19th April and 27th April). Also on 20th May the plants from the edges of the plastic-house were treated with acrinatrin. For the control of the other pests *Bacillus thuringiensis* (6 treatments after 24th April) and Piriproxifen (2 treatments, 5th & 18th June) were applied.

Plastic-house B was treated with Lufenuron (27th Dec.) and acrinatrin (5th & 13rd Jan.). Later the following natural enemies were released: *A.cucumeris* (2nd Feb. & 26th April), *O.laevigatus* (16th Feb. & 1st March) and *O. albidipennis* (23rd & 31st May) at the same rates as in the plastic-house A. Also the plastic-house was treated with *Bacillus thuringiensis* (6 times from 28th May), hexitiazox (2nd June) and piriproxifen (21st June). sulphur was applied too (9 times) and specific fungicides.

In the plastic-house C, 19 treatments were used with specific products against thrips making use of metamidofos (14 times), formetanato (4 times), metiocarb (twice), clorpirifos (once) and acrinatrin (once). Other treatment were: *B.thuringiensis* (10 times), lufenuron (3 times), imidacloprid (once), fufenoxuron (once), mtomilo (3 times), priproxifen (3 times), pridafention (once), slphur (19 times) and secific fungicides. In Fig. 5 the dates of the specific applications against thrips are indicated.

A.cucumeris was distributed in sachets; the first release was placed in the fork of the plants while the following releases were placed on the apical branch, near to the flowers. The

Orius were distributed on the leaves regularly, throughout the plastic-house. *Amblyseius californicus* was released on 30th March, distributing it on the leaves.

To follow the population evolution of the thrips and their natural enemies, 6 samples of 10 flowers and 6 samples of 10 leaves were taken weekly from the third apex of the plants. These samples were placed into plastic bags hermetically seated and carried to the laboratory in a refrigerated container.

The extraction of the thrips and the predators was done in Berlese-Tullgren funnels with incandescent lamps of 25 w. The individuals that remained in the bags were collected with a brush. All the individuals were collected in alcohol at 10%, to which had been added a wetter (Agral ®) at 1 part per 1000. The adults and larvae of *F.occidentalis* and both species of *Orius*. were counted .Separate counting was carried out of the adults of *A.cucumeris* the other *Amblyseius* species spontaneously associated with the crop, or those that had been released for the control of tetranychid mites (*A.californicus*).

RESULTS

In the plastic-house A the first larvae of *F.occidentalis* were recorded on 22td February, with a 5 weeks delay prior to finding adults in the flowers. The mean density of thrips in the flowers remained low until early May (Fig. 1). The highest numbers were present between the middle of May and the end of June, with a maximum of 10'7 Thrips/flower.

The populations of *A.cucumeris* reached a maximum of 15 - 20 mites/flower between the first week of February and the first of March (Fig. 1), but later the population decreased until it disappeared in early July. The release made in May had no apparent effect on the level of *A.cucumeris* inside the flowers. *Amblyseius barkeri* occurred naturally, although at much lower numbers than *A.cucumeris*.

The first larvae of *Orius laevigatus* were seen two weeks after the release. The total population increased at the same time as those of *A.cucumeris* , were decreasing (Fig. 1), reaching a relative maximum of 0'4/flower on the 7th of March. They later decreased, registering minimum numbers at the begining of April (Fig. 2). The first larvae of *O.albidipennis* were found two weeks after the release; later the population increased as did the number of thrips. After that moment, the population of *Orius* was mainly made up of *O.albidipennis* (Fig. 2), with over 0,4 *Orius* per flower from the begining of June until mid August. Numbers peaked at nearly 1 *Orius/*flower in the middle of July. Thrips populations were kept at low levels from early July.

The number of flowers/plant increased progessively from transplanting until 7th March, when a maximum of 10 flowers/plant was recorded. After that moment the level decreased until values of 1 - 1'5 flowers/plant were reached between the last week of March and the third week of April. Later there were slight oscillations of the flowering level, but it did not fall below 3 flowers/plant (Fig. 7).

In the plastic-house B adults of *F.occidentalis* were found from the start of the crop. With larvae appearing from the middle of February. The total populations were under 0'6 thrips/flower until early May. Later, the thrips density increased quickly (Fig. 3), at the same time as the numbers of flowers/plant, reaching a maximum of 4'7 thrips/flower. After that moment the level decreased drastically due to predation by *Orius spp*. and was maintained under 0'6 thrips/flower until the end of August when it increased slightly (Fig. 3).

The behaviour of *A.cucumeris* was very similar to that in plastic-house A. The populations increased from the time of release (Fig. 3), maintained over 3 predatory

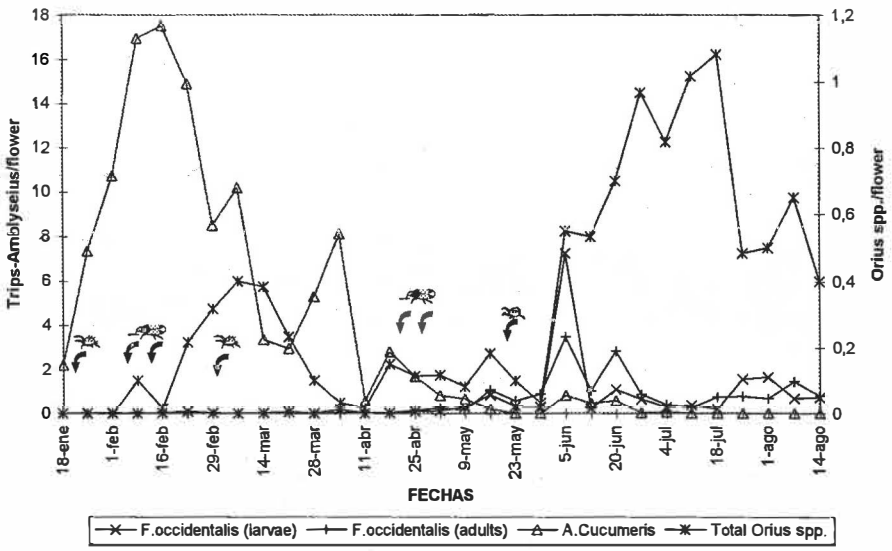


Fig. 1. Plastic-house A. Evolution of *F.occidentalis, Orius spp.* and *A.cucumeris.* Natural enemies release . *Orius spp.* . *Amblyseius cucumeris* .

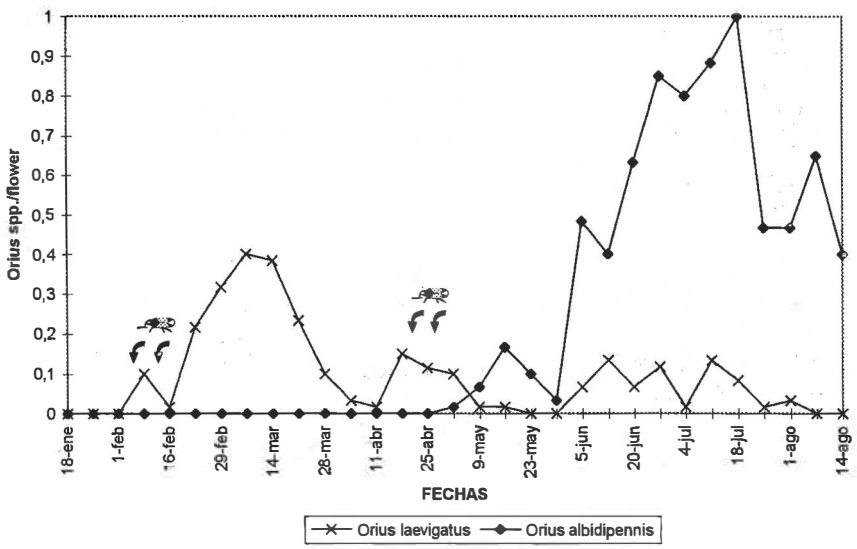


Fig. 2. Plastic-house A. Evolution of *Orius laevigatus* and *Orius albidipennis.*

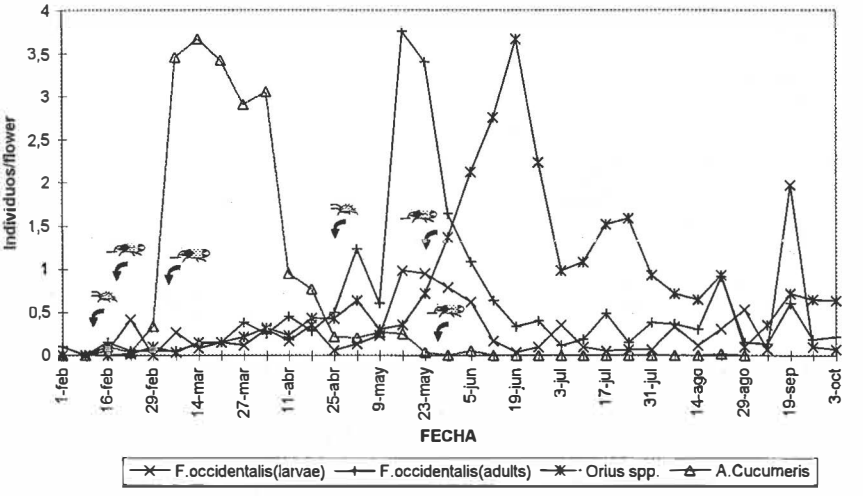


Fig. 3. Plastic-house B. Evolution of *F.occidentalis, Orius spp.* and *A.cucumeris.* Natural enemies release . *Orius spp.* . *Amblyseius cucumeris* .

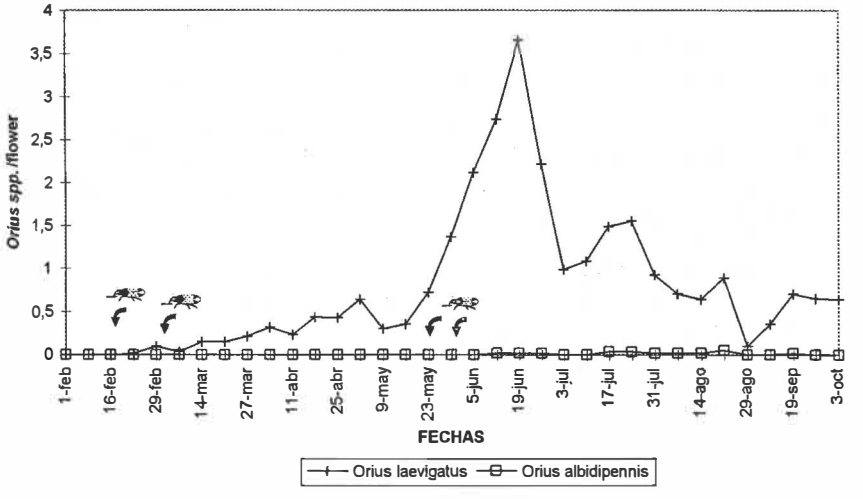


Fig. 4. Plastic-house C. Evolution of *Orius laevigatus* and *Orius albidipennis.*

mites/flower from early March until the middle of April, and then decreased drastically. Later the decline continued until the middle of June, when they disappeared. The release of *A.cucumeris* made at the end of April had no impact in the population level registered inside the flowers.

The first larvae of *O.laevigatus* were found two weeks after the release. The total population of this *Orius sp*. was maintained between 0'2-0'3 Orius/flower from the middle of March until the middle of April, while the level in the middle of May fluctuated between 0'4-0'6 Orius/flower. After that moment numbers increased parallel to the increase of the density of thrips/flower, reaching a maximum of 3'5 Orius/flower on 20th of June. Later the population decreased drastically and continued at the level of 1 Orius/flower until the middle of August, mantaining the level of thrips low from the middle of June until the end of the crop. *O.albidipennis* appeared in the flowers a few weeks after the release, but the population of Orius was composed mainly, of *O.laevigatus* (Fig. 4). In general, the level of flowering in this plastic-house was less variable, with from 6 to 12 flowers/plant from early May until the end of the crop (Fig. 7).

In the plastic-house C, the first few thrips appeared in the flowers at the end of February, not appearing continously until the middle of April (Fig.5). By the middle of May a maximum of 6'6 thrips/flower was reached numbers remained between 2 and 5 thrips/flower from early June until the end of the crop when a maximum of 20 thrips/flower was reached. Even with the treatments, natural populations of *A.barkeri* occurred inside the flowers. These were high during the spring and reduced in the summer, when the treatments were intensified (Fig. 5). The fluctuations in the level of flowering were similar to the plastic-house A.

For the control of *Tetranychus spp. (T.urticae, T.turkestani*) in the plastic-houses of integrated pest management, *A.californicus* was released preventively. In plastic-house A pest hot spots occurred, requiring specific interventions with hexitiazox. In B, *A.californicus* and *Phytoseiulus persimilis*, which appeared spontaneously, achieved a good control of the tetranychids. The control of *Spodoptera exigua* was good, but the control of the caterpillars *Pyrausta nubilalis*, which bore into the fruits was more difficult. In the plastic-houses A and B the control of *Bemisia tabaci* was good, however the population in the plastic-house C was very high after the middle of June. During spring some colonies of Macrosiphum euphorbiae were found in plastic-house B.

DISCUSSION AND CONCLUSIONS

The predators released have established in the crop. They have multiplied and have achieved a good control of the populations of thrips, in comparation with the plastic-house where the chemical control was used.

The phenological evolution of the plant, particularly the level of flowering and the temporal evolution, affects the population evolution of the thrips as well as that of the predators. The decrease of the number of flowers that usually occurs before the first harvest can bring a decrease and in extreme situations nearly cause the extinction of the population of *Orius*. In these cases a second release would be recommended and a change in the plant handling to obtain a level stable of flowering.

The minimum average temperature of the plastic-house is over 10°C after the middle of March, which ensures continued development of the sweet-peppers and of *F.occidentalis*. Between end of April and early May there is a critical period, coinciding with the decrease in the number of flowers/plant. In May the increase of flowering, after the first harvest, is accompanied by an important increase in the population of *F.occidentalis* and a delay of two or three weeks in the increase of the population of *Orius*. The same phenomenom has been

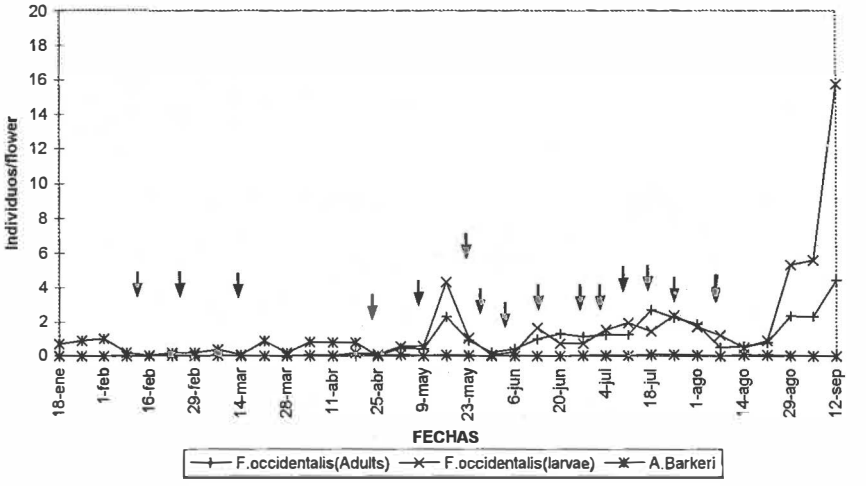


Fig. 5. Plastic-house C. Evolution of *F.occidentalis* and *Amblyseius barkeri*. Treatments for thrips control ⇩.

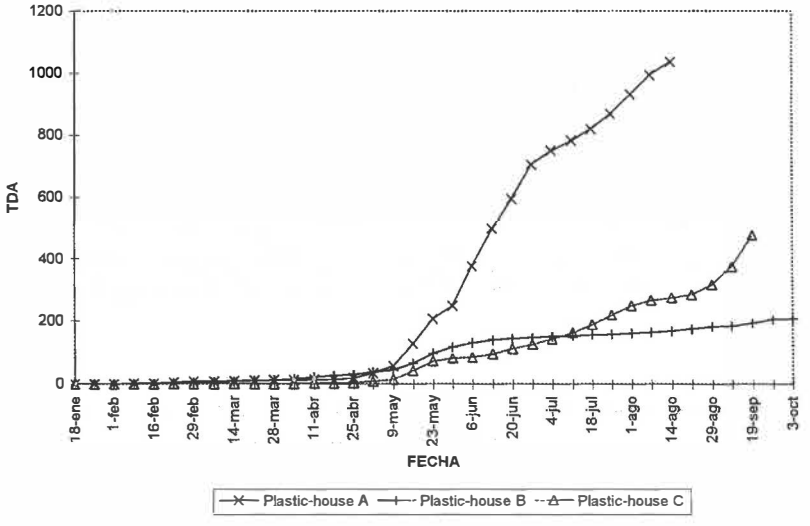


Fig. 6.Evolution of TDA (Acumulated Thrips Day) in the plastic-houses A,B and C.

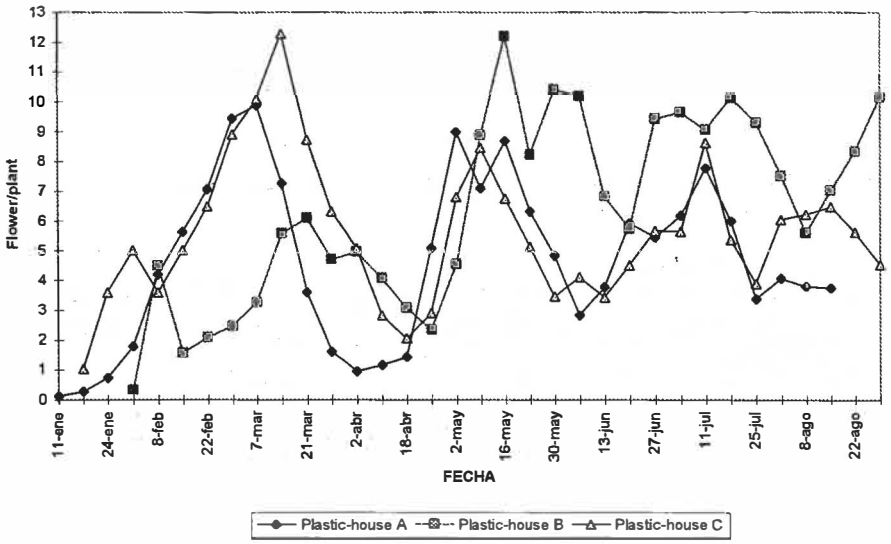


Fig. 7. Flowerin evolution in the plastic-houses A,B and C.

observed by VAN DE VEIRE and DEGHEELE (1993) limiting the growth of *A.cucumeris* populations.

The biological control of F.occidentalis can be achieved by the combined use of *A.cucumeris* and species of *Orius*, as has been stated by RAMAKERS (1993) and SORENSSON and NEDSTAM(1993) in crops under plastic. *A. cucumeris* gives adequate thrips control at the beginning of the crop: it delays the establishment and reduces the multiplication of the thrips, and at the same time it facilitates the establishment of the first releases of Orius.

The drastic decrease of the *A.cucumeris* populations may be a result of the high temperatures and the low humidity which are recorded at the end of April, and by the predatory action of *Orius* (GILLESPIE Y QUIRING, 1992), whose population increased at the same time, when the population of thrips are still low. This direct or antagonistic action of competition for the prey cannot be considered negative in the joint use of both predators (RAMAKERS, 1993).

The evolution of the populations of *F.occidentalis* expressed in TDA (Acummulated thrips day) (Fig. 6) was very close in the plastic-houses B and C, and showded a direct relation with the level of incidence of TSWV, expressed in acummulative percentage of infected plants (LACASA, *et al.* 1994). The proportion of infected plants at the end of the crop was similar in B and C; in plastic-house B the evolution was at progressive intervals from February, while in plastic-house C the progression was higher in the last phases of the crop.

ACKNOWLEDGMENTS

We are grateful for the collaboration of the growers D. Juan Cartagena, D.Miguel Jimenez, Hnos Garcia de la Paca, The technical support of D.Francisco Torró, D. David Hernandez, D M Carmen Martinez and D.Francisco Ferragut for the determination of tetranichids. The financial support of Novartis BCM and the project INIA SC93-183 C5.

REFERENCES

GILLESPIE, D.R. & QUIRING, D.J.M. 1992. Competition between *Orius tristicolor* (White) (Hemiptera: Anthocoridae) and *Amblyseius cucumeris* (Oudemans) (Acari: Phytoseiidae) feeding on *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Canadian Entomologist, **124:** 1123-1128.

LACASA, A.; CONTRERAS, J.; TORRES, J.; GONZALEZ, A.; MARTINEZ, M.C.; GARCIA, F. & HERNANDEZ, A. 1994. Utilización de mallas en el control de *Frankliniella occidentalis* (PERGANDE) y el virus del bronceado del tomate (TSWV) en el pimiento. Bol.San.Veg.Plagas. **20:** 561-580.

LACASA, A. & CONTRERAS, J. 1993. Comportamiento de *Frankliniella occidentalis* en la transmisión del virus del bronceado del tomate: planteamientos para su control. Phytoma-España, **50:** 33-39.

RAMAKERS, P.M.J. 1993. Coexistence of two thrips predators, the anthocorid *Orius insidiosus* and the phytoseiid *Amblyseius cucumeris* on sweet pepper. Bulletin OILB SROP, **16:** 133-136.

RAMAKERS, P.M.J. & VAN DEN MEIRACKER, R.A.F. 1990. First-rate control of Californian thrips with *Orius*. Groenten en Fruit, **46:** 44-45, 47.

SANCHEZ, J.A.; LACASA, A.; CONTRERAS, J. & LORCA, M. 1995. Datos preliminares sobre la utilización de *Orius laevigatus* (Fieber) en el control de *Frankliniella occidentalis* (Pergande) en pimiento de invernadero. Phytoma-España, **68:** 32-38.

SORENSSON, A. & NEDSTAM, B. 1993. Effect of *Amblyseius cucumeris* and Orius insidiosus on *Frankliniella occidentalis* in ornamentals. Bulletin OILB SROP, **16:** 129-132.

TAVELLA, L.; ALMA, A. & ARZONE, A. 1994. Attività predatrice di *Orius spp.* (Anthocoridae) su *Frankliniella occidentalis* (Perg.)(Thripidae) in coltura protetta di peperone. Informatore fitopatologico, **1:** 40-43.

TEILLER, A.J. & STEINER, M.Y. 1990. Control of the western flower thrips, *Frankliniella occidentalis* (Pergande), with a native predator *Orius tristicolor* (White) in greenhouse cucumbers and peppers in Alberta, Canada. Bulletin OILB SROP, **13(5):** 209-211.

VAN DE VEIRE, M. & DEGHEELE, D. 1993. Control of western flower thrips, *Frankliniella occidentalis* with the predator *Orius insidiosus* on sweet peppers. Bulletin OILB SROP, **16:** 185-188.

DISTRIBUTION PATTERN AND BINOMIAL SAMPLING FOR *Frankliniella occidentalis* AND *Orius spp.* IN SWEET PEPPER CROPS.

J.A. SANCHEZ[(1)]; A. LACASA[(1)]; L. GUTIERREZ[(1)] & J. CONTRERAS[(2)]

[(1)] Dept. Protección Vetetal. Centro de Investigación y Desarrollo Agroalimentario. C/Mayor, s/n. 30.150 La Alberca (Murcia-ESPAÑA).
[(2)] Dept. Producción Vegetal. Escuela Técnica Superior de Ingenieros Agrónomos. C/Alfonso XII, 34. Cartagena (Murcia-ESPAÑA).

ABSTRACT

Sampling methods are needed in Integrated Pest Management (IPM) to know the quantity and evolution of the pest or predator population in the crop. Binomial Sampling makes it possible to carry out an estimation of mean density and it is less expensive than counting all the organisms present in a sample unit. *Frankliniella occidentalis* is the main pest in sweet pepper in greenhouses in the Southeast of Spain, and *Orius spp.* the most frequent auxiliary used against it. During 1995/96 several greenhouses were sampled for both species, from the appearance of the first flowers until the growth cycle was completed. The variance-mean relationship was established by Taylor's power law, and the distribution pattern explained by the negative binomial and Poison distribution. An empirical model was used as well to establish the relationship between mean and spatial distribution; a few tally thresholds have been considered. Taylor's aggregation index in *F.occidentalis* was 1,36 in larvae and 1,30 in adults. Most of the data fitted the negative binomial. Taylor's aggregation index in *Orius spp.* was 1,03 in larvae and 0,92 in adults. Most of the data fitted the Poisson distribution.

INTRODUCTION

The knowledge of the population density of a determined pest or natural enemy present in a crop in a precise moment, turns out to be a fondamental aspect when integrated pest management is chosen, as it is important to know the pest state and the levels of the natural enemies associated with it. In sweet pepper crops in greenhouses in South East of Spain, *Frankliniella occidentalis* is the main pest and *Orius spp.* one of the natural enemies most frequently found in our ecosystems and used to control it.

Counting all the individuals present in a sample unit is expensive, above all when population density is high. Binomial sampling of presence-absence constitutes a good alternative, provided that a good relationship is established between the average population density and the species distribution, and it implies a remarkable saving of time.

In this work the distribution patterns of *F. occidentalis* and *Orius spp.* are studied for their different development phases, and Taylor indexes, the fitting to theoretical distribution functions, as the negative binomial and Poisson distribution, are studied. An empirical model has been also used to establish the relation between the proportion of non infested organs and mean density (KONO Y SUGINO, 1958; GERRARD Y CHIANG, 1970; NACHMAN, 1984). For the empirical model a few tally thresholds (T) above zero have been essayed, that means, to consider that a sample unit is occupied when it presents more than a certain T number of individuals. The number of samples required was given by each model.

The main goal of our work was to develop a precise, accurate and fast sampling programme, which could be used in IPM sweet pepper crops. We considered that the acceptation of the sampling programme by farmers and technicians was a fundamental aspect, therefore we have made an effort to design simple and undestructive samplings.

MATERIALS AND METHODS

The trials were carried out during 1996 in 5 sweet pepper greenhouses: 2 of them were experimental greenhouses and 3 were commercial ones. Both experimental greenhouses had a surface of 300 m^2 and were divided in two sectors. In 3 of the sectors an IPM was applied, employing *Orius laevigatus* and *Orius albidipennis* to control *Frankliniella occidentalis*, and other natural enemies to control secondary pests or *Bacillus thuringiensis* to control Lepidoptera. Fungal deseases were treated with chemical products compatible with the natural enemies. The only difference among the three sectors was that two of them had a 14x10 threads/cm mesh in lateral openings and not the third. The difference between the two that had the mesh was the date of release of the anthocorids. The fourth sector was used as control and no treatment was made against *F.occidentalis*, and for the other pests and diseases products with the least possible influence on the natural enemies which could have colonised spontaneously the crop were used. In all sectors the variety grown was "Atol".

The three commercial greenhouses had a surface of about 3,000 m^2. In two of them an IPM was realized employing *O. laevigatus* and *Orius albidipennis* and a mesh in lateral openings to control *F.occidentalis*. For the other pests or diseases natural enemies or compatible products were used. In one of them the type "lamuyo" was grown and in the other one the type "Orlando". In the third greenhouse the type "Atol" was grown and traditional chemical control was used for all pests and diseases.

191 samplings were carried out in all, which consisted of visual observation of 100 flowers randomly taken along the greenhouse. The number of thrips and *Orius* found was registered, distinguishing between larvae and adults. Samplings were weekly since the first flowers appeared towards the end of the crop.

RESULTS AND DISCUSSION

Frankliniella occcidentalis DISTRIBUTION PATTERN.

Taylor's power law establishes a relationship between the variance and the mean (1) which is fulfilled for a great number of species, and that is considered constant and characteristic for each of them (TAYLOR, *et al.* 1978). When b=1 the distribution is considered at random, when the values are <1 regular and when b>1 aggregative. Nevertheless b=1 values must not be interpreted as aleatory distribution patterns unless the value of the constant "a" is near to the unit (GEORGE, 1974, en (Southwood. 1978)).

$$s^2 = am^b \qquad (1)$$

"b" aggregation index was of 1,36±0,02 for the larvae (r^2=0,97), 1,30±0,02 for the adults (r^2=0,98) and 1,35±0,02 for adults and larvae taken together (r^2=0,97), which shows an aggregative tendence both in larvae and in adults.

In *F.occidentalis* Taylor aggregation index has been used by STEINER (1990) to describe the distribution of larvae and adults in cucumber crops, in flowers, fruits and leaves of different strata of the plant. In all the cases she obtains an aggregative distribution, significantly higher in larvae than in adults, although she finds no differences in aggregation index in leaves of different strata. In strawberries RIBES Y COSCOLLA (1992) find an aggregative distribution for larvae and adults, similar to the one calculated by GARCIA-MARI *et al.* (1994). In sweet pepper crops BELDA *et al.* (1992) find the same kind of

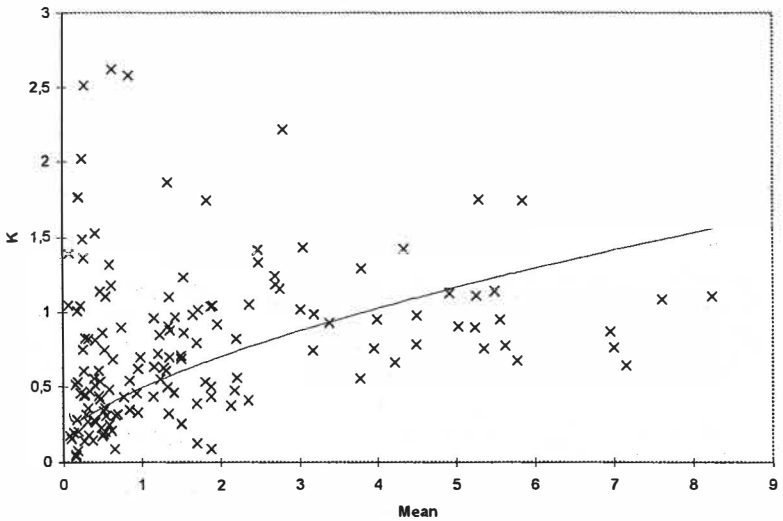
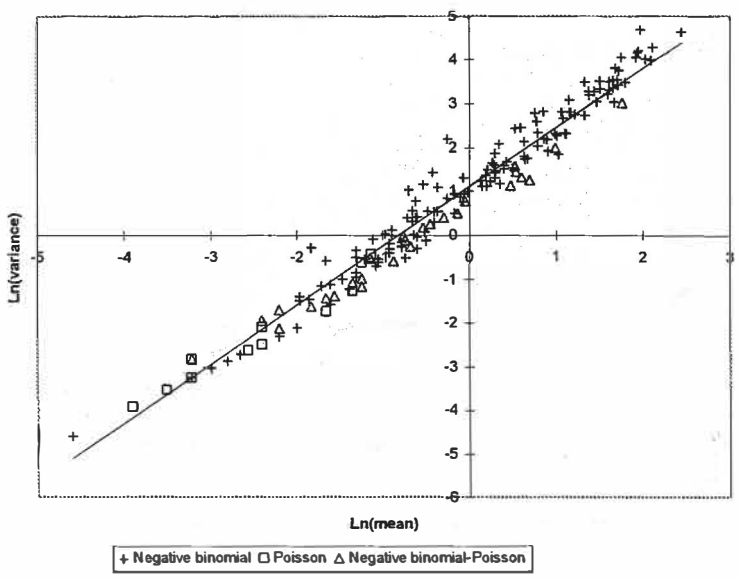


Fig.1: Plot of the mean and "k" values. The curve represents the value of "k" acording to the expresion (4)



.Fig.2: Spatial distribution of *F.occidentalis* to different theoretical distributions: negative binomial, Poisson or negative binomial and Poisson at the same time. The straight line represents the relationship between the variance and the mean according to Taylor's power law.

distribution in flowers, leaves and fruits although they use the Iwao index.

One of the most used distribution functions in ecology to describe aggregative patterns has been the negative binomial (BLISS Y FISHER, 1953), even though it is not expectable to find a unique function that fits perfectly to all the range of species density (TAYLOR, 1984). Th efrequency of organs with x individuals is calculated by the equation (2) and the frequancy of organs with T or less individuals by the expression (**3**) being T the tally threshold considered.

$$P_x = \frac{\Gamma(k+x)}{x!\Gamma(k)}\left(\frac{\bar{x}}{\bar{x}+k}\right)^x\left(\frac{k}{\bar{x}+k}\right)^k \quad (2) \qquad P_T = \sum_{x=0}^{T} P(x) \quad (3)$$

$\Gamma(x)$= function gamma, $\bar{x}$ is the mean population density and k the value of the parameter considered.

"k" has been calculated by using the 2nd and 3rd results proposed by (Southwood. 1978). The second method has been used for law densities ($\leq$ 1 thrips/flower), and the third for higher densities. Even if in some occasions it is possible to find a range of densities in which "k" remains constant the value of "k" generally varies with the density. In this case we decided to make the value of "k" vary in function of the mean as is given by the expression (4), (Fig.1) in which the variance has been sustituted in function of the mean according to the Taylor's power law (WILSON Y ROOM, 1983).

$$K = \frac{m^2}{a\,m^b - m} \qquad (4)$$

12 of the 191 samples, all tested by the χ^2 with a significancy level of 95%, fitted to Poisson distribution, while 38 followed both Poisson distribution and the negative binomial, and the remaing only the negative binomial (Fig.2). Except 5 samples the random distribution is found in law densities. under 0,07 thrips/flwer. It can be said that the binomial distribution describs satisfactorily the species distribution for a wide range of densities.

For *F.occidentalis* the negative binomial has been used in order to describe the species distribution in strawberries by GARCIA-MARI *et al.*(1994) and in sweet pepper by BELDA *et al.*(BELDA, *et al.* 1992). The latters find a common value of "k" for leaves, flowers and fruits.

SAMPLING METHODS

When then counting of the total of the individuals found in each sampling unit is realized, the number of sample is expressed in function of the mean and of the coefficient of variation (CV_m) (5). In the equation (6) the variance has been sustituted by the Taylor's Power Law (GREEN, 1970).

$$CV_m = \frac{S/\sqrt{n}}{m} \qquad (5) \qquad n = \frac{a \cdot m^{b-2}}{CV_m^2} \qquad (6)$$

A binomial sampling can be used when the distribution of a species is known. Once the relationship between the distribution and the abundance is established it is possible to make an estimation of the population density on the basis of the proportion of occupied organs. This reduces considerably the cost of the sampling (Fig.3). The sample numbers (n) have been

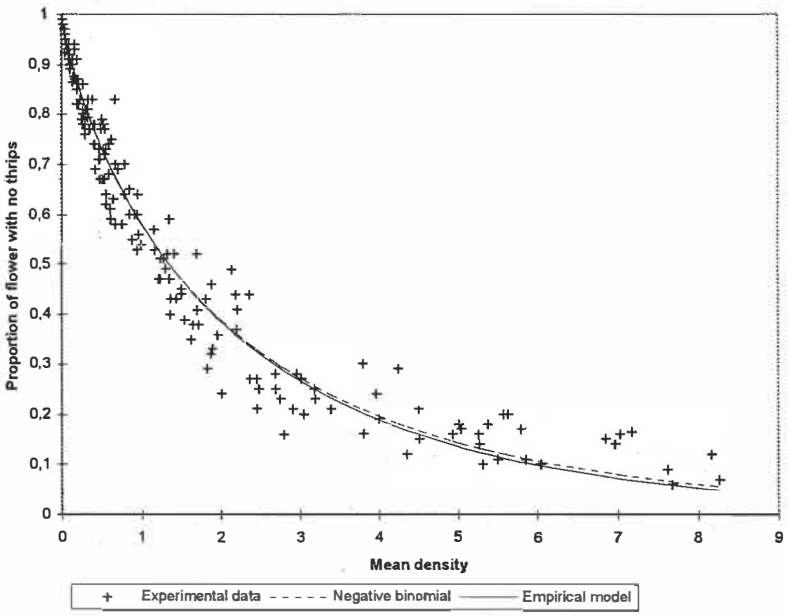


Fig.3. Relationship between the proportion of non occupied flowers (P_T) and the mean population density according to the negative binomial distribution and the empirical model.

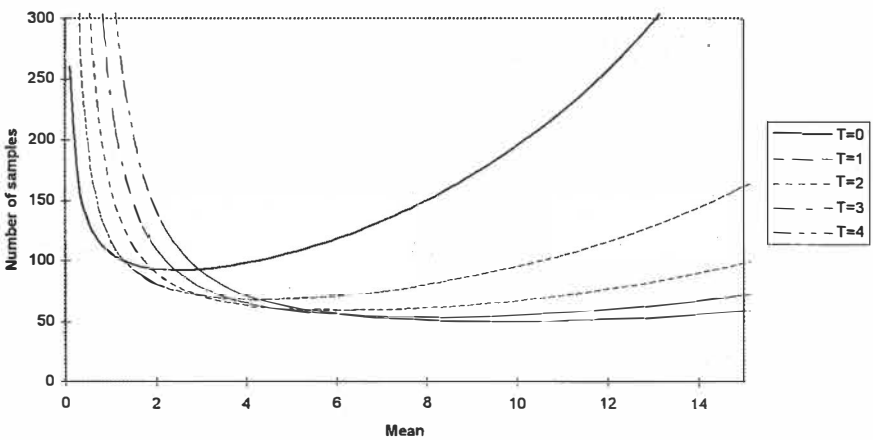


Fig.4. number of samples for a binomial sampling considering various tally thresholds.

calculated, in function of the mean according to the expression (7) proposed by KUNO (KUNO, 1986), and are shown in Fig.4, for a coefficient of variation of the mean estimation of 0,25.

$$n = \frac{1}{CV_m^2}(1 - P_T) \cdot P_T^{\left(-\frac{2}{k}\right)-1} \cdot \left[K\left(P_T^{-\frac{1}{k}} - 1\right)\right]^{-2} \qquad (7)$$

n= number Sample, CV_m= Coefficient of variation of the mean estimation, "k" according to expression (4).

EMPIRICAL MODEL

The relationship between the mean population density and the proportion of organs with T or less individuals can also be established with an empirical model (KONO Y SUGINO, 1958; GERRARD Y CHIANG, 1970; NACHMAN, 1984) (**Fig.3**), in which it is given by the expression (8).

$$m = e^{a'}\left(-\ln P_T\right)^{b'} \qquad (8)$$

NACHMAN expresses the relationship by a logarithm.

$$\ln(m) = a' + b' \ln\left(-\ln P_T\right) \qquad (9)$$

a' and b' = regression constants. P_T= probability of finding T or less individuals in a sample unit.

Once established the regression parametres (Table 1), starting from the expression (8) we can estimate of the mean population density on the bases of the proportion of organs that have T or less individuals, whose variance can be calculated by the Nachman equation (10) (NACHMAN, 1984):

$$Var\left(\ln(m)\right) = s^2\left\{\frac{1}{N} + \frac{\left(\ln\left(-\ln P_T\right) - \bar{P}\right)^2}{SSM}\right\} + \frac{b'^2(1 - P_T)}{n P_T (\ln P_T)^2} + \frac{a}{n} m^{(b-2)} \qquad (10)$$

N= n° of pairs of data used in the regression, $\bar{P}$ = Mean value of pairs ln(-ln P_T) used in the regression, SSM= Sum of squares of the desviations Ln(-Ln(P_T), m= Estimation of the mean according the expression (8).

T	a'	b'	s^2	N	$\bar{P}$	SSM	r^2
0	0.723	1.210	0.063	191	-1.009	344.18	0.977
1	1.301	0.896	0.069	163	-1.478	286.44	0.954
2	1.613	0.748	0.067	151	-1.999	319.99	0.945
3	1.843	0.703	0.069	141	-2.247	283.90	0.935
4	2.054	0.668	0.062	124	-2.409	240.23	0.933

Table 1: Parametres of the relationship ln(m)= a'+b'ln(-ln(P_T)) for *F.occidentalis*.

The determination of the number of sample can be calculated starting from NACHMAN equation (1984) with the approximation for the variance of the equation (11), according to the coefficient of variation considered (12).

$$Var(m) = m^2 \text{ var}(\ln m) \qquad (11)$$

$$CV_m = \frac{\sqrt{Var(m)}}{m} \qquad (12)$$

The predictions obtained by the empirical method show very little differences from the ones realised with the negative binomial (Fig.3). The binomial sampling is not very reliable when more than 80% of the sample units are occupied (Southwood. 1978). The fact of considering a flower occupied when it has more than a determined number of insects (T), makes it possible to estimate when we have high population densities and in some occasions to improve the accuracy of the proceedings (GERRARD Y CHIANG, 1970; BINNS Y BOSTANIAN, 1990).

The estimation of the mean density is entirely based on the relationship between the proportion of organs with T or less individuals and the mean population density. The increase of the tally thresholds can be associated with a decrease of the mean square error, with which the accuracy of the estimations improves remarkably. In our case when increasing the tally threashold the mean square error remains nearly the invariable, although the precision improves as the slope of the relationship decreases.

When the densities are low, it is convenient to take a tally threshold zero, because it takes less time than counting up a number of individuals (T), before deciding wether or not a flower is occupied, and lower number sample are required (Fig.4). For more elevated densities it is convenient to take tally threasholds above zero, which for the same precision in the estimation, require a lower number of samples (Fig.4).

The number of samples calculated for the empirical model are slightly higher than those calculated for the negative binomial, according to the KUNO expression (1986) and much higher than those calculated when the total counting is made. This is due to the fact that when the binomial sampling is used to make an estimation of the mean, the variance associated to the relationship between P_T and m must be added to the one associated to the population dispersion (NYROP, *et al.* 1989).

Orius spp. DISTRIBUTION PATTERNS

Taylor's aggregation index of *Orius spp.* was b=1,03±0.02 for larvae (r^2=0,95), b=0,99±0.02 for the adults (r^2=0,96), and b=0,995±0.011 for the total (r^2=0,97). According to Taylor index *Orius spp.* presents a random distribution in sweet peppers flowers; "b" is not significantly different from 1 (P<0,05) and "a" is also near the unit. In all the 151 samples tested with the χ^2 except 3, the species distribution fitted to Poisson distribution (Fig.5). In the Poisson distribution the proportion of organs with x individuals is given only in function of the mean following the expression (13).

$$P_x = e^{-\bar{x}} \frac{\bar{x}^x}{x!} \qquad (13)$$

Random distributions are not generally very frequent in nature, nevertheless, in the group hemiptera several predators species have been described with random distribution patterns. Among them *Nabis spp.* and *Geocoris spp.* in beans (WADDILL, *et al.* 1974) and *Orius laevigatus* in strawberries (GARCIA-MARI, *et al.* 1994).

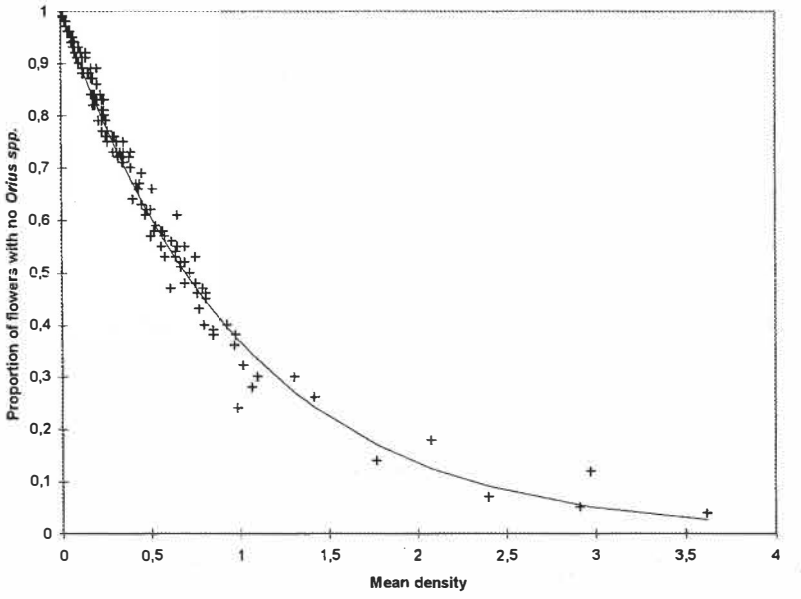


Fig.5. Relationship between the proportion of non occupied flowers (P_T) and the mean population density in *Orius spp.* the straigh line represents the estimates acording to the Poisson distribution.

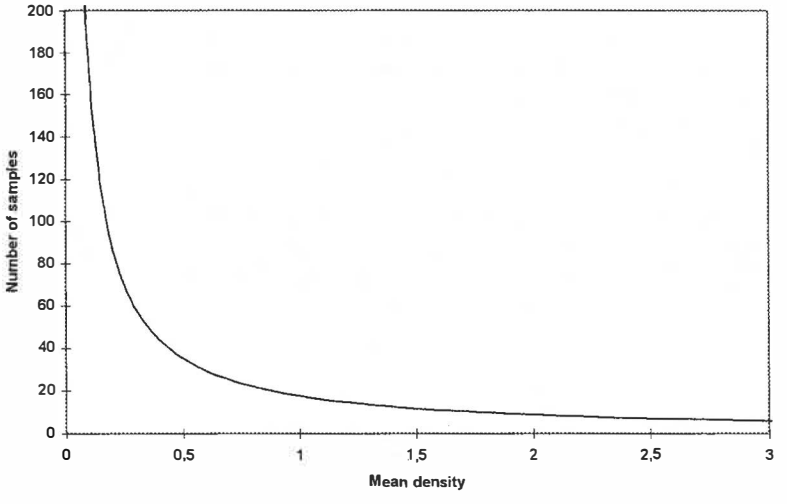


Fig.6. Number of samples for a sampling method when all the individuals are counted for *Orius spp.*

SAMPLING METHODS

When then counting of the total of the individuals found in each sampling unit is realized, the number of samples is given by the expression (6). Fig.6 represents the sample number for a coefficient of variation CV_m=0,25.

In case of adopting a presence-absence sampling method, the estimation of the mean population density can be done on the bases of the proportion of non occupied organs, by Poisson distribution (13). The number of samples can be calculated from the expression proposed by KARANDINOS (14)(KARANDINOS, 1976).

$$n = \frac{p}{(1-p)CV_m^{\ 2}} \qquad (14)$$

Using the binomial presence-absence method involves a considerable saving of time when the species sampled is present at high densities and counting the total of the individuals requires a considerable effort. In the case of *Orius spp.* the mean density per flower is not generally high, so, even though the use of a presence-absence sampling method involves a spare of time against a total one, the difference is not as remarkable as in the case of *F.occidentalis*.

ACKNOWLEDGMENTS

We are grateful for the collaboration of Novartis BCM , The technical support. Dª M Carmen Martinez, D.Matias Oncina y Jerónimo Torres. Dª Umberta Pennaroli and Dª Faith Birtwell for helping with the translation. The financial support of the project INIA SC93-183 C5.

REFERENCES

BELDA, J.; CABELLO, T.; ORTIZ, J. & PASCUAL, F. 1992. Distribución de *Frankliniella occidentalis* (Pergande) (Thys.: Thripidae) en cultivo de pimiento bajo plástico en el sureste de España. Boletin de Sanidad Vegetal, Plagas, **18:** 237-252.

BINNS, M.R. & BOSTANIAN, N.J. 1990. Robust binomial decision rules for integrated pest management based on the negative binomial distribution. American Entomologist, **36:** 50-54.

BLISS, C.I. & FISHER, R.A. 1953. Fitting the negative binomial distribution to biological data. Biometrics, **9:** 176-200.

GARCIA-MARI, F.; GONZALEZ-ZAMORA, J.E.; RIBES, A.; BENAGES, E. & MESEGUER, A. 1994. Métodos de muestreo binomial y secuencial del trips de las flores *Frankliniella occidentalis* (Pergande) (*Thysanoptera, Thripidae*) y de antocóridos (*Heteroptera, Anthocoridae*) en fresón. Bol.San.Veg.Plagas. **20:** 703-723.

GERRARD, D.J. & CHIANG, C.H. 1970. Density estimation of corn rootworm egg populations based upon frequency of occurrence. Ecology, **51:** 237-245.

GREEN, R.H. 1970. On fixed precision level sequential sampling. Res Popul Ecol, **12:** 249-251.

KARANDINOS, M.G. 1976. Optimun sample size and comments on some published formulae. Bull Entomol Soc Am, **22:** 417-421.

KONO, T. & SUGINO, T. 1958. On the estimation of the density of rice stems infected by the rice stem borer. Japanese Journal of Applied Entomology and Zoology, **2:** 184-188.

KUNO, E. 1986. Evaluation of statistical precision and design of efficient sampling for the population estimation based on frequency of ocurrence. Res Popul Ecol, **28:** 305-319.

NACHMAN, G. 1984. Estimates of mean population density and spatial distribution of *Tetranychus urticae* Acarina: Tetranychidae) and *Phytoseiulus persimilis*

(Acarina:Phytoseidae) based upon the proportion of empty sampling units. Journal of Applied Ecology, **21:** 903-913.

NYROP, J.P.; AGNELLO, J.; KOVACH, J. & REISSIG, H.W. 1989. Binomial sequential clasification sampling plans for european red mite (Acari:Tetranychidae) with special reference to performance criteria. Journal of Economic Entomology, **82:** 482-490.

RIBES, A. & COSCOLLA, R. 1992. Notas sobre el seguimento poblacional de *Frankliniella occidentalis* Perg. en el cultivo del fresón. Boletin de Sanidad Vegetal, Plagas, **18:** 569-584.

SOUTHWOOD, T.R.E. (1978). Ecological methods with particular reference to the study of insect populations. 2nd ed, London: Chapman & Hall. 524 p.

STEINER, M.Y. 1990. Determining population characteristics and sampling procedures for the western flower thrips (Thysanoptera: Thripidae) and the predatory mite Amblyseius cucumeris (Acari: Phytoseiidae) on greenhouse cucumber. Environmental Entomology, **19:** 1605-1613.

TAYLOR, L.R. 1984. Assesing and interpreting the spatial distributions of insect populations. Annual Review of Entomology, **29:** 321-357.

TAYLOR, L.R.; WOIWOD, I.P. & PERRY, J.N. 1978. The density-dependence of spatial behaviour and the variety of randomnes. J Anim Ecol, **47:** 383-406.

WADDILL, V.H.; SHEPARD, B.M.; TURNIPSEED, S.G. & CARNER, G.R. 1974. Sequential sampling plans for Nabis spp. and Geocoris spp. on soybeans. Environmental Entomology, **3:** 415-419.

WILSON, L.T. & ROOM, M.P. 1983. Clumping patterns of fruit and arthropod in cotton, with implication for binomial sampling. Environmental Entomology, **12:** 50-54.

BIOLOGICAL PEST CONTROL IN SWEET PEPPER IN SPAIN: INTRODUCTION RATES OF PREDATORS OF *FRANKLINIELLA OCCIDENTALIS*

JAN VAN DER BLOM[1], MARCELINO RAMOS RAMOS[1] & WILLEM RAVENSBERG[2]

[1] Koppert Sistemas Biológicos S.L. Apdo. Postal 38, 04738 VICAR, Almería
Tel. (9)50-554464, Spain
[2] Koppert Biological Systems, P.O.Box 155, 2650 AD Berkel en Rodenrijs
The Netherlands.

Key words: IPM, sweet pepper, *Frankliniella occidentalis*, *Amblyseius cucumeris*, *Orius laevigatus*, population development

Summary

The control of Western Flower Thrips, *Frankliniella occidentalis*, is the key to the success of biological control in sweet pepper in Spain. Present study was performed in order to evaluate the development of the populations of both *Amblyseius cucumeris* and *Orius laevigatus* at different introduction rates. The populations of *A. cucumeris* maintained a clear relation to the introduction rate for about two months. *O. laevigatus* initially also developed according to the introduction rate, but its population started to grow differently after about 4 weeks. The determining factor for population growth in this case seemed to be the availability of prey (thrips). The most complicating factors that were encountered for the use of natural enemies against thrips were the low Relative Humidity (RH) in several greenhouses, the difficulty in control of *Spodoptera exigua* and the presence of chemical residues on the plants. These factors are discussed.

Introduction

The application of Integrated Pest Management (IPM), including biological control of the key pests, is rapidly increasing in the protected crops in the South of Spain. In technical terms, IPM has gone beyond the experimental stage and commercially, IPM has become an important selling point for fruit and vegetable producers. With over 10.000 ha, pepper is one of the most important crops in greenhouses in the South of Spain. Since there are many pest and virus problems in this crop, chemical pest control requires a considerable cost input. The use of natural enemies against the most important pests may result less expensive for the grower and more favourable for plant growth. Furthermore, IPM facilitates the use of bumblebees for pollination, a factor which recently has proven to increase production with a significant percentage.

The most important pest against which natural enemies are introduced is Western Flower Thrips (*Frankliniella occidentalis*). Two predators are commonly used, *Amblyseius cucumeris* and *Orius laevigatus* (Ramakers, 1990, Lacasa & Contreras, 1993, Rodríguez & Fidalgo, 1994, Dissevelt et al., 1995)). Other important pests are aphids (*Aphis gossypii*) and red spider mite (*Tetranychus urticae*). These pests can also be controlled by introducing natural enemies.

There are two different cropping cycles of pepper in Southern Spain. The largest acreage, in the province of Almería, is planted in summer and the crop lasts until the end of winter. A smaller extension, mainly in Alicante-Murcia, is planted in winter, and is maintained until the end of summer. There are important differences between these cycles with respect to pest control. In the winter planted crop, the pest pressure is usually low during the first months. The experiences of the past years have shown that IPM is very feasible in this crop, in spite of a severe virus (TSWV) pressure. *A. cucumeris* works very well from the beginning, and *O. laevigatus* may take over thrips control by the months of April or May. Once a big population has become established, *Orius* also contributes significantly to the control of other pests, such as whitefly or caterpillars. Aphids, mainly *Myzus persicae* can usually be controlled with *Aphidius colemani*. The spontaneous appearance of predatory mites is of great help in the control of red spider mite.

In the summer planted crops, in Almería, the young plantations are immediately threatened by many insect and mite pests. Efficient pest control is necessary from the outset. A few basic problems may complicate the biological control of thrips:
- Low Relative Humidity (RH) in summer, so the eggs of *A. cucumeris* do not hatch. It is difficult to indicate precisely at what level of greenhouse RH the eggs start to suffer mortality, since the microclimate around the leaves is difficult to measure and the eggs do have a tolerance for shorter periods of drought (Van Houten & Van Lier, 1995, 1996). However, under the harsh conditions of Spanish greenhouses in summer, low RH certainly is a factor in limiting the population growth of this mite. The problem of low RH may be resolved by the installation of a simple type of fog system.

- *Spodoptera exigua*. Caterpillars may be difficult to control with *Bacillus thuringiensis*. Since other biological agents against this pest are hard to find, the use of IGR's (i.e. teflubenzuron and flufenoxuron) may become inevitable. These products are very harmful for *O. laevigatus*, so they are incompatible with an adequate biological thrips control. Other, more specific, products against caterpillars (or newly found natural enemies) will have to be used to resolve this problem. The use of light traps, surrounded by sticky traps, seems to help in eliminating moths and limiting the damage.

- Inconsistent establishment and development of *Orius* and *Amblyseius*. In several greenhouses where *O. laevigatus* was introduced in the month of September, a significant population had developed by the fourth week following the initial release. However, in other greenhouses the establishment took considerably longer, or did not take place at all. Although we suspect that in some places the presence of chemical residues may have seriously harmed the population of *Orius*, there have been other factors of importance as well. A general problem in pepper is that the flowering of the plants is not very constant. If *Orius* is released in a period in absence of prey, such as thrips, and with little availability of pollen as an alternative food source, it will be severely limited in its reproduction.

Although there is ample experience with respect to the optimal introduction rates of the natural enemies used, these studies have almost always been performed in other regions of Europe and may not always be representative for the specific conditions we meet in the South of Spain. The influence of introduction rate of both predators, *A. cucumeris* and *Orius laevigatus* on the population development of these species, as well as on the population of *Frankliniella occidentalis*, will be the subject of the rest of this paper.

Material and methods

Three identical neighbouring greenhouses, each with a surface of 7.000 m^2 and belonging to the same grower, were selected. They contained the same pepper variety (NASSAU), transplanted at the same time (August 15th 1996) with a density of slightly less than 2 plants per m^2. The irrigation system and the composition of the nutritional solution was the same in the three greenhouses. The greenhouses were equipped with a simple humidification system, which was automatically brought into action once the RH dropped below 55%. Prior to the introduction of the natural enemies, several chemical treatments were performed in all three greenhouses at the same time.

A. cucumeris was introduced in sachets, containing a substrate with initially 500 A.c. mites. Since the conditions are optimal for the reproduction of *A. cucumeris* inside the sachets, these serve as a slow release system, producing a large number of predatory mites throughout the weeks after introduction. *O. laevigatus* was introduced in the adult and fifth instar stage in vermiculite, dispersed over the crop. The rates of introduction can be found in Table 1.

Table 1.

Introduction rates of A. cucumeris *(sachets) and* O. laevigatus *(individuals) per greenhouse*

	A. cucumeris (sachets) 13 Sept.	***Orius laevigatus*** (individuals) 13 Sept.	19 Sept.	26 Sept.	**Total *Orius* per m^2**
Plot A:	8.500 (= 2 per 3 plants)		3.500	3.500	1
Plot B:	5.400 (= 1 per 2 plants)		7.000		1
Plot C:	2.600 (= 1 per 5 plants)	7.500	7.000	7.000	2,6

Weekly counts were performed in order to follow the population development of the two predators and of *Frankliniella occidentalis*. 50 flowers dispersed over the entire greenhouse were examineded in order to detect presence of *Frankliniella* and *Orius*. For both species, adults and nymphs were separately recorded. From 25 plants, three leaves were taken (an upper leaf, a leaf from the middle of the plant and a lower leaf) in order to detect presence of *A. cucumeris*.

Results

The development of the three populations of thrips, *Orius* and *Amblyseius* are represented in Figures 1 - 3. Figure 4 shows the development of *O. laevigatus* in the three greenhouses. *Orius* maintained itself and kept reproducing without entering into diapause. All nymphal stages were found, until the end of the crop cycle, even though the months of November and December were relatively cold and dark. The development of *A. cucumeris* in the three plots is given in Figure 5. *O. laevigatus* has been was in all nymphal and adult stages until the end of the crop cycle.

In none of the greenhouses, there has been a need to interfere chemically against thrips after the introduction of the predators. The thrips level remained sufficiently low so as not to cause economic damage throughout the entire trial. Chemical treatments were applied against whitefly, with an IGR, and against fungal diseases. Caterpillars were controlled with *Bacillus thuringiensis*. In plots B and C, there was a chemical correction with pirimicarb against aphids (well controlled with *Aphidius colemani* in plot A) on November 29th.

Figure 1 *Thrips & predators, Greenhouse A*

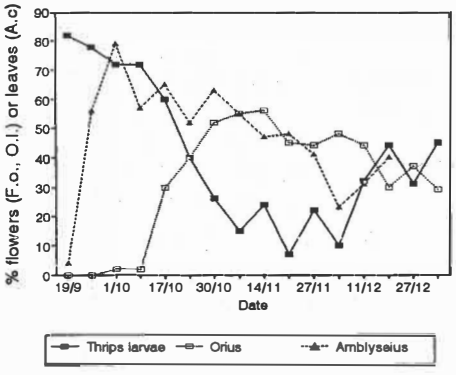


Figure 2 *Thrips & predators, Greenhouse B*

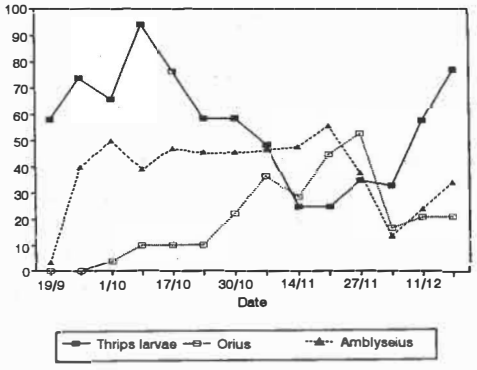


Figure 3 *Thrips & predators, Greenhouse C*

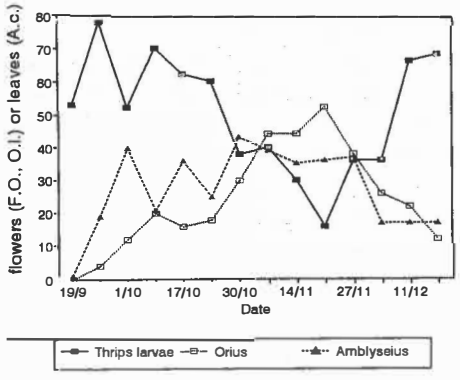


Figure 4 *Population development Orius*

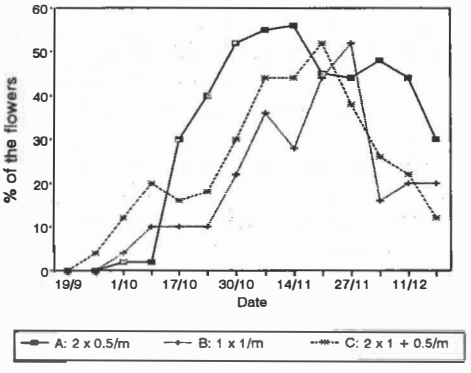


Figure 5 *Development Amblyseius*

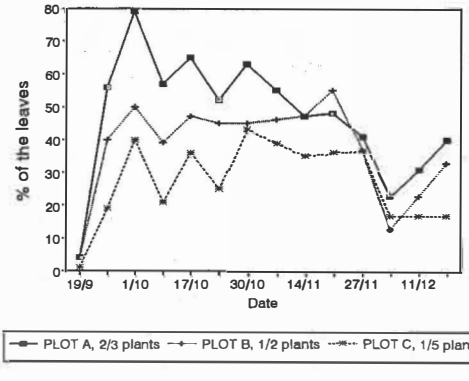
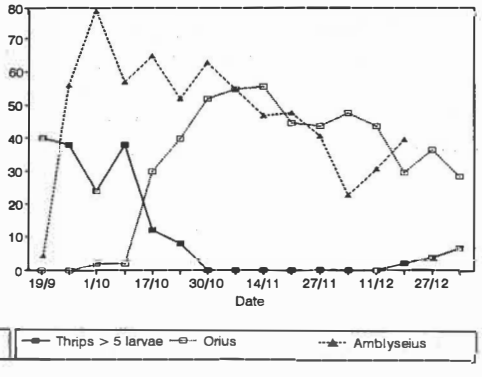


Figure 4 *Thrips & predators, Greenhouse A*



Discussion

In the first three figures, it is clearly demonstrated that *Orius laevigatus* is the most important predator species against thrips. As soon as the *Orius* population starts to grow, thrips is virtually eliminated. Favoured by the humidification system, *Amblyseius cucumeris* reproduced well from the beginning onwards in the three greenhouses. However, due to the form of representing the data in these figures, the role of *A. cucumeris* is rather obscured. Figure 6, however, shows the importance of *A. cucumeris* as predator of thrips larvae. In this figure, the thrips population is given using another parameter, i.e. the percentage of flowers with more than 5 thrips larvae (Greenhouse A). Clearly, after *A. cucumeris* established itself on a massive scale, the percentage of flowers with more than 5 thrips larvae did not increase, but decreased, before *O. laevigatus* became active. This proves that *A. cucumeris* has to be considered a vital tool against thrips before *O. laevigatus* has reached the desired population size. In the three greenhouses, the *A. cucumeris* population drops at the end of November (Figure 4). This may be explained by several factors. In the first place, there was hardly any thrips and very little flowering at that time, so pollen as an alternative food source was no longer available. Secondly, it is highly probable that *A. cucumeris* has served as an alternative prey for *O. laevigatus*, in absence of thrips and pollen.

In the comparison between the three greenhouses, some interesting phenomena were observed. In the first three weeks after the *Orius* introductions, the counts reflected the initial introduction rates (Figure 4). However, the first greenhouse where a significant growth of the population was observed was Greenhouse A, where considerably less *Orius* had been introduced than in Greenhouse C. This can probably be explained by the availability of thrips. Figures 1-3 show that there was considerably more thrips in Greenhouse A than in the other plots. Apart from that, the much higher introduction rate of *A. cucumeris* may have resulted in the availability of a much more abundant alternative food source. The drop of the *Orius* population in Plots B and C after the 27th of November has to be attributed to the Pirimicarb treatment, which did not take place in Plot A. No young stages (until the fifth instar) of *Orius* were found in two weeks after the treatment in plots B and C, but abundantly in A (before the treatment, there was no such difference).

The initial introduction rates were reflected in the population sizes of *A. cucumeris* over a long period. This is logical given the fact that the mites were constantly appearing from the paper sachets during a long period of time. Remarkably, the three populations show peaks and declines at the same moments. This seemed to be correlated with rainy periods, as a result of which the favourability of the RH for egg eclosion varied over the weeks, but was the same for the three greenhouses. The maintenance of a high RH has resulted to be vital for the functioning of *A. cucumeris*. Humidification systems will be recommended next year in all places where IPM will be started in summer.

From the results obtained, it may be concluded that thrips control can effectively be achieved by the combined application of *A. cucumeris* and *O. laevigatus*. It does not seem to be very useful to increase the introduction rate of *O. laevigatus* above the level of 1 per m^2. The introduction of this quantity may be done in two steps, since a good population build-up was observed in the greenhouse where this was practised. By introducing in two steps, an important advantage may be that the risk of any problem (presence of residues, mortality during handling) is reduced. It is very important to introduce *Orius* as early as possible, when

there is availability of food. Apart from ample flowering, the presence of prey, be it thrips or any other prey (springtails and some pollen eating mites were observed to be predated by *Orius*) is an important factor in the speed of establishment of this species. Since *A. cucumeris* initially is the most important predator, it is recommended to adjust the introduction rate of this predatory mite according to the level of thrips pressure in the beginning.

Acknowledgements

The authors wish to thank the growers of AGRONIVI and the Cooperative CAMPOVICAR for their collaboration in the experiment and their hospitality. Folker Cantalapiedra, Inmaculada Segura and Lidia Lara Acedo are thanked for their help during the counting.

References

DISSEVELT, M, ALTENA, K, & RAVENSBERG, W.J., 1995. Comparison of different *Orius* species for control of *Frankliniella occidentalis* in glasshouse crops in The Netherlands. Med. Fac. Landbouww. Rijksuniv. Gent **60/3a**: 839-846

HOUTEN, Y.M. VAN & LIER, T. VAN, 1995. Influence of temperature and humidity on the survival of eggs of the thrips predator *Amblyseius cucumeris*. Med. Fac. Landbouww. Rijksuniv. Gent **60/3a**: 879-883

HOUTEN, Y.M. VAN & LIER, T. VAN, 1996. Effect of temperature and humidity on survival of the thrips predators *Amblyseius cucumeris* and *Amblyseius limonicus* in a cucumber crop. Proc. Exper. & Appl. Entomol., N.E.V. Amsterdam **7**: 95-99

LACASA, A. & CONTRERAS, J. 1993. Comportamiento de *Frankliniella occidentalis* en la transmisión del virus del bronceado del tomate: planteamientos para su control en cultivos hortícolas. PHYTOMA, España **50**: 33-39

RAMAKERS, P.M.J., 1990. Control of Western Flower Thrips, *Frankliniella occidentalis*, mediante depredadores. Cuadernos Phytoma España **6**: 46-49

RODRIGUEZ RODRIGUEZ, J.M. & FIDALGO SOSA, B. 1994. Aplicación de un esquema de lucha integrada para el control de *Frankliniella occidentalis* en cultivo de pimiento bajo cierro. Cuadernos de Fitopatología **2º trimestre 1994**: 71-77

THE APHID PARASITOID *APHELINUS ABDOMINALIS* (HYM.: *APHELINIDAE*) FOR BIOLOGICAL CONTROL OF *MACROSIPHUM EUPHORBIAE* ON TOMATOES GROWN IN UNHEATED PLASTIC GREENHOUSES

O. Alomar, R. Gabarra and C. Castañé
IRTA, Unitat d'Entomologia Aplicada. E-08348 Cabrils (Barcelona), Spain.

Abstract

The aim of this work was to evaluate the effectiveness of releasing *Aphelinus abdominalis* in order to control aphids in unheated greenhouses growing spring tomatoes in our area. Although aphid populations developed in some foci, no sooty mold appeared. *A. abdominalis* did establish in the crop, altogether with indigenous predators (*Aphidoletes aphidimyza*, *Macrolophus caliginosus*, *Dicyphus tamaninii*). Aphid control could clearly be related to abundance of *A. aphidimyza* larvae, although *A. abdominalis* and mirid bugs also contributed.

Introduction

IPM programs are being used for pest control in tomato greenhouses in our area. Secondary pests like the aphid *Macrosiphum euphorbiae* (Thomas) (Hom.: *Aphididae*) are controlled by localized applications with pirimicarb (Albajes *et al.*, 1994). However, application of chemical treatments may affect the effectiveness of natural enemies released to control other pests. Our objective was to evaluate the effectiveness of releasing *Aphelinus abdominalis* Dalman (Hym.: *Aphelinidae*) in unheated greenhouses growing spring tomatoes and to evaluate the spontaneous presence of indigenous entomophages in the crop.

Material and methods

Two commercial greenhouses containing 2200 (C1) and 1600 (C2) tomato plants were monitored in 1994. Plants were transplanted in mid-February, and *Encarsia formosa* and *Diglyphus isaea* were released to control whiteflies and leafminers. For sampling purposes, each greenhouse was divided into a grid of 36 (C1) and 24 (C2) contiguous quadrats. Two monitoring systems were used to assess insect populations. First, up to three plants ('random plant') were selected at random within each quadrat. Second, and in order to better evaluate the control of aphid foci, those random plants that had aphid colonies were fixed ('fixed plant') and monitored over the following weeks until the aphids disappeared. No more than three plants were fixed per quadrat. At least one random plant was always chosen from each quadrat. For monitoring, each random and fixed plant was observed during one minute, and an abundance class was estimated (**0**, no insects; **1**, 1 to 3; **2**, 4-10; **3**, 11-30; **4**, 31-100; **5**, 101-300, and **6**, >300). The same index was used to record abundance of aphids and their specific entomophages. The abundance index of black parasitoid mummies (*Aphelinus* spp.) was recorded separately. The number of mirids was also recorded.

A. abdominalis were inoculatively released in the greenhouses at the rate of 20 mummies per fixed plant. Releases lasted six weeks according to the progress of the aphid infestation

Results and Discussion

M. euphorbiae

In greenhouse C1, *M. euphorbiae* colonies were already found in week 5 after transplanting. Initially, few plants had aphids, and aphid abundance classes up to 3 were

found. Throughout the cropping period, populations in samples of random plants stayed around the class 2 average abundances. During the dispersion phase, an increasing number of new aphid foci appeared each week in other parts of the greenhouse; by week 13, up to 40% of the quadrats had aphids in them, which then decreased to 10% in two weeks.

The aphid infestation in C2 was lower. Although the first aphid foci were located earlier, the number of new foci did not increase as much in C1, and most of the time only ~10% of the quadrats had aphids on them. After an increase in aphid populations, these stayed at less than class 2 average abundance on aphid-infested random plants.

Monitoring fixed plants indicated that most aphid foci in C1 lasted several weeks, but were controlled sooner in C2. No sooty mold was recorded in either greenhouse, which indicated that these average aphid abundances are tolerable.

Aphelinus abdominalis

Introductions of *Aphelinus* were started the same week aphids were first detected (Fig. 1a, 1b). But the first 4 releases failed to establish on time because of unexpected heavy predation by ants and poor emergence of the mummies introduced. Mummies had been placed in small vials, which were put on canes stuck in the ground close to the aphid colonies. Ant predation continued even after painting the canes with Fluon® to prevent ants from crawling up. Therefore, the last two releases (weeks 10 and 11, ▼, Fig. 1a, 1b) were hung and separated from plants. These two releases were made at overall rates of 1.2 (C1) and 0.8 (C2) mummies / m^2, with the vials distributed at known aphid foci.

As a result, there is a clear increase in the average abundance of black mummies on fixed plants by weeks 13 and 14 in both greenhouses (Fig. 1a, 1b). In C1, some parasitism was also recorded three weeks before, presumably due to partial establishment of earlier *Aphelinus* releases. On the whole, black mummies were recorded on ~50% (C1) and <20% (C2) of the aphid foci. But *Aphelinus* mummies were also found in new foci after the releases were made, which indicated that *Aphelinus* was dispersing throuhgout the greenhouse. By week 16, all sampled aphid-infested random plants in C1, and ~30% in C2, had *Aphelinus* mummies.

Spontaneous aphid entomophages

Other than black mummies of *Aphelinus* spp., there were almost no aphid parasitoids present in either greenhouse. However, gall-midges and mirids were prevalent in both.

Aphid control in both greenhouses can clearly be related to abundance of *Aphidoletes aphidimyza*. Spontaneous populations of *Aphidoletes* larvae were already detected 5 and 6 weeks after the first apterous aphids were found (greenhouses C1 and C2 respectively, Fig. 1c, 1d). On average, ~40% of fixed plants in C1, though less in C2, had *Aphidoletes* larvae on them. Average abundances on these plants (class 1~2, i.e. between 1 and 10 larvae) clearly indicate the great impact of these predators on aphid populations. Moreover, *Aphidoletes* larvae were also well extended throughout greenhouse C1 (present on ~20% of aphid-infested random plants), which indicated this predator's searching and dispersion ability when spontaneously colonizing greenhouses. In C1, gall midge larvae were abundant for 5 weeks and had almost disappeared by week 16, once aphids on fixed plants were controlled (Fig. 1c). In greenhouse C2, gall midge larvae were present for one month and no longer found after week 13 (Fig. 1d).

Predatory mirids were also located very soon in both greenhouses. *Macrolophus caliginosus* (Wagner) adults were detected in the same week as aphids (C2, week 4) or one week later (C1, week 6). *Dicyphus tamaninii* Wagner, and *D. errans* (Wolff) were recorded later. From then on, mirid populations increased steadily. Ten weeks after locating the first

VI. Mites, aphids, sciarid flies, slugs and leafminers
Acariens, pucerons, sciarides, escargots et mineuses

adult, greenhouse C1 had an average of 1.5 mirids per random plant (adults and nymphs of all species) and were present on 50% of the plants. Mirids presumably contributed to maintain aphid populations at low densities, since *M. caliginosus* and *D. tamaninii* can feed and complete their development on young *M. euphorbiae* under laboratory conditions (Alvarado *et al.*, 1997).

Conclusions

Results show how *A. abdominalis* does establish in unheated tomato greenhouses and new mummies can be found three weeks after releases of parasitoid mummies. However, when mummies were first recorded, aphid populations were being restricted to steady levels. It is unlikely that in this experiment *Aphelinus* were key controllers of aphid populations. Nevertheless, *Aphelinus* species are known for their predatory activity, and parasitized aphids tend to move away from the plant, which means that parasitoid efectiveness can be underestimated (e.g. Lykouressis *et al.*, 1983).

The spontaneous presence and abundance of indigenous aphid predators in our open greenhouses (*Aphidoletes* and mirids) indicates the importance of conservative biocontrol strategies of pest control. Augmentative releases which complement the action of natural populations of these beneficial insects, wether generalist predatory mirids or more specific aphid entomophages (*Aphidoletes*), may be worth pursuing as a viable strategy for *M. euphorbiae* control in tomato greenhouses.

Acknowledgments

Research in this paper was funded by the European Union (project AIR3-CT93/0842). We wish to thank Dr. Rabasse for preparing and sending the *Aphelinus* parasitoids.

References cited

ALBAJES, R., GABARRA, R., CASTAÑE, C., ALOMAR, O., ARNÓ, J., ARIÑO, J., BELLAVISTA, J., MARTÍ, M., MOLINER, J. & RAMIREZ, M., 1994. Implementation of an IPM program for spring tomatoes in mediterranean greenhouses. IOBC/WPRS Bulletin **17(5)**: 14-21.

ALVARADO, P., BALTÀ, O. & ALOMAR, O. 1997. Efficiency of four heteroptera as predators of *Aphis gossypii* Glover and *Macrosiphum euphorbiae* (Thomas) (Hom.: Aphididae). Entomophaga **42 (1/2)**: 217-228.

LYKOURESSIS, D.P. & EMDEN, H.F. VAN. 1983. Movement away from feeding site of the aphid *Sitobion avenae* (F.) (Hemiptera: Aphelinidae) when parasitized by *Aphelinus abdominalis* (Dalman) (Hymenoptera: Aphelinidae). Entomologia Hellenica **1(2)**: 59-63.

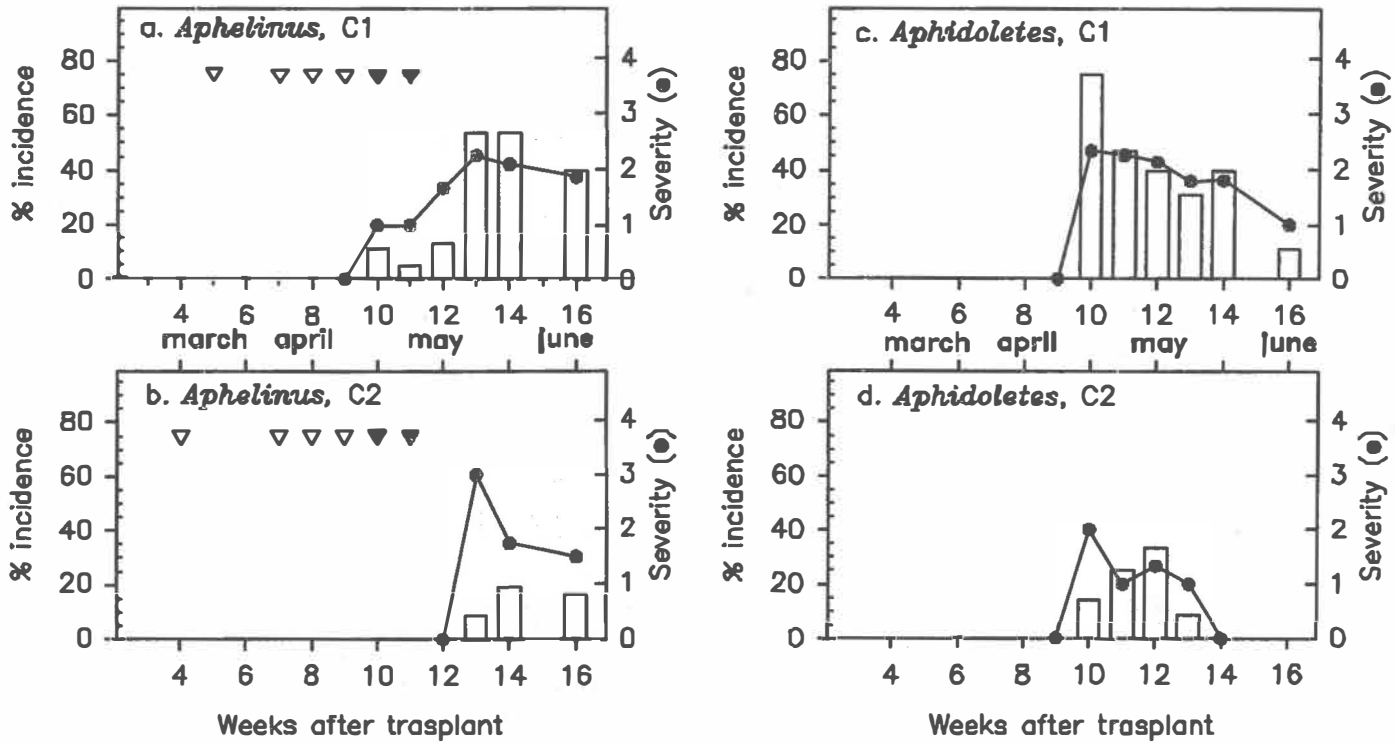


Fig. 1. Population development of aphid *Aphelinus* spp. and *Aphidoletes aphidimyza* on fixed tomato plants in two greenhouses (C1 and C2). Incidence (bars) is the percentage of aphid-infested plants that also had *Aphelinus* mummies (a, b) or *Aphidoletes* larvae (c, d). Severity (●) is the average abundance class on those plants. Triangles indicate releases of *Aphelinus abdominalis* mummies (see text).

DEVELOPMENT OF A METHOD FOR TESTING ADULT-FLY CAPACITY OF *Aphidius colemani* VIERCK (Hymenoptera: Braconidae)

Z. Ilovai[1] & J.C. van Lenteren[2]

[1]Biological Control Laboratory, Plant Health and Soil Conservation Station of Csongrád county, Hodmezovásárhely, P.O. Box 99, H-6801 Hungary, email:hl0958hod@ella.hu
[2]Department of Entomology, Wageningen Agricultural University, P.O.Box 8301, 6700 EH Wageningen, The Netherlands, email: joop.vanlenteren@medew.ento.wau.nl

Abstract

The inoculative and inundative programs of biological control are based on mass-reared beneficial insects. In greenhouse application a high number of individuals and also a high quality is required for this aim. In this case the capability for host searching and flying capacity of adults must be taken into consideration. Aphidius colemani Vierck (Hymenoptera: Braconidae) is one of the most effective species controlling aphids. The goal of our work was to develop a standard method for the estimation of fly-capacity of this parasitoid. For testing the flying capacity on short-distance a glass-cylinder was used. Its inside wall was covered with a layer of repellent substance. All this aparatus was shaded. The adults enforced to fly were caught by a glass sheet coated with insect-glue. The testing facility was applied for examination of parasitoids commercially packed and distributed. The best repellent effect was reached by covering the wall of cylinder with cosmestic lipstick up to 10 cm height. Screening of the container made a light sensitive adults to fly in the direction of catching sheet. As a result of experiment it was concluded that conditions of a very closed apparatus had no toxic effect on parasitoids. It was also considered that there is no need for feeding adults. The commercial product contains more mummies than emerged adults. In spite of high number of emerged insects tha rate of adults able to fly varied in-between 40-65%. The different reasons of inability for fly were also determined. These were as the following: poor physical condition, body injury got during hatching from mummy, some of the wasps could not emerge at all and die inside. On the base of the results positive correlation was found between the number of emerged adults and that able to fly.

Introduction

Aphidius colemani Vierck is one of the most effective bioagents controlling aphids. It parasites *Myzus persicae* and *Aphis gossypii* Glover equally well. In the last years application of this aphid parasitoid has increased rapidly. The initial design and preliminary results of flight test were done on *Aphidius matricariae* by Enkegard and Rietzel (1991). This method was based on using a plastic-container with lids, which was covered by black plastic on its outside. To prevent the crawling out of the beneficials, the inside wall was coated with politetrafluorethylen (Fluon GP-1). On the base of presented results the method seems to be usable for the emergence and combined flight-test. Authors determined a high percentage of individuals being able to fly, though emergence rate was 71,9%.

An Aphidius colemani flight-test was conducted to revise the suggested guidelines and to carry out more tests for improving the method (van Lenteren *et al.* 1993). In difference from the previous method for the test of A. colemani a ventilated cage with sticky yellow trap was suggested. The colour trap was put into dish filled with water, which worked as a barrier for walking insects. In this bioassay not a commercial product was examined, but naturally infected aphids collected from cucumber leaves. By this experiment the product control criteria were suggested: flight activity in

comparison with emerged wasps >90%. In the test at least 500 beneficials should be examined, in 10 replicates. (steinberg and Cain, 1993). This method could be appropiate only for short range flight assay.

During the 2nd IOBC workshop on quality of bioagents held in 1994 at Evora (Portugal) the development of the short-range flight-test was discussed. It was considered that the method hadn't been tested on other species yet, so it is necessary to prepare a method suitable for other small-bodied beneficials (van Lenteren, 1994).

According to this new approach a method developed for Encarsia flight-test was examined from the view of its suitability for *A. colemani*. The aim was to develop a standard test method for fly-capacity of *Aphidius* spp.

Materials and Methods

Testing of repellent effect

In this experiment a glass cylinder was used, its size was 16 cm in diameter and 21 cm height. Its top was covered with glass sheet (20x20cm), which was sprayed with sticky insect glue (Souverode aerosol) in the midle, on an area of 16 cm in diameter, inside. For delimiting of a part of the cylinder's wall a lipstick (Blistex) was applied. The wall was smeared with the lipstick in a thin, semitransparent layer of 10 cm in height. The isolation zone was formed on the UPPER and UNDER part of the cylinder. In the test a commercial, ready for sale product was performed. The one day old adults of *A. colemani* were collected fron their container by vacuum pipe into a Petri-dish of 5 cm in diameter. Than the dishes were placed into the cylinder-set and the upper part of the dish was removed. The observation of animals was done visually during their whole activity period. After they finished, the cylinder was opened and the wasps were counted on different items: the glass cover-sheet, the repellent layer and the bottom of the cylinder. The distribution of *A. colemani* was exprssed in percentage.

Development of testing set

The results of trapping in the previous test were infleuntial for the next steps. At first necessity of comparison of the different shading-degree was considered. The cylinder was covered by black paper around the wall and the glass plate except a hole on the top of 10 cm in diameter (Figure 1). A commercial product of A. colemani was used for testing: pupa in the host animal body or sometimes emerged adults, which could be always found in the shipper container. The mummies were first collected into a small Petri-dish and after that placed into the cylinder. Every time the same number of mummies was collected, but as mummies could be empty sometimes, the effective number of emerged insects varied. That's why five replicates were applied.

The series of tests were carried out in the climate-room, under the following environmental conditions: temperature 25ºC, RH 70%, photoperiod (L:D) 16:8 hrs. Evaluation was carried out after all mummies had emerged, or the adults finished their activity. The glass plate was taken off, and all the adults were counted on different items of the testing apparatus. The mummies were also checked under binocular for separation of empty mummies, or not emerged wasps. The evaluation was done for determination of the emergence and trapping effect. The effectiveness of different testing sets was established on the base of distribution in percent.

Examination of mass-reared, commercial product

After establishing of the most effective method some commercial samples were examined completely for verification of its suitability for testing. The product was divided into 5 parts, each of

them was placed into the testing set. All this was examined under climatic conditions described above. On the base of the number of mummies and emerged wasps a percentage of emerged and trapped deneficials was determined. The observation lasted for 5 days.

Results

Testing of the repellent effect of lipstick

The results of lipstick probe are shown in Table 1. The wasps generally kept themselves away from the surface smeared with the lipstick. That adults, which were in a bed condition, tried to walk on it, maximally on a distance of 6 cm. The insects, which occasionally landed on it, in a short time escaped. The lipstick did not show trapping nor attractive effect.

Place of repellence	Number, per cent	Adults/cylinder			
		Total	Trapped	Repelled	On the bottom
UNDER	number %	45,0 100,0	23,0 51,1	9,0 11,1	17,0 37,8
UPPER	number %	34,5 100,0	12,8 36,9	0,3 0,8	21,5 62,3

Table 1. Distribution of *Aphidius colemani* adults in the repellent test (average).

When the repellent layer was located on the upper part of the glass, much more wasps were found on the bottom of the cylinder. In this situation the insects preferred to fly diagonally and did not aimed the top. If the repellent surface was formed on the under part of the glass, the number of adults found on it was higher, but these were mostly deficient.

Development of the set for testing

As the previous test shown the wasps didn't prefer to fly in the vertical direction, so for making them to do it, the cylinder was covered with black paper. Results of this assay are shown in Table 2.

The experiment proved also a high photosenvity of A. colemani. The compulsion of flying into the direction of light was stronger than that of in food's one. The food was not so important for them; the wasps emerged were trapped by honey. Results of this observation are presented in Table 3 (see aldo Figure 1).

Lighting	Number, per cent	Adults/ cylinder			
		Total	Trapped	Bottom	Repellent
Normal	number %	19,2 100,0	6,2 32,3	10,6 55,2	2,4 7,1
Shaded wall	number %	16,8 100,0	7,2 42,9	8,4 50,0	1,2 7,1
shaded wall and top	number %	52,6 100,0	27,2 51,7	23,0 41,9	3,4 6,5

Table 2. Influence of the lighting on the trapping of *Aphidius colemani* adults (average of 5 replicates)

Treatment	number per cent	adults/cylinder				
		Total	Trapped	Bottom	Repelled	Honey
No honey	number %	52,6 100,0	27,2 51,7	23,0 41,9	3,4 6,5	- -
honey (drops)	number %	24,6 100,0	12,0 48,8	8,0 32,5	0,4 1,6	4,2 17,1

Table 3. Influence of honey on the trapped adults of *Aphidius colemani* (average of 5 replicates)

The different variations of the test-method are shown in the Figure 1.

Evaluation of the commercial product

During the evaluation of the commercial product shipped directly by the producer it was established that the number of mummies divided into testing sets varied between 86 and 238. The percentages of animals, which hadn't emerged was determined as 6,7 and 19,5 %. 89,3 and 79,8 % of insects emerged, but the number of that's able to fly was comparably low. Though the number of active wasps warranted by the producer in the shipped material was actually present.

Testing period	Samples	
	№1	№2
	02-07 Dec 1994	07-12 Dec 1994
Total number of mummies - empty (emerged) -not emerged	701 653 47	630 507 123
Total number of wasps -flown out -trapped	438 391 255	609 486 196
Percentage of emerged wasps	98,3	79,8
Percentage of trapped (:able to fly) wasps	65,2	40,3

Table 4. Results of two series of evaluation of fly ability of commercial *Aphidius colemani* product.

The results of comparison of probable toxicity of closed and opened system are shown in Table 5.

Conditions of the observation	Opened Petri-dish		Closed testing set	
Series	1	2	1	2
Number of mummies	116	87	252	281
Total number of wasps	58	43	159	210
Number of wasps flown out	35	19	123	148
Percentage of wasps flown out	60,3	44,2	77,7	70,4

Table 5. Results of evaluation of *Aphidius colemani* adults in conditions of opened and closed testing system.

Conclusions

As a result of the present work it was proved that the Blistex lipstick was suitable for using as a repellent material for A. colemani, too. The series of tests shown, that the repellent should be semitransparent, otherwise the frequency of diagonal flying will increase, which is not desired from the view of the test. Aiming to reduce the walking, resting and diagonal flying it is necessary to shadow the testing set-up with a dark material (e.g. paper).

The flying test should be done during short time (max 7 days). This makes superfluous the feeding the of parasitoids with honey. The more, due to honey the number of individuals able to fly can decrease as some of emerged wasps can drown. This way the effectiveness of trapping can also decrease.

In accordance to results of evaluation of different testing sets (see Figure 2) the application of shaded wall and top (with the exception of hole in the centre of it) provided the highest rate of trapped parasitoids.

Analysis of the results made possible the quantitative evaluation of individuals unable to fly. On the base of observations and examinations the enable insects could be classify as follow:

1) Weak, bed conditioned individuals:
smaller than normal parasitoids, they mostly walk and hardly fly; in the testing set they walk onto repellent zone.

2) Injured, limited in movement:
in many cases the adult's wing can get wounded during the emergence; it can also happened, that the mummy's skin remain on the beneficial making it unable to fly.

3) Abnormal emergence:
some of adults don't emerge at all or are not able to finish this process and die inside the mummy.

The examination of effect of closed and opened testing system hadn't shown any negative influence on wasps. In the opened Petri-dish the percentage of emerged insects was not higher than in the closed set. The closed space had no harm effect. It was also considered that there was no toxicity either from the lipstick or from the insect glue.

Results of test-series were analysed mathematically, using the data of emerged and trapped parasitoids (see Figure 3). It was shown that the higher is the number of emerged adults, the higher will be the number of individuals able to fly.

Acknowledgements

The first author appreciates the possibility provided by the bilateral Hungarian-Dutch Agreement on scientific co-operation, thanks to which he could take part in the developing work on "Quality control of mass-reared arthropods". Grateful acknowledgements for J.A.M. Doodenann for his kind technical assistance in experiments and Koppert B.V. for supplying the parasitoids. Authors are also much obliged to Mrs. M. Regos and E. Ceglarska Hódi for their kind help.

References

ENKEGAARD A. & REITZEL J. 1991. A simple method for quality control of Aphidoletes aphidimyza, *Aphidius matricariae* and *Encarsia formosa*. Proceedings of the 15th workshop of the Global IOBC working group "Quality Control of Mass Reared Organisms". Wageningen, March 1991. pp.102-110.

LENTEREN J.C. van, BIGLER F.& WADDINGTON C. 1993. Quality control guidelines for natural enemies. Report 3rd meeting on guidelines for natural enemies applied in greenhouses.

Proceedings of the 7th workshop of Global IOBC working group "Quality Control of Mass Reared Organisms". Rimini, Italy, 13-16 Sept. 1993. pp. 222-230.
LENTEREN J.C. van 1994. Designing and implementing quality control of beneficial insects: towards more reliable biological pest control. Report of the second workshop. Evora, Portugal, 17-20 Sept. 1994 24pp.
POSTHUMA-DOODEMAN C.J.A., LENTEREN J.C. van, SEBESTYÉN I. & ILOVAI Z. 1996. Short-range flight test for quality control of *Encarsia formosa*. Proc. of Exp. and Appl. Entomol. N.E.S. Amsterdam, vol.7 1996. pp.153-158.
STEENIS M.J. van 1993. Intrinsic rate of increase of *Aphidius gossypii* Glov. (Homoptera: Aphididae) at different temperatures. J. Appl.Ent. **116**: 192-198.
STEINBERG S. & CAIN H. 1993. Apects of product control in *Phytoseiulus persimilis*, *Aphidius colemani* and *Diglyphus isaea*. Proceedings of the 7th workshop of Global IOBC working group "Quality Control of Mass Reared Organisms". Rimini, Italy, 13-16 Sept. 1993. pp. 179-190.

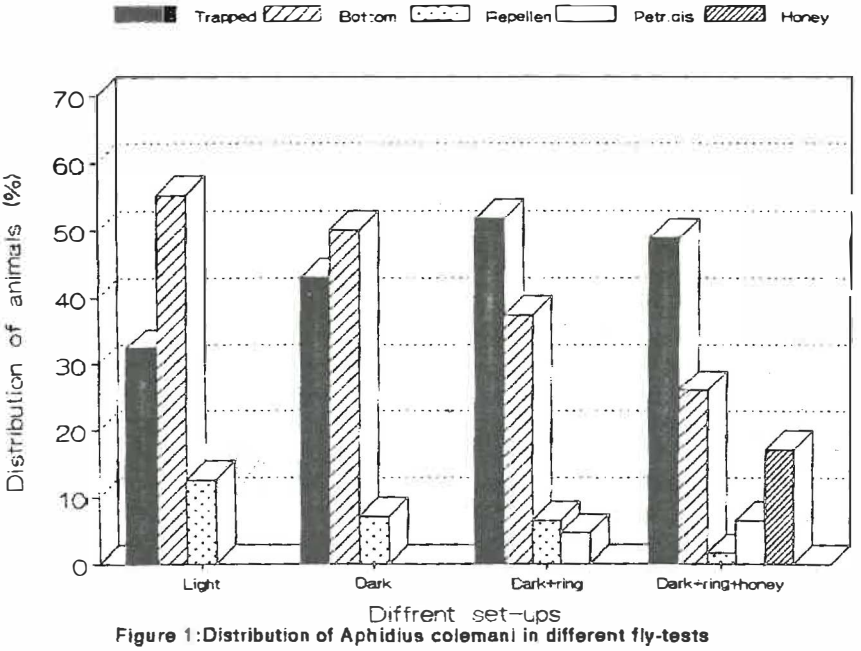


Figure 1:Distribution of Aphidius colemani in different fly-tests

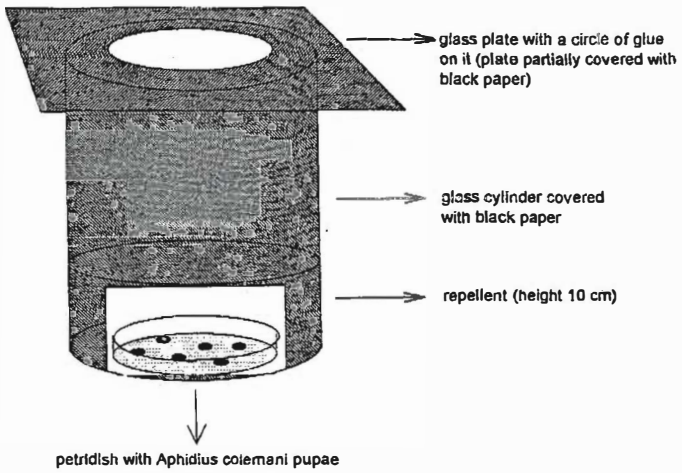


Figure 2. The developed flight-test set-up for Aphidius colemani

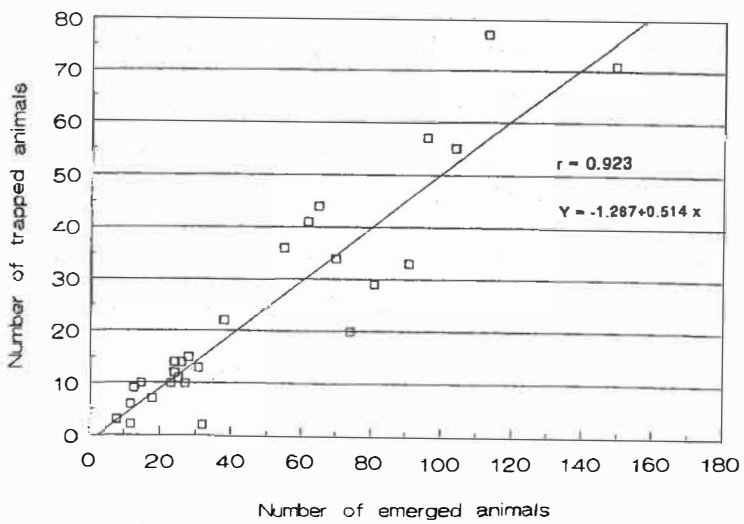


Figure 3: Relationship between the number of emerged and able to fly individuals of *Aphidius colemani*

BIOLOGICAL CONTROL OF *Tetranychus cinnabarinus* BY *Phytoseiulus persimilis* ON GREENHOUSE CUCUMBERS.

C. Kazak[1] T. Colkesen[2] K. Karut[1] E. Sekeroglu[1]

[1]Dept. of Plant Protection, C. U. Agricultural Faculty. 01330 Adana, Turkey
[2]Dept. of Plant Protection, K.S.U. Agricultural Faculty. 46060 Kahramanmaras, Turkey

Abstract

Effectiveness of *Phytoseiulus persimilis* to control *Tetranychus cinnabarinus* was studied both in two experimental glasshouse sections. In each section 5 plots (control, acaricide treated one week after spider mite infection, predator released before prey, predator released together with prey, and predator released one week after spider mite infection) were established. Each plot consisted of six plants, and release rate was 5:1 (prey:predator) per leaf. Counts were made on four 2.25 cm^2 areas of each of three leaves (from top, middle and bottom) of each plant by hand-lens. Experiment was carried for six weeks and then terminated due to the death of all plants in control plots. In prey-predator released together plot *P. persimilis* consumed the prey readily and therefore no prey and predators were observed afterwards. The prey density was 4-5 fold less in chemical and late predator release plots than that of control plot. Spider mite populations declined almost to zero level after acaricide treatment by fifth week. In late predator release plot spider mite populations were always present but at very low levels.

Introduction

Despite its world-wide use as a succesful biological control agent (Cross, 1984; Goodwin, 1990; Jarosik & Pliva, 1990; Zang & Sanderson, 1995) *Phytoseiulus persimilis* Athias-Henriot is only studied at experimental basis in Turkey (Kazak & Þekeroðlu, 1990; Kazak *et al.*, 1992; Kýlýnçer *et al.*, 1992). Spider mites are one of the main problems in greenhouse vegetables in Turkey (Kazak, 1991; Yiðit & Erkýlýç, 1992) and their control is mainly depended on use of pesticides, creating well known undesirable effects. The experimental studies indicated that *P. persimilis* is good candidate for biological control of spider mites all local conditions in Turkey (Kazak *et al.*, 1992, Öncüer *et al.*, 1994). Recent discovery of natural strains of *P. persimilis* in Turkey (Þekeroðlu & Kazak, 1993; Kazak, 1996), increases the importance *P. persimilis* for use of controlling the spider mites at local greenhouse conditions.

Various release methods for *P. persimilis* was reported in literature (Parr & Hussey, 1966; Havelka & Kindlman, 1984). Here, it was aimed to study the effect of different release methods on activity of *P. persimilis* on controlling *Tetranyhus cinnabarinus* Boisduval before attempting to use it growers conditions.

Materials and Methods

Experiment was set up in two different sections (3x5 m each) of a glasshouse. In each section, 5 plots (untreated control, acaricide treated one week after spider mite infection, predator released before prey, predator released together with prey, and predator released one week after spider mite infection). For acaricide treatment propargite %30 was used at a rate of 120 g/100 l. Each plot consisted of six cucumber plants which were seeded in 23x23x35 cm tin containers (one plant in each container). Both prey and predator were introduced on to every plant at four leaf stage, and release rate was 5:1 (prey:predator) per leaf. First release was on

24 January 1994. First count was made 7 days after releasing and continued weekly on four 2.25 cm^2 areas of each of three leaves (top, middle, and bottom leaves) from each plant by hand-lens. Experiment was carried for six weeks and then terminated due to the death of all plants in untreated, predator free control plot.

Results and Discussion

Fig. 1 and 2 present the population development of *P. persimilis* and *T. cinnabarinus* in two different chambers of the glasshouse on different predator-released and predator-free cucumbers. On the predator-free, untreated plants *T. cinnabarinus* population was increased very sharply with time and all host plants were blighted by the 6th week, however the number of eggs and motile forms in Chamber 1 was almost two times as high as than that of Chamber 2 (Fig. 1-A and B).

The estimation of predator efficiency were made in comparison with the development of prey populations on predator-free and predator-released plots. As seen in Fig.1 C and D acaricide application kept the *T. cinnabarinus* populations at very low levels during the extent of experiment, the number of eggs never exceeded 10 eggs/2.25 cm^2, and the number of motile forms were even lower. In predator-prey released together plots both prey and predator were disappeared one week after the release and never observed again, presumably *P. persimilis* consumed all the prey available readily and migrated for a search of food. When two chambers are compared the number of predators was very low in one and higher in the other for unexplained reason, but *P. persimilis* was still able to keep *T. cinnabarinus* populations under control (Fig. 2).

Population density of *P. persimilis* in pre-release plot was low early in the beginning of the experiment then started to increase, the early low population density was probably due to death of the released individuals. Following increase in predator population might have been resulted from development of the offspring of released individuals. In post-release plot *P. persimilis* responded readily and kept *T. cinnabarinus* populations under control. When prey populations compared, the population development and density was very similar both acaricide treated and predator released plots indicating the predator efficiency of *P. persimilis.*

Hussey *et al.*, (1965) have shown that if the predator is introduced when prey population is low, elimination of the mites occurs without leaf injury becoming high on cucumbers. They also reported that if the number of predators introduced is too high in relation to the prey available, rapid extermination of the prey and subsequent loss of the predator will follow. Gould (1967, 1970) showed that *P. persimilis* could be used to control *Tetranychus urticae* Koch if the predator is introduced after an even, artificial infestation of *T. urticae* has been established soon after planting. The results obtained in this study also gave similar indications. When *P. persimilis* released together with prey both the predator and prey were diminished in a very short time, although no infestation occurred during experiment the system was unprotected for the spider mite infestation. *P. persimilis* seemed to be most successful when it was released one week after the spider mite inoculation. Although the results showed that *P. persimilis* is very promising, trials should be set up in a large scale commercial greenhouses to draw further conclusions for the effectiveness of *P. persimilis* to control *T. cinnabarinus* at local conditions.

Acknowledgement

This research was supported in part by TUBITAK (Scientific and Technical Research Council of Turkey) and by NATO Science for Stability Program, Project TU-POOLINATION

References

CROSS, J. V., 1984. Biological control of two-spotted spider mite (***Tetranychus urticae***) by ***Phytoseiulus persimilis*** on strawberries grown in 'walk-in' plastic tunnels, and a simplified method of spider mite population assessment. Plant Pathology, 33: 417-23.

GOODWIN, S., 1990. Seasonal abundance and control of spider mites (Tetranychidae) infesting commercial strawberries in coastal New South Wales. J. Aust. ent. Soc., 29: 161-66.

GOULD. H. J., HUSSEY N. W. & PARR W. J., 1967. Large scale commercial control of ***Tetrancyhus urticae*** Koch on cucumbers by the predator ***Phytoseiulus persimilis*** A-H. Proceedings of the 2nd International Congress of Acarology. pp. 383-388.

GOULD. H. J., 1970. Preliminary studies of an integrated control programme for cucumber pests and evaluation of methods of introducing ***Phytoseiulus persimilis*** Athias-Henriot for the control of ***Tetrancyhus urticae*** Koch. Ann. appl. Biol. 66: 505-513.

HAVELKA, J. & KINDLMANN P., 1984. Optimal use of the "pest in first" method for controlling ***Tetrancyhus urticae*** Koch (Acarina, Phytoseiidae) on glasshouse cucumbers through ***Phytoseiulus persimilis*** A-H. (Acarina: Phytoseiidae). Z. ang. Ent. 98: 254-263.

HUSSEY, N. W., PARR, W. J. & GOULD H. J., 1965. Observation on the control of ***Tetranychus urticae*** Koch on cucumbers by the predatory mite ***Phytoseiulus riegeli*** Dosse. Ent. exp. appl., 8: 271-81.

JAROSIK, V. & PLIVA, J., 1990. Efficient control of twospotted spider mite (***Tetranychus urticae*** Koch) by ***Phytoseiulus persimilis*** A.-H. on glasshouse peppers. J. Appl. Ent. 110: 270-74

KAZAK, C. & ÞEKEROÐLU, E., 1990. Life table studies of two strain of ***Phytoseiulus persimilis*** Athias-Henriot (Acarina: Phytoseiidae) under laboratory conditions. Procedings of the 2nd Turkish National Congress of Biological Control. Publications of Entomological Society of Turkey (4): 203-210.

KAZAK, C., 1991. The effect of limited and optimal food densities on the development and reproduction of ***Phytoseiulus persimilis*** Athias-Henriot under different temperature and humidity levels. M. Sc. Thesis. Çukurova University (unpublished).

KAZAK C., Çölkesen T., Zaman K. & Þekeroðlu E., 1992. The effect of ***Phytoseiulus persimilis*** Athias-Henriot (Acarina: Phytoseiidae) on ***Tetrancyhus cinnabarinus*** Boisduval (Acarina: Tetranychidae) on greenhouse grown strawberry. Proceedings of the 2nd Turkish National Congress of Entomology. Publications of Entomological Society of Turkey (5): 145-157.

KAZAK, C., 1996. Studies on the biological characteristics of Hatay strain of ***Phytoseiulus persimilis*** Athias-Henriot (Acarina: Phytoseiidae) under laboratory conditions and its population development in field. Ph. D. Thesis. Çukurova University (unpublished).

KÝLÝNÇER N., ÇOBANOÐLU S. & HAS A., 1992. Studies on the possibilities of predatory mite ***Phytoseiulus persimlis*** Athias-Henriot (Acarina: Phytoseiidae) as a biological control agent on various crops in a greenhouse conditions. Proceedings of the 2nd Turkish National Congress of Entomology. Publications of Entomological Society of Turkey (5): 109-122.

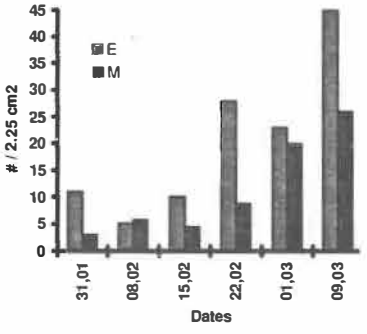
ÖNCÜER, C., YOLDAÞ Z., MADANLAR, N. & GÜL, A., 1994. Biological control practices against insect pests of greenhouse vegetables in Aegaen region. Proceedings of the 3rd Turkish National Congress of Biological Control. Publications of Entomological Society of Turkey (7): 395-407.

PARR, W. J. & HUSSEY, N. W., 1966. Biological control of red spider mites on cucumbers: effects of different predator densities at introduction. Rep. Glasshouse Crops Res. Inst. 135-39.

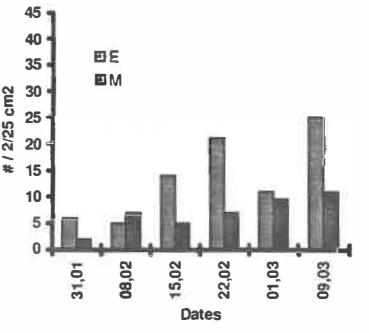
ÞEKEROĐLU, E. & KAZAK, C., 1993. First record of ***Phytoseiulus persimilis*** (Athias-Henriot) in Turkey. Entomophaga 38: 343-345.

YIĐIT, A. & ERKÝLÝÇ, L., 1992. Studies on the chemical control of ***Tetrancyhus cinnabarinus*** Boisd. (Acarina: Tetranychidae), a pest of strawberry in the East Mediterranean region of Turkey. Crop Protection. (11) 433-438.

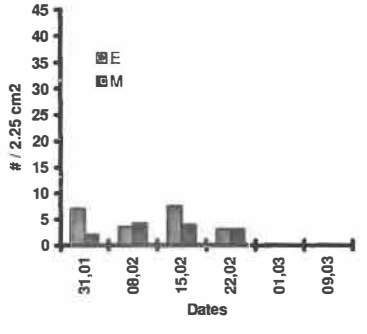
ZANG, Q. Z., & SANDERSON, J., 1995. Twospotted spider mite (Acari: Tetranychidae) and ***Phytoseiulus persimilis*** (Acari: Phytoseiidae) on greenhouse roses: spatial distribution and predator efficacy. J. Econ. Entomol., 88 (2): 352-57.



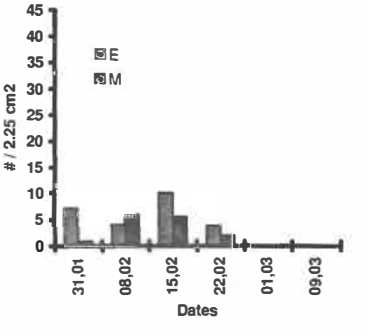
A. Untreated control plot 1.



B. Untreated control plot 2.

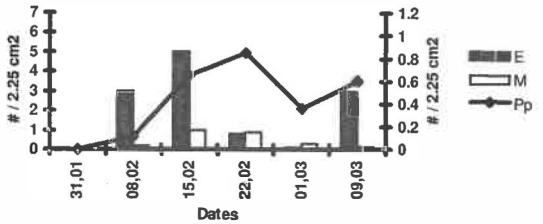


C. Treated control plot 1.

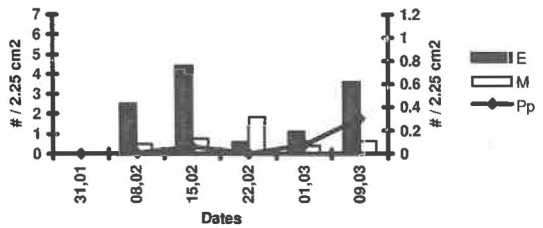


D. Treated control plot 2.

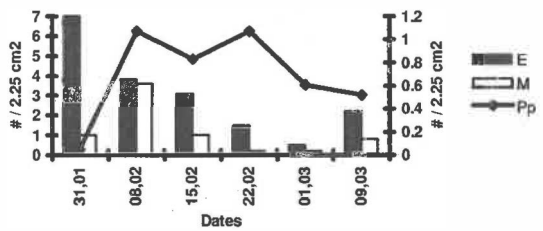
Figure 1. Number of *Tetranychus cinnabarinus* (E:Eggs, M:Motile forms) per 2.25 cm^2 leaf area on cucumber.



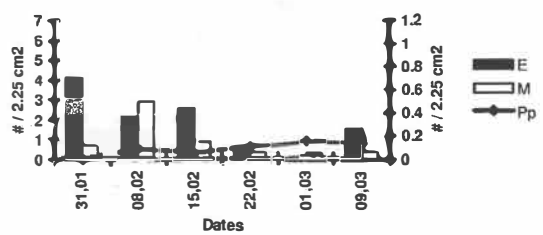
A. Pre-release plot 1.



B. Pre-release plot 2.



C. Post-release plot 1.



D. Post-release plot 2.

Figure 2. Number of *Tetranychus cinnabarinus* (E:Eggs, M:Motile forms) and *Phytoseiulus persimilis* (Pp) per 2.25 cm^2 leaf area on cucumber.

BIOLOGICAL CONTROL OF SCIARID FLIES (BRADYSIA SPP.) WITH PREDATORY MITES HYPOASPIS ACULEIFER ON POINSETIA CROPS IN GREENHOUSES

Janusz Piatkowski
Institute of Plant Protection, 60-318 Poznan,ul. Miczurina 20 Poland

Introduction

Predators belonging to Hypoaspis species are the arthropodes living in the soil, and also in glasshouse substrates. Sciarid flies live in the same enviroment, being one of numerous organisms attacked by such the predators and among them by *Hypoaspis aculeifer* Can. (Acari:Laelapidea). The role of *H.aculeifer* in biological control was underlined earlier by Barker (1968), Ignatowicz (1974). The experiments carried out by Gillespie and Quiring (1990), Piatkowski and Lindquist (1993), Piatkowski (1995) and Lindquist and others (1995) confirmed the usefulness of this species for biological control of sciarid flies as well as for the reduction of numerousness of other species, such as among others Western flower thrips (*Frankliniella occidentalis* Pergande). Basing on the results of the laboratory tests in 1995 - 1996 the trials of practical use of the predator *H.aculeifer* for sciarid flies control on poinsetia crops have been undertaken.

Materials and methods.

Experiments were carried out on poinsetia crops in the glasshouses belonging to Horticultural Farm in Zlotów.
Plant seedlings have been divided into groups depending on the method of protection:
1995.
I - Biological - application of the predator *H. aculeifer* (65 individuals per plant per season in three application rates),
II- Biological - application of entomoparasitic nematodes *Steinernema feltiae* (3 millions per plant per season in three application rates),
III- Chemical - watering with 0,05% solution of Nomolt 15 SC (teflubenzurone) - 4 treatments,
IV- Biological - application of predator *H.aculeifer* as in the variant I and one treatment using nematodes (1 million/m^2)
1996
I - Biological - one introduction usin predator *H.aculeifer* (7,5 individuals per plant) shortly after plant seedling and one application of nematodes *S.feltiae* (1 million per m^2) 2 weeks after predators introduction.
II- Chemical - three waterings with 0,1% Trigard (cyromazine).
During the transplantation and transferring of plants to a permanent place the conformability of variants has been maintained, placing the plants in uniform blocks.
The density of sciarid flies (adults) population was controlled every week placing horizontally on substrate surface in the pots 50 yellow sticky traps (2,5 x 2,5 cm) in each variant. After controlling and noting the number of caught flis - the tables were changes for the new ones.

Results and discussion.

In 1995 first adults of sciarid flies have been observed after approximately one month from the seedling (Tabl 1, Figure 1). In the first period of plant growth the development of sciarid flies population was similar. Considerable differentiation in the density of pest population took

place in the second period of plant growth (June). Three introductions of the predator was more effective than chemical method and after the increase of population density stable decrease tendency have been stated. In the variant with chemical protection population density of sciarid flies increased during the whole period of experiment. In the variant with nematode application after relatively high increase of sciarid flies population density considerable decrease has been observed. The dynamics of sciarid flies population density in the variant predator + nematodes was unexpected. Scinc the moment of nematode application the population density of the pest began to increase faster than in the variant with the predator only. In further observations considerable decrease of population density has been observed, however. It can suggested that the nematodes introduced to the substrate became additional attractive food for the predator, which caused temporary increase of sciarid flies population density.

In 1996 cyromazine has been applied in the variant with chemical control, and in the variant with biological control only single introduction of predator and single nematode application have been performed. It should be underlined, that in 1996 population density of sciarid flies in the glasshouses in Zlotów was lower than in 1995. Apart from the decrease of predator and nematode population density no significant differences in the increase of sciarid flies population density have been stated (Table 2, Figure 2).

During plant rooting and growth, i.e. in the period when sciarid flies are the most dangerous for the plant, no significant noxiousness of such the pest have been observed. It can be suggested that when the risk of losses caused by sciarid flies is relatively low, it is possible considerably reduce the number of introduced predators and nematodes. It is, of course, of high importance for the cost of protection, which causes that the horticulrists are more interested in this method. It has been also confirmed that early introduction of the predator assures and stable control of sciarid flies population density.

References

BARKER P.S. 1968. The response of a predator *Hypoaspis aculeifer* (Canestrini)(Acarina: Laelapidae) to two species of pray. Can.J.Zool.,**47: 343-345**.

GILLESPIE D.R., QUIRING M.I. 1990. Biological control of fungus gnats Bradysia sp. (Diptera, Sciaridae) and western flower thrips, *Frankliniella occidentalis* (Pergande)(Thysanoptera: Thripidae) in greenhouses using a soil-dwelling predatory mite Geolaelaps sp.nr.aculeifer (Canestrini)(Acarina: Laelapidae). Canad.Entomol., 122:975-983.

IGNATOWICZ S. 1974. Observation on the biology and development of *Hypoaspis aculeifer* Canestrini (Acarina: Gamasides). Zoologica Poloniae, **24:41 - 59**.

LINDQUIST R.K., BUXTON J., PIATKOWSKI J. 1995. Biological control of sciarid flies and shore flies in glasshouses. Proc. of the Brighton, Brighton Crop Prot. Conf. - Pest and Diseases, **3:1067 - 1072.**

PIATKOWSKI J. 1995. Potential possibilities of using a predatory mite Geolaelaps sp. (Acarina: Laelapidae) in practice. Proc. of the Conf. on "Actual and potential use of biological pest control on plants". Skierniewice: **135 - 138**.

PIATKOWSKI J., LINDQUIST R.K. 1993. Stosowanie drapiezcy Geolaelaps sp. (Acarina:Laelapidae) i entomofilnych nicieni w zwalczaniu ziemiórek na uprawach roœlin ozdobnych. Mat.XXXIII Sesji Nauk. Inst. Ochr. Roslin, Referaty: **136 - 139**.

Table 1 -Dynamics of development of sciarid flies population density on poinsetia crops (Zlotów 1995).

Date	Biological control Hypoaspis - I		Biological control *S.feltiae* - II		Biological control Hypoaspis sp. + *S.feltiae*-IV		Chemical control Nomolt 15 SC-III		Treatments
	Aver. numb./ trap	Stand. error	Aver. numb/ trap	Stan. error	Aver. numb./ trap	Stan. error	Aver. numb./ trap	Stan. error	
16.03.	-	-	-	-	-	-	-	-	Seedling
30.03	-	-	-	-	-	-	-	-	Hypoaspis(15 indiv/plant) *S.feltiae*(1 million/m^2) Nomolt 15 SC(0,05%)
7.04	0	0	0	0	-	-	0	0	
14.04	0	0	0	0	-	-	0	0	Nomolt 15 SC(0,05%)
21.04	0,08	0,08	0,2	0,1	-	-	0,12	0,06	Hypoaspis(35 indiv/plant)
25.04	0,12	0,06	2,04	1,29	-	-	0,08	0,05	Planting of rotted plants Nomolt 15 SC(0,05%)
28.04	0,88	0,44	2,04	1,29	-	-	0,12	0,06	*S.feltiae*(1 million/m^2)
3.05	5,2	1,36	4,76	0,98	-	-	2,96	0,63	
13.05	10,08	1,85	4,68	1,55	-	-	3,76	0,6	
20.05	7,84	1,14	4,52	1,08	-	-	5,48	1,51	Hypoaspis(15 indiv/plant) *S.feltiae*(1 million/$m.^2$) Nomolt 15 SC(0,05%)
26.05	7,6	1,07	5,08	0,68	-	-	7,48	1,7	
5.06	25,0	2,46	15,52	2,54	-	-	16,6	2,68	
12.06	23,16	2,15	29,28	2,8	23,16	2,15	23,76	2,37	*S.feltiae*(1 million/m^2) - in variant IV only
21.06	12,24	1,4	18,52	2,05	15,12	2,0	18,84	1,82	
28.06	16,72	3,09	31,48	3,97	35,96	3,99	30,24	3,08	
5.07	39,48	3,91	74,56	6,68	57,84	5,1	50,92	4,82	
12.07	22,56	2,54	56,2	6,37	37,8	5,35	50,72	5,55	
19.07	29,04	2,62	48,08	4,73	34,76	4,04	51,68	4,52	
26.07	25,76	2,85	48,84	4,73	34,52	4,9	54,72	5,91	

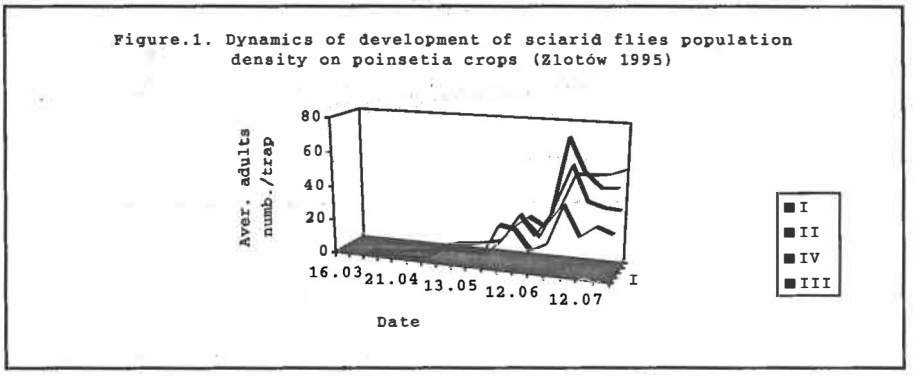
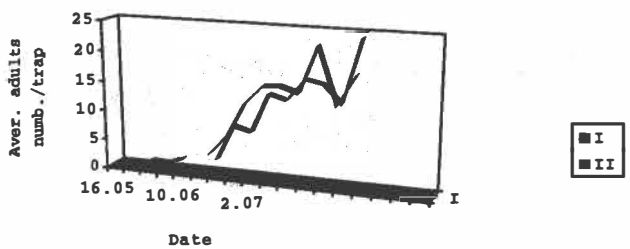


Table 2.
Dynamics of development of sciarid flies population density on poinsetia crops(Zlotów 1996).

Date	Biological control (H.aculeifer + S.feltiae)-I		Chemical control (Trigard) - II		Treatments
	Aver. numb./ trap	Stand.error	Aver. numb./ trap	Stand.error	
16.05.	-	-	-	-	Seedling
17.05	-	-	-	-	Hypoaspis (7,5 indiv./plant)
27.05	0.96	0,22	0,78	0,15	Trigard (0,1%)
4.06	0,44	0.15	0.52	0.17	
10.06	2.48	0.81	1.8	0.51	
15.06	-	-	-	-	S.feltiae(1 million/m^2)
17.06	--	-	-	-	Planting of rotted plants Trigard(0,1%)
18.06	4.08	1,58	2.33	0.91	
24.06	7.32	1.23	8.22	1.24	
2.07	12.76	1.74	7.44	0.85	Trigard (0,1%)
8.07	15.88	2.27	14.08	2.19	
15.07	16.12	1.75	13.13	1.72	
22.07	15.26	1.98	16.86	1.79	
29.07	23.04	2.56	16.24	1.62	
5.08	13.76	2.12	13.31	1.78	
12.08	18.77	2.09	24.04	3.05	

Figure.2. Dynamics of development of sciarid flies population density on poinsetia crops (Zlotów 1996)



BIOECOLOGICAL STUDIES ON SOUTH AMERICAN LEAFMINER *Liriomyza huidobrensis* (BLANCHARD) IN CRETE.

N. E. Roditakis & N.G. Golfinopoulou

NAGREF-Plant Protection Institute Heraclion Crete, 71 110 Heraclion Crete Greece

Abstract

The south american leafminer *Liriomyza huidobrensis* (Blanchard) was firstly noticed on greenhouse and field crops in 1992 in Crete. Up to now it becames the dominant leafminer species.

In 1993-1994 crop season, bioecological studies were carried out on experimental greenhouses of ca. 50 m^2 on beans cv. barbouni at Katsabas Heraclion.

The results obtained were as following:

1. *L. huidobrensis* developed four generations during Oct-April crop season.
2. Percentage of leaves bearing mines increased up to 40% while the damage of leaf surface was 5%.
3. Pupation took place mainly in leaves (98%) while on soil only 2%.
4. Natural parasitism increased up to 70% till March. The main parasitoids identified were the native ones *Diglyphus isaea* (Walker) and *Dacnusa sibirica* Telenga which relative abundance was depended on period (winter-sping).
5. Yellow trap captures were much higher on those traps hunged close to top leaves of plant at early plant growth stages.
6. Dead adults on leaves beared hyphae of non pathogenic xerophilic fungi species *Penicillium brevicompactum* and *Cladosporium cladosporioides* .

Introduction

The south american leafminer *Liriomyza huidobrensis*, a guarantine insect (Anon. 1980), has just became the dominant leafminer species as in field as in greenhouse crops in Greece, five years since its invasion in Crete and it almost substituded native species *L. bryoniae*, which was a pest of secondary importance (Roditakis, 1996). Its spread was favoured by winter outdoors hosts as lettuce, onions, faba beans, potatoes and artichokes, causing heavy damage and crop loss. Hitherto, its control was mainly based to new insecticides (98%) e.g. abamectin, cyromazine, imidachlorpid, thiocyclam - hydrogenoxalate and much less in IPM programmes. The latest years, more and more cases of failures of new insecticides were noticed, resulted an increasing interest on IPM programmes. In IPM programmes, the hymenoptera parasitoids used, *Dacnusa sibirica* Telenga (Hymenoptera: Eulophidae) and *Diglyphus isaea* (Walker) (Hymenoptera: Braconidae), controlled it more or less succesful. These parasitoids are included in the list of biological agents of *L. huidobrensis* (Grenuillet *et al.,* 1993) and noticed as native in France (Martin and Shoem, 1993) and in Holland (Linden, 1993). There are not data about the existence of these parasitoids in Greece. These species are mainly distributed in Holarctic (*D. isaea*) and Palearctic realm (*D. sibirica*) (Grenuillet *et al.,* 1993) and normally these species should be included in beneficial fauna of Crete.

This study is aiming to collect data on bioecology and population dynamic of *L. huidobrensis* in Crete, aiming to develop a strategy of pest management suitable for local conditions.

Materials and methods

The experiments were carried out in two experimental greenhouses with wood frame and plastic polyethylene cover of ca. 50 m^2 , at Plant Protection Institute Heraclion experimental field. 48 bean plants/ greenhouse cv. Barbouni were transplanted early October 1993. 100 adults of *L. huidobrensis,* coming from a laboratory colony, were released 15 days after transplanting. 45 days later when first pupae were visible on the leaves, 8 small yellow sticky traps (8 cm x 10 cm) arranged each greenhouse for monitoring. The number of adults trapped were recorded twice a week. The following observations were additionally taken at biweekly intervals from 12 sampled plants / greenhouse (1 sampled plant: 4 plants): 1) number of leaves / plant, 2) number of leaves bearing mines / plant, 3) discoloration (%) of leaf blade due to mines, 4) number of pupae / infested leaf, 5) number of pupae / m^2 of ground, 6) mortality (%) of pupae per month, 7) parasitism (%) and 8) speed of development of pupal stage.

60 random sampled infested leaves, 20 each of top, middle and lower part of plant, were put on plastic trays in the laboratory. The trays were covered with fine nylon film extended over its lips for 4-6 days and the number of pupae per case was recorded.

100 pupae collected from infested leaves were put on a glass Petri dish at 20 °C and 16h light daily in a controlled temperature and light cabinet and their mortality (%) was recorded 15 days later.

Up to 100 infested leaves were dissected under a stereomicroscope to record larval parasitism. Collected parasitoids were identified. Parasitoids *D. isaea* was identified by Dr. Stefan Vidal, Institute fur Pflanzenkrakheiten und Pflanzenschutz Universitat Hannover.

Eight plastic trays of 43 cm x 28 cm were put underneath plant canopy and the number of pupae collected was recorded four days later. 80 newly formed pupae, in four groups of 20, were put on glass tubes CAN, plugged with cotton wick lightly moistured. Then they put on controlled temperature and light cabinets at four constant temperatures 12, 16, 20 and 24 ± 0.5 °C and 16h light daily. The exits of adults were recorded daily.

Four yellow sticky traps, each one consisted one replication, were hunged at 40 cm (just over the top of new plants), 1.00m and 2.00m over the ground. The number of adults trapped were recorded 48h later. This repeated four times, in 10 days period.

Leaf blade whitening due to mines was measured by image analysis computer programme on four samples of leaves (10 leaves / sample) by SKY instrument. Temperature and relative humidity were recorded by electronic thermohygrograph RATONA.

Results

The number of generations developed during automn - winter crop season on greenhouse beans cv. Barbouni are presented in Fig.1. Data presented in Fig.1 showed that *L. huidobrensis* developed four generations (the 4th was on the pun), amongst 2nd and 3rd were very dense midwinter. A weak natural parasitism (7%) was noticed early winter, 60 days after artificial infestation, which increased up to 70% following three months (Fig. 1). The reciprocal (1/p) of natural parasitism (%) was linearly related to time past from the first notice of mines of *L. huidobrensis* (x_a) ($1/p=0.280826-1.819\ x_a$, $r=0.99$, $R^2=99.8$) on greenhouse beans in automn greenhouse conditions in Crete.

The main parasitoids identified were *D. isaea* (Walker) and *D. sibirica* Telenga. Samples of parasitoids taken during winter as from experimental field as from South Crete (Antiskari) belonged only to *D. isaea*, while samples taken early winter and late spring had a mixture of species *D. isaea* and *D. sibirica.*

Pupae were usually formed underside of leaves (98%) half inserted in the exit hole while only 2% were fallen on the ground (Table 1). The mean number of pupae per infested bean leaf was increased from 3.5 early winter to 11 late winter and the leaves bearing mines increased from 7% up to 40% (Fig. 1). The number of pupae (y_p) of *L. huidobrensis* produced on greenhouse beans was linearly related to time past from the first notice of mines of *L. huidobrensis* (x_a) (y_p =0.055679-4.18452 x_a, r=0.99, R^2=98.7).

Table 1. Mean number of pupae of 12 bean leaves cv. Barbouni and the mean number of pupae fallen on a plastic trays 43 cm x 28 cm underneath plant canopy (standard deviation in parenthesis).

Mean number of pupae on 12 bean leaves	Mean number of pupae on plastic disc
44.25 (21.71)	1.0 (0.81)
98%	2%

The mean number of pupae / leaf was varied to the height of the plant. This is more sound on older plants. Most pupae formed close to the top leaves of plant (Table 2).

Table 2. Mean number of pupae / leaf on greenhouse bean plant cv. Barbouni sampled from top (2.00 m), middle (1.00 m) and low part of plant (0.4 m) in Crete in 1994 (20 leaves / sample per case).

Date	Low	Middle	Top	Mean
28 Dec	2.3	4.1	4.1	3.5
11 Jan	1.5	2.9	6.7	3.6
27 Jan	2.1	6.7	11.5	6.7

Pupal mortality differentiated in winter months. The mortality was lower (12.5%) early winter and much higher late winter (63%) (Table 3). The speed of development of pupal stage was $1/y_p$=0.0390854+0.0252983t, (r=0.96, R^2=93.16, y=days, t=temperature °C) (Table 4). According to data presented in Table 4 the threshold temperature for pupae was 6.4 °C.

Table 3. Pupal mortality of *Liriomyza huidobrensis* during winter - spring months under greenhouse conditions in Crete - 1994 (number of pupae 100 / treatment) at constant temperature 20±0.5 °C.

Months	Pupal mortality %
January	12.5
February	31
March	63

Table 4. Speed of development ($1/y_n$) of pupal stage of *Liriomyza huidobrensis* at constant temperatures (number of pupae 80 / treatment) (standard deviation in parenthesis).

Temperature °C	Mean duration in days	Mortality %
12	15.67 (5.1) *	57.5
16	11.57 (4.2)	41.2
20	8.8 (2.8)	18.7
24	7.12 (1.3)	30.0
$1/y_n$=0.0390854+0.0252983t, r=0.96, R^2=93.16, t=temperature °C y=days		

The height of yellow sticky traps hunged affected adults capture. The yellow sticky traps hunged just over the top of new plants (40 cm height) captured much more adults than those hunged 1.00 m and 2.00 m over the ground (Table 5).

Table 5. Mean number of adults of leafminer *Liriomyza huidobrensis* (Blanchard) trapped in yellow sticky traps at various heights of young bean greenhouse plants of 30 cm height (standard deviation in parenthesis).

Height of trap (cm)	Mean number of adults
40	71.00 (13.03) *
100	12.75 (5.73) *
200	7.25 (3.77) *

ANNOVA Test, LSD (P=0.05)

Leaf blade whitening due to mines was varied from 4 to 7% during crop season. The leaf surface of bean leaflets varied from 6.000 mm^2 to 10.000 mm^2 and the mean number of leaves per bean plant varied from 280 to 425.

Late winter, we observed dead adults on leaves bearing rich whitish or brownish mycelium on the body. These fungi, isolated with PDA, tested and prooved non pathogenic. They belonged to xerophilic species *Penicillium brevicompactum* Dierckx one of the most xerophilic species of Penicillia and *Cladosporium cladosporioides* (Fresen) (IMI No 360984, 360985).

Discussion

The south american leafminer *L. huidobrensis* is known leafminer species inhabited on winter crops (potatoes, artichokes, lettuce, etc) in high altitudes at which they can survive and multiply (Lange *et al.*, 1957, Spencer, 1973, Vercambre & Crozals, 1993). This is mainly due to lower thresholds temperature of development of its life stage than other known harmfull leafmineres (Vercambre & Thierry, 1985, Parrella, 1987, Vercambre & Crozals, 1993). Pupal stage of *Liriomyza* species spend 50% at least of total development time and it is a reason of its particular importance. Temperature thresholds of development of pupal stage of known harmfull leafmineres are over 8°C. Among the factors affecting this threshold are host plants and relative humidity (30-70% optimum) (Parrella, 1987). Mcdonald & Walters (1993) found that pupal threshold temperature was 7.4 °C, lower than *L. trifolii* (Miller & Isger, 1985) at constant temperature in the laboratory. Our data showed one degree lower threshold temperature (6.4 °C) (Table 4) which should be rather dued to different methodology. We used different host plant and more precise temperatures.

During crop season 1993-1994, the mean temperature was always over the threshold temperature of development (Fig.1). These mild winter conditions in Crete favoured obviously very dense populations of *L. huidobrensis* during winter resulted high crop losses as on outdoor crops potatoes, lettuce, onions, articholes, fava beans etc. as on greenhouse crops melons, beans, cucumbers and zerberas. According to regression $y_p=(0.055679t-4.18452) \times 10^6$, concerning pupal production of *L. huidobrensis* / $1000m^2$, this leafminer can produce 4×10^6 pupal / 1000 m^2 late winter which clearly shows how favorable are the greenhouse conditions during winter in Crete.

The third larval substage of leafmineres usually leaves the mine by cutting a semi-circular exit slit in the leaf epidermis, dropped on the ground and pupated 5cm in the soil (Minkenberg & Lenteren, 1986, Parrella, 1987). Our data are in controversy with those of Minkenberg & Lenteren, (1986), Parrella, (1987). Most pupae of *L. huidobrensis* were

pupated on leaf half inserted in the exit hole (Table 1). This lead to particular care on haevily infested plants at the end of crop season. Their destroy after removal is considered necessary to avoid reinfestation of following or neighbouring crops.

Generally leafminers larvae feed on different sections of messophyll dependent on species e.g. *L. trifolii* in messophyll (Parrella *et al.*, , 1985), *L. brassicae* in the palissade and spongy messophyll (Spencer, 1973) while *L. huidobrensis* in the spongy messophyll (Parrella *et al.*, 1985). This is the reason that mines of *L. huidobrensis* are not completely visible but only through a source of light. This is also the reason that leaf blade discoloration was not propotional to density of population and it can not be an index of damage.

Liriomyza leafminers can damage crops with several ways e.g. crop yield, aesthetic value of ornamentals, destroying young seedlings etc.. The mining activity is not always proportional to crop losses (Parrella, 1987). Greenhouse tomatoes can tolerate in high levels of leaf damage without serious crop losses (Linquist, 1974). We observed a significant leaf drop of bean leaves in 3rd generation of *L. huidobrensis* late winter but we didnt' collect data on crop yield.

D. isaea and *D. sibirica* has been succesfully used for biological control *of L. bryoniae, L. trifolii* and *L. huidobrensis* as well (Minkenberg & Lenteren, 1986, Linden, 1993). We found that these parasitoids included in beneficial fauna in Crete and they are very suitable for biological control of *L. huidobrensis*. We observed that ectoparasitoid *D. isaea* was much more abundant and rather dominant parasitoid species in winter in Crete. It might be due to hyperaparasitism which noticed in some cases. or it ability to overwintered outdoor on several leafminers species on herbaceous plants (Boucek & Askew, 1968). Concerning biological control of *L. huidobrensis* in Crete, *D. isaea* should be itself effective so mixed releases of *D. isae*a and *D. sibirica* may be not necessary.

Native parasitoids could be rapidly established under greenhouse conditions late automn, causing a weak parasitism initially which could be increased up to 70% following three winter months. This period is quite long to wait for a good control of *L. huidobrensis* so a mass release of sufficient number of parasitoids is suggested just after the first appearance of mines on lower leaves in order to achieve an ecxellent control very fast.

In cases of mass trapping with yellow sticky traps, a method which used sometimes in Crete, yellow sticky traps should be hunged just over the top of new plants very early. In practice this method was not succesfull as well as on *L. bryoniae*. We observed adults *L. huidobrensis* to be more sluggish than *L. bryoniae* and it seems to be the main reason of lower effectiveness. We suggested a combination of yellow traps and soil treatment with abamectine or cyromazine at early plant growth stages which prooved very effective. In the following the absense of insecticides application permitted the establishment of natural parasitoids which contributed to a very good control in the rest of the crop season.

Acknowledgements

We are grateful to Dr. Stefan Vidal, Institute fur Pflanzenkrakheiten und Phlanzenschutz Universitat Hannover, for identification of hymenoptere parasitoids *Diglyphus isaea*.

References

ANONYMOUS, 1980. The Import and Export (Plant Health) (Great Britain) Order 1980. Statutory Instruments 1980 no. 420 HMSO, London.

BOUCEK, Z. & ASKEW, R.R., 1968. Index of palearctic Eulophidae (excl. Tetrastichinae) Paris: Le Francois.

GRENUILET, C., MARTINEZ, M. & RASPLUS, J.Y., 1993. Liste des parasitoïdes et des predateurs des *Liriomyza* d' importance économique dans le monde (DIPTERA:

Agromyzidae). "Lyriomyza" Conference on leaf mining flies in cultivated plants. 24-26 March, Montpellier: 143-156.
LANGE, W.H., GROGARICK, A.A. & CARLSON, E.C., 1957. Surpentine leafminer damage. Californian Agriculture **11**: 3-5.
LINDEN A. van der, 1993. Biological control of leafminer in glasshouse lettuce. "Lyriomyza" Conference on leaf mining flies in cultivated plants. 24-26 March, Montpellier: 157-162.
LINQUIST, R.K., 1974. Effect of leafminers larvae on yields of greenhouse tomatoes: a preliminary report. Ohio Agric. Dev. Cents Res. Summ. **73**: 25-29.
MARTIN, C. & SCHOEN, L., 1993. Observations comparées d' epidemiologie et de methodes de lutte contre les mouches mineuses *Liriomyza trifolii* Burgess et *Liriomyza huidobrensis* (Blanchard) sur cultures horticoles en Roussillon."Lyriomyza" Conference on leaf mining flies in cultivated plants. 24-26 March, Montpellier: 93-104.
McDONALD, O.C. & WALTERS, K.F.A., 1993. Overwintering potential of *Liriomyza huidobrensis* in the U.K. Populations and patterns in biology. K.F.A. Walters and W.A.C. Kidd. (Eds) Intercept Press, Andover U.K.
MILLER, G.A. & ISGER, M.B., 1985. Effects of temperature on the development of *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae). Bulletin of Entomological Research **75**: 321-328.
MINKENBERG, O.P.J.M. & LENTEREN van, J.C., 1986. The leafminers *Liriomyza bryoniae* and *L. trifolii* (Diptera: Agromyzidae), their parasites and hosts plants: a revew. Agricultural University Wageningen papers 86-2.
PARRELLA, M.P., 1987. Biology of *Liriomyza.* Ann. Rev. Entomol. **32**: 201-24.
PARRELLA, M.P., JONES, V.P., YOUNGMAN, R.R.& LEBECK, L.M., 1985. Effect of leaf mining and leaf stippling of *Liriomyza* spp. on photosynthetic rates of chrysanthemum. Ann. Entom. Soc. Am. **78**: 90-93.
RODITAKIS, N., 1996. First record of south american leafminer *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae). *Entom. Hellenica* (under publication).
SPENCER, K.A., 1973. Agromyzidae (Diptera) of economic importance. Dr. W. Junk La Hague *Series entomologica* **9**: 1-418.
VERCAMBRE, B. & A. de CROZALS, 1993. Relation entre *Liriomyza huidobrensis* et *L. trifolii* a l' Ile de la Reunion. "Lyriomyza" Conference on leaf mining flies in cultivateal plants. 24-26 March 1993, Montpellier: 71-78.
VERCAMBRE, B., & THIERRY A., 1985. Donées bio-écologiques sur le *Liriomyza trifolii* Burgess (Dipt: Agromyzidae) et de son principal parasite Hemitarsenus semi -albiclava (Hym: Eulophidae). *Acta Horticulture* **153**: 259-266.

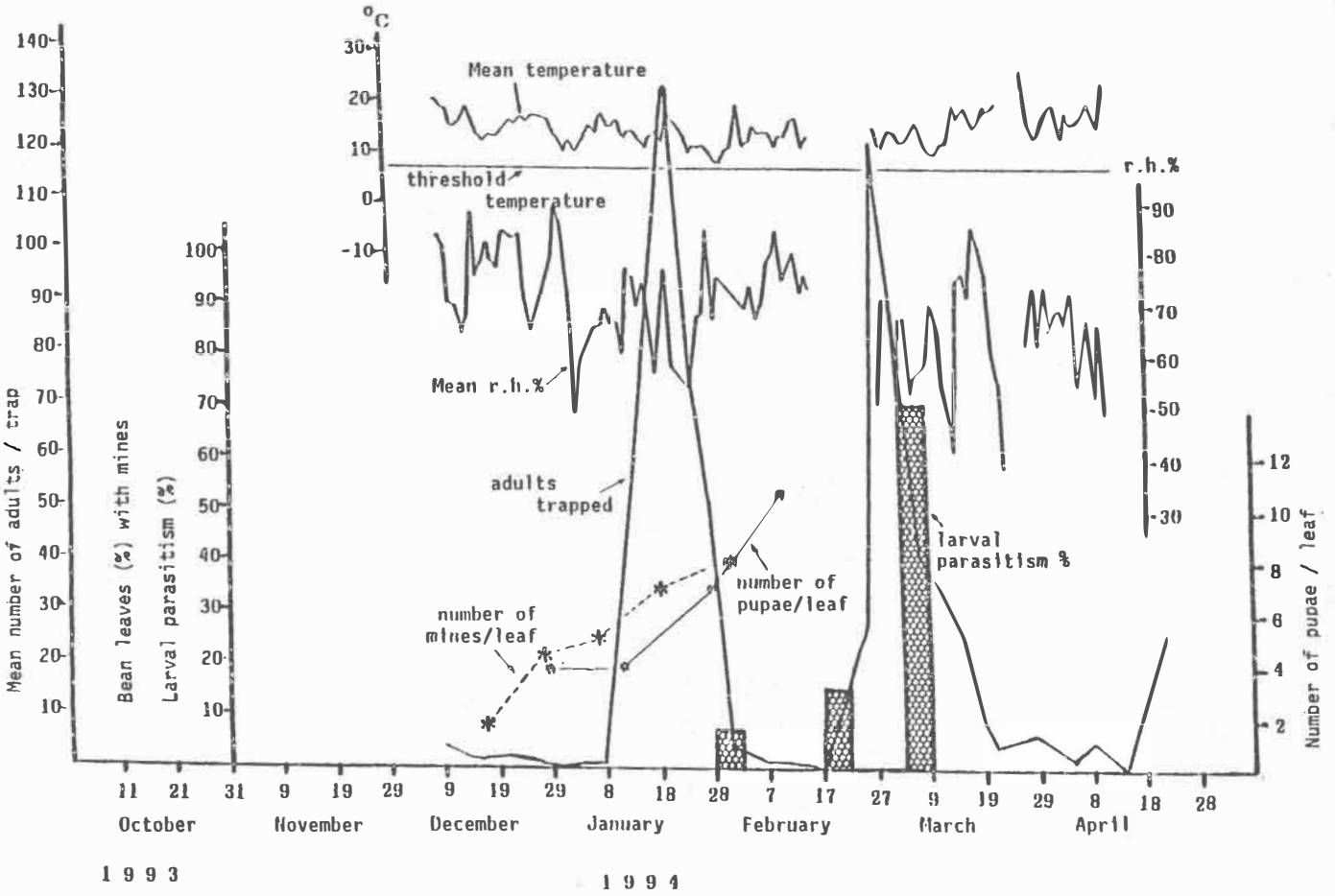


Fig.1. Seasonal flights of Liriomyza huidobrensis (Blanchard), larval parasitism (%) and number of pupae and mines/leaf on greenhouse beans cv. Barbouni, under greenhouse conditions in Crete in 1993-94 crop season.

BIOLOGICAL CONTROL OF *Liriomyza trifolii* BY *Diglyphus isaea* ON UNHEATED GREENHOUSE TOMATOES IN ADANA, TURKEY

Asuman Ulubilir[1] & Erdal Sekeroglu[2]

1 Plant Protection Institute, Adana, Turkey
2 Dept of Plant Protection, C. U. Agricultural Faculty, 01330 Adana, Turkey
e-mail seker@pamuk.cu.edu.tr

Abstract

The percentage of parasitization by *Diglyphus isaea* was studied on greenhouse tomatoes (400 m^2) by comparing parasite release plots with insecticide applied and untreated control plots. Plots were established by dividing the greenhouse into three section by use of polyethylene sheets. Release rate was 100 adults/100 m^2 and done by releasing parasites at 10 different spots (10 adults/10 m^2). For chemical control Cyromazin 75 was applied at rate of 20 g/d. The mean number of *Liriomyza trifolii* larva was 1.26, 1.05 and 1.59 per leaf in control, release and insecticide plots, respectively, before any treatment. When compared with non-chemical control plots, parasitoid was as effective as chemical control. Populations of *L. trifolii* larva increased a peak of 1.75 larva/leaf by the fourth week and stayed more or less steady between 1.0 and 1.5 larva/leaf until the end of experiment. Larval population decreased to less than 1 larva per leaf both in release and Cyromazin plots by the fourth week and stayed at very low levels.

Introduction

Although its pest status is not known exactly, *Liriomyza trifolii* Burgess showed a very rapid dispersion along the Mediterranean cost of Turkey since 1990 and rapidly rising to the major pest status of greenhouse vegetables (Anonymous 1994). One of the strategies for control of *L. trifolii* has been use of parasites as biological control agents (Allen & Charlton 1981, Price 1981, Parella 1987) and species in the genus *Diglyphus* constitute one of the important groups of parasites attacking *Liriomyza* spp. leaf miners (Minkenberg & van Lenteren 1986, Parella *et al.* 1989). Ulubilir & Yabas (1996) reported that *Diglyphus isae* was observed at varying population densities in fall and spring greenhouse vegetables along the Mediterranean cost of Turkey.

Here we report a study in which the release of *D. isae* was compared with chemical application to evaluate the feasibility of the parasite to control *L. trifolii*.

Materials and Methods

An unheated tomato greenhouse (plastic covered, 400 m^2) in Yumurtalik, Adana, Turkey was the study site. The greenhouse was sectioned into two half by using polyvinyl sheet. One side (200 m^2) was received application of cyrozamine 75 (20 g/d). Another half again was divided into two half (100 m^2 each) one of which served as control without any treatment, and other half received parasite release. In all three sections four plots were established to serve as replicates, and 25 leaves (one from each plant) were sampled randomly

on every sampling date and taken to the laboratory in brown bags. Larval counts of *L. trifolii* were done under steromicroscope. Live and death larva were counted separately. Pretreatment counts were done first on 12 November 1996 and then parasites were released on some day at a rate of 100 adults/100 m^2, releases were done at 10 different points and 10 adults were released at each point. Cyrozamine was also applied at same day. Parasite release and cyrozamine application were repeated two weeks later again.

Laboratory cultures of *D. isae* were used for release treatments which were cultured at 25 ± 2^{0}C and 80±10 % RH in an insect rearing room with *L. trifolii* as host. All rearing were under long-day conditions (photoperiod of 16:8 [L:D]).

The statistical analysis between treatments were done using ANOVA. One-way ANOVA and Duncan's Multiple Range Test (MSTAT-C) were used to separate the means for each sample dates.

Results and Discussion

The population density of *L. trifolii* larvae for each treatment is given in Table 1.

Table 1. Larval density of *L. trifolii* on tomato (Nos./leaf)*

	Sampling Dates						
Treatments	12/11	19/11	26/11	02/12	10/12	17/12	24/12
Parasite	1.05 a	1.30 a	1.38 a	0.62 b	0.23 b	0.34 b	0.40 c
Cyrozamine	1.59 a	1.26 a	1.40 a	0.27 b	0.24 b	0.26 b	0.13 b
Control	1.26 a	1.24 a	1.59 a	1.77 a	1.47 a	1.20 a	0.92 a

*Means within columns followed by the same letter are not significantly different (P>0.05).

Population density of *L. trifolii* larva was similar for all plots before the treatments and there was no statistical differences among them. Populations started to increase steadily but pulled down after the third week in cyrozamine applied and parasite release plots. The highest larval density was observed by the fifth week in control plots. The number of larva in cyrozamine applied and parasite release plots was statistically lower after the third week, but no statistical differences observed between cyrozamine and parasite plots except the seventh week on which larval density was statistically lower in cyrozamine plot than parasite release plot (Table 1).

Percent reduction in larval density of the leefminer observed to be similar in both cyrozamin applied and parasite release for the duration of the trial. The highest reduction was more evident (74.1% in parasite release) when larval populations reached to a peak in control plot. *D. isae* has been reported to be an important in several European countries, in both glasshouses and open fields as a biological control agent of agromyzid leafminers in vegetable crops (Lyon 1986, Nedstam 1987, Pena 1983 , Van Lenteren & Woets 1988). But it was reported to be only predominant during summer, causing substantial mortality of leafminers in glasshouse crops in Scandinavia and western Europe (Hendricks *et al.* 1980, Wardlow 1985, Woets and Van der Linden 1983). Minkberg (1989) reported that the reproduction of *D. isae* was not limited by the temperatures regimes specific for western European glasshouses. The location where this study was conducted possess typical Mediterranean climate with a mild winter, offering a high potential for biological control of leafminers by *D. isae*, and our results reveal that. However, it seems to early to draw definite conclusions about the effectiveness of

D. isae for controlling leafminers by just depending on the results of this trial. Since the population density of *L. trifolii* was very low even in unsprayed control plot. Further studies with varying population densities of leafminers should be carried to evaluate the effectiveness of *D. isae* against *L. trifolii* 'n Turkey.

References

ALLEN, W. W., & CHARLTON, C. A. (1981). The biology of *Diglyphus begini* and its performance in caged release on chrysanthemums, pp. 75-81. In D. J. Schuster [ed.], Proceedings, institute of Food and Agricultural Science Conference on Biology and Control of Liriomyza Leafminers, Lake Buena Vista, Fla.ANONYMOUS, (1994). T. K. B. Tarým Ýl Müdürlükleri verileri (Adana Zir. Müc. Araþt. Enst.).

HENDRICKSE, A., ZUCCHI, R., VAN LENTEREN, J. C., & WOETS, J. (1980). *Dacnusa sibirica* Telenga and *Opius pallipes* Wesmael (Hym., Braconidae) in the control of the tomato leafminer *Liriomyza bryoniae* Kalt. IOBC/WPRS Bulletin **3. (3),** 83-98.

LYON, J. P. (1986). Specific problems posed by *Liriomyza trifolii* Burgess (Diptera: Agromyzidae) and biological control of this new pest of protected crops. RAE.: **75** (8-12) Abst: 4891.

MINKENBERG, O. P. J.M. & VAN LENTEREN, J. C. (1986). The leafminers *Liriomyza bryoniae* and *L. trifolii* (Diptera: Agromyzidae), their parasites and host plants: a review. Agricultural University Wageningen Papers **86 (2),** 1-50.

MINKENBERG, O. P. J. M. (1989). Temperature effects on the life history of the eulophid wasp *Diglyphu isaea,* an ectoparasitoid of leafminers (*Liriomyza* spp.),on tomatoes. Ann. Appl. Biol. **115**:381-397.Life history of the agromyzid fly *Liriomyza bryoniae* on tomato at different temperatures.

NEDSTAM, B., STENGARD HANSEN, L. & VAN LANTEREN, J. C. (1987). Proceedings combined working groups on integrated control in glasshouses EPRS/WPRS [Eds.] Budapest, Hungary, 26-30 April 1987. IOBC/WPRS Bulletin **10**, 202 pp.

PARELLA, M. P. (1987). Biology of *Liriomyza.* Ann. Rev. Entomol. **32**: 201-204.

PARELLA, M. P., YOST, J. T., HEINZ K. M. & FERRENTINO, G. W. (1989). Mass rearing of *Diglyphus begini* (Hymenoptera: Eulophidae) for biological control of *Liriomyza trifolii* (Diptera: Agromyzidae). J. Econ. Entomol. **82**: 420-425.

PENA, M. A. (1983). *Diglyphus isae* (Wolker), una nueva especie de Eulophidae parva las Islas Canarias, con interes en el control biologico de Liriomyza spp. Xoba **4**, (1), 31-34.

PRICE, J. F. (1981). Pesticides effect on parasitoids of *Liriomyza* spp. in flower crops, pp. 197-205. In D. J. Schuster [ed.], Proceedings, institute of Food and Agricultural Science Conference on Biology and Control of Liriomyza Leafminers, Lake Buena Vista, Fla.

ULUBILIR, A. & YABAÞ, C. (1996). Akdeniz Bölgesi'nde örtüaltýnda yetiþtirilen sebzelerde görülen zararlý ve yararlý faunanýn tespiti. Türk. Entomol. Derg. **20 (3):** 217-228.

VAN LENTEREN, J. C. & WOETS, J. (1988). Biological and integrated pest control in greenhouses. Ann. Rev. Entomol. **33**: 239-269.

WARDLOW, L. R. (1985). Control of life-miners on chrysanthemums and tomatoes by parasites. In biological pest control: the glasshouse experience, pp. 129-133. Eds N. W. Hussey and N. E. A. Scopes. Poole: Blandford.

WOETS, J. & Van der Linden, A. (1983). Observations on *Opius pallipes* Wesmael (Hym.; Braconidae) as a potential candidate for biological control of the tomato leafminer *Liriomyza bryoniae* Kalt. (Dipt.; Agromyzidae) in some large greenhouses in the Netherlands. IOBC/WPRS Bulletin **6**: 134-141.

VII. Polyphagous predators
Prédateurs polyphages

BIOLOGICAL CONTROL OF GREENHOUSE CUCUMBER PESTS WITH THE MIRID BUG *DICYPHUS TAMANINII.*

C. Castañé, O. Alomar & J. Riudavets
IRTA, Centre de Cabrils, 08348-Cabrils (Barcelona), Spain.

Abstract

Our research on the use of the predatory bug *Dicyphus tamaninii* (Heteroptera, Miridae) for the biological control of greenhouse cucumber pests (greenhouse whitefly, western flower thrips and cotton aphid) is reviewed. A 3:10 ratio of *D. tamaninii* to prey gave a good control of western flower thrips and greenhouse whitefly in different greenhouse experiments and exclusion cages. An average daily predation of 46 young aphid nymphs by females and a type II functional response when feeding on the cotton aphid was found in the laboratory. This mirid bug is a good candidate in augmentative or inoculative release strategies for the control of greenhouse cucumber pests in the Mediterranean region.

Introduction

Cucumbers grown in greenhouses of our area have several pests, such as the western flower thrips *Frankliniella occidentalis*, the greenhouse whitefly *Trialeurodes vaporariorum* and the cotton aphid *Aphis gossypii*. Damage caused by these pests consists of loss of crop yield, fruit scarring in the case of the western flower thrips and development of sooty mold on the honeydew excreted by whiteflies and aphids. Fruits become unmarketable or they produce a low quality yield with the corresponding loss of income by the grower.

Integrated pest management programs in this crop are not working properly since available biological control agents show gaps in their pest control efficiency. A traditional agent for whitefly control is *Encarsia formosa*, but this aphelinid does not work well when temperatures are high and now *Eretmocerus californicus* is also released in the warmer periods of the crop. The mirid bug *Macrolophus caliginosus,* which controls whiteflies in tomato, is not commercially used in cucumbers apparently due to poor establishment. As candidates for western flower thrips control there are anthocorid bugs and phytoseiid mites. These predators are pollenophagous and work well in peppers due to the continuous production of pollen by this plant (Ramakers, 1995). But pollen is virtually absent from most of the greenhouse cucumber varieties. Anthocorids (*Orius laevigatus* and *O. majusculus*) do not establish in cucumbers unless a high density of prey is available, and by then fruits are already damaged (Chambers *et al.* 1993; Jacobson 1995). Phytoseiids, as *Amblyseius cucumeris,* are used in cucumber but they are not very efficient since they only feed on first larval stages and therefore they have to be introduced continuously and in high numbers to maintain the thrips population under control (Bennison *et al.* 1990). For this same reason, they cannot respond effectively to sudden increase in prey from outside the greenhouse, so common in our area. *A. cucumeris* is very sensitive to low humidity, which affects hatching of their eggs (van Houten & van Stratum, 1993) and this is a handicap for their establishment in greenhouses of our area in spring and summer.

In the Mediterranean region several natural enemies which may have an impact on these pests are present in outdoor cucumber crops that are not heavily sprayed with insecticides. These natural enemies will also colonize cucumber greenhouse crops if they are not heavily sprayed, although late in the season or in low numbers for effective pest control. Among them are some polyphagous heteropteran predators such as the mirid bug *Dicyphus tamaninii* (Riudavets *et al.* 1993). This predator feeds on thrips, whiteflies and aphids and lays eggs and reproduces in cucumber crops (Albajes *et al.* 1996).

In this paper we summarize our recent work on *D. tamaninii* for the control of cucumber pests in inoculative or conservative strategies of biological control.

Exclusion cage experiments

Initial trials with exclusion cages were performed and the impact of *D. tamaninii* on greenhouse whitefly and western flower thrips populations was evaluated for the first time in this crop (Gabarra *et al.* 1995). The mirid bug did establish on the crop when released as a late instar nymph, producing a new generation in 3-4 weeks if prey were available. It was distributed all over the plant, since the prey was present in most of the leaves. The 3:1 ratio of *D. tamaninii* to prey caused a consistent reduction in the whitefly population after several weeks of predator:prey interaction, and a dramatic reduction in western flower thrips population at the initial weeks of the interaction. No damage on fruits was detected due to feeding punctures of the bug after finishing feeding on the prey as has been described in tomatoes (Alomar & Albajes, 1996).

Greenhouse experiments

More trials were performed in greenhouse conditions in order to test the effectiveness of two predator:prey ratios in keeping western flower thrips populations under the Economic Injury Level (EIL) of 16 thrips/leaf. This EIL is described for avoiding fruit scarring on Dutch type cucumber varieties (Welter *et al.* 1990). When the thrips population was low (1 adult per leaf) at the beginning of the crop, a release of a 1:10 predator:prey ratio was effective in keeping *F. occidentalis* under control. But, when the thrips population was initially higher (5 adults per leaf) the release of a 3:10 ratio of *D. tamaninii* to prey was needed to avoid reaching the EIL throughout the growing season (Castañé *et al.* 1996).

D. tamaninii was also successful in reducing whitefly populations when released at a 3:10 predator:prey ratio (unpublished results). From the 6th week after transplanting onwards (3 weeks after predators were released) more than 50% reduction in the whitefly nymph and adult population was achieved when comparing with a control treatment without predatory mirids (figure 1). No sooty mold was detected in the predator treatment. Once established in the crop, the predator population was able to significantly reduce (up to 50%) a late western flower thrips infestation (from the 7th week onwards) while still maintaining whiteflies at a low level.

Prospects for aphid control

Laboratory experiments with *D. tamaninii* showed that they can complete their nymphal development when fed with young nymphs of *A. gossypii*. The average daily predation rate for *D. tamaninii* females was 46.5 young nymphs at 25°C. These females exhibited a type II functional response when preying on various densities of *A. gossypii* nymphs (Alvarado *et al.* 1997). No greenhouse test has yet been performed in order to evaluate the impact of this predator in the control of this aphid.

Conclusions

D. tamaninii is a promising candidate for biological control of greenhouse whitefly and western flower thrips on greenhouse cucumber in the Mediterranean region. When it is released as a late instar nymph, it establishes in the crop reproducing and giving rise to a new generation.. It can control greenhouse whitefly populations throughout the growing season, without the need for introducing another natural enemy. It can effectively control western flower thrips even when the thrips population is low. It is found mainly on the leaves where the majority of the thrips are located, and is not dependent on pollen as anthocorids and

phytoseiids are. No damage of the fruit has been detected. *D. tamaninii* can also respond to sudden increases of prey from outside, as frequently occurs in our region.

D. tamaninii can colonize Mediterranean greenhouses from mid April onwards, because greenhouses are open for ventilation during the day. However, their numbers are not enough for adequate pest control and augmentative or complementary releases should be done.

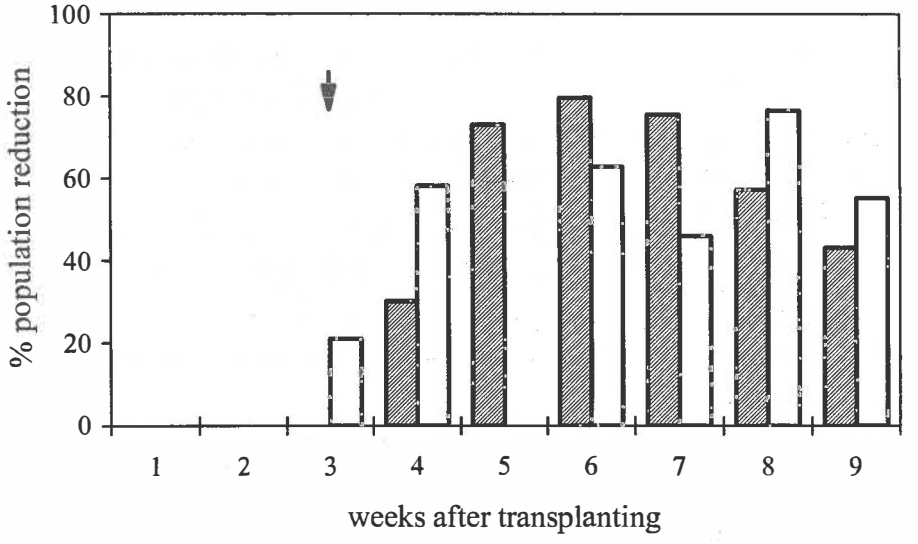


Figure 1. Percentage of population reduction of greenhouse whitefly nymphs (□) and adults (▩) when comparing a 3:10 ratio of *D. tamaninii:T. vaporariorum* with a control treatment without predators. ⇩ release of predators.

Acknowledgments

Research in this paper was funded by the Spanish Research Agencies CICYT (project AG 89-159) and INIA (project SC 95-052), and by the European Union (project CAMAR 90-0026). We grateful acknowledge Dr. Ramon Albajes for his suggestions.

References cited

ALBAJES, R., ALOMAR, O., RIUDAVETS, J., CASTAÑË, C., ARNÓ, J. & GABARRA, R. 1996. The mirid bug *Dicyphus tamaninii* : an effective predator for vegetable crops. Bull. IOBC/WPRS **19(1)**: 1-4.

ALOMAR, O. & ALBAJES, R. 1996. Greenhouse whitefly (Homoptera: Aleyrodidae) predation and tomato fruit injury by the zoophytophagous predator *Dicyphus tamaninii* (Heteroptera: Miridae). *In*: Zoophytophagous Heteroptera: Implications for Life History and Integrated Pest Management (O. Alomar & R. Wiedenmann, eds.). Thomas Say Publications in Entomology: Proceedings. Entomological Society of America, 155-177.

ALVARADO, P., BALTÀ, O. & ALOMAR, O. 1997. Efficiency of four heteroptera as predators of *Aphis gossypii* Glover and *Macrosiphum euphorbiae* (Thomas) (Hom.: Aphididae). Entomophaga, **42 (1/2),** 217-228.

BENNISON, J. A., HOCKLAND, S. & JACOBSON, R. 1990. Recent developments in integrated control of thrips on cucumbers in the United Kingdom. Bull. IOBC/WPRS **XIII/5,** 19-26.

CHAMBERS, R. J., LONG, S. & HEYLER N. L. 1993. Effectiveness of *Orius laevigatus* (Hem. Anthocoridae) for the control of *Frankliniella occidentalis* on cucumber and pepper in the UK. Biocontrol Science and Technology, **3,** 295-307.

JACOBSON, R. J. 1995. Egg laying sites of *Orius majusculus*, a thrips predator on cucumber, pp. 241-244. *In:* B.L. Parker *et al.* (eds.), Thrips biology and Management, Plenum Press, New York.

CASTAÑÉ, C., ALOMAR, O. & RIUDAVETS, J. 1996. Management of western flower thrips on cucumber with *Dicyphus tamaninii* (Heteroptera: Miridae). Biological Control, **7,** 114-120.

GABARRA, R., CASTAÑÉ, C. & ALBAJES, R. 1995. The mirid bug *Dicyphus tamaninii* as a greenhouse whitefly and western flower thrips predator on cucumber. Biocontrol Sci. Technol., **5,** 475-488.

HOUTEN, Y. M. VAN & STRATUM P. VAN 1993. Biological control of western flower thrips in greenhouse sweet pepper using non-diapausing predatory mites. Bull. IOBC/WPRS **16(2)**: 77-80.

RIUDAVETS, J., GABARRA, R. & CASTAÑÉ, C. 1993. *Frankliniella occidentalis* predation by native natural enemies. Bull. IOBC/WPRS **16(2)**: 137-140.

WELTER, S. C., ROSENHEIM, J. A., JOHNSON, M. W., MAU, R. F. L. & GUSUKUMA-MINUTO, L. R. 1990. Effects of *Thrips palmi* and western flower thrips (Thysanoptera: Thripidae) on the yield, growth and carbon allocation pattern in cucumbers. J. Econ. Entomol. **83(5),** 2092-2101.

RATE OF DEVELOPMENT AND MORTALITY OF NYMPHAL STAGES OF THE PREDATOR *Macrolophus pygmaeus* RAMBUR FEEDING ON VARIOUS PREYS AND HOST PLANTS

D. CH. Perdikis & D. P. Lykouressis

Laboratory of Agricultural Zoology and Entomology,
Agricultural University of Athens, 118 55 Athens, Greece

Abstract

The rate of development and mortality of the predator *Macrolophus pygmaeus* Rambur was studied on various host plants with and without prey. The host plants were eggplant, cucumber, melon and broad beans. On the first three host plants, the previous mentioned parameters of *M. pygmaeus* were studied in the presence and absence of prey, *Aphis gossypii* Glover, whereas on broad beans in the presence and absence of prey, *Aphis fabae* Scopoli. Experiments were carried out in a growth cabinet at 25^{0}C, 65% R.H. and 16:8 hours L:D. In each experiment, 40 plastic petri dishes, bearing a leaf of the respective host plant, were used. Only in the half of the petri dishes aphid prey was available to the predator. The results showed that on all host plants tested, the period of development of *M. pygmaeus* was shorter when prey was available than in cases it was not. When prey was provided, the shortest period of development of *M. pygmaeus* was found on eggplant followed by cucumber broad beans and melon. In the absence of prey, the developmental period of the predator on eggplant and cucumber was similar and this was also the case for melon and broad beans. However, the period of development on melon and broad beans was much longer than that on eggplant and cucumber. Large differences in the total mortality of nymphal stages of the predator were found on the various host plants. Mortality on eggplant either with or without prey and on broad beans with prey was low, whilst the highest mortality was observed on melon without prey. In the presence of prey, mortality of the predator was higher at the first and second than at the third, fourth and fifth nymphal stages on cucumber and melon. Among the nymphal stages on the various hosts, with and without prey, the fifth stage was the longer one. In conclusion, eggplant and cucumber were found as the most suitable host plants for *M. pygmaeus* as far as its developmental period is concerned whereas mortality of nymphal stages was the lowest on eggplant.

Introduction

Species of the genus *Macrolophus* (Hemiptera : Miridae) are thought as effective biological control agents, occurring either outdoors or in glasshouses. The most well known species in that genus are *Macrolophus caliginosus* Wagner and *Macrolophus pygmaeus* Rambur. These two species are very similar to each other so they are considered by some authors that they consist a complex species (Alomar *et al.*, 1994; Goula & Alomar 1994). These predatory bugs feed on major insect pests as species of whiteflies and aphids. Also, they feed on plant juices but in tomato they don't cause damage to the fruit (Malausa, 1987).

M. caliginosus has been proved as very effective when it was used for the control of whitefly on tomato (Roditakis and Legakis, 1989; Sampson and King, 1996). *M. caliginosus* has been recorded on cucumber, french bean and eggplant (Alomar *et al.* 1990; Goula & Alomar, 1994). The main biological parameters of *M. caliginosus* on some host plants and preys in laboratory conditions were studied by Fauvel *et al.* (1987) who found *Trialeurodes vaporariorum* Westwood, *Myzus persicae* Sulzer and *Aulacorthum circumflexum* Buckton as the most suitable preys among others for nymphal development of the predator.
Large numbers of nymphs and adults of *M. pygmaeus* have been recorded on tomato plants in central Greece towards the end of the growing season (Perdikis & Lykouressis, 1996; Lykouressis, Perdikis & Chalkia unpubl. data). In both studies, this predator was the most important natural enemy for suppressing population of aphids on processing and fresh market tomato. *M. pygmaeus* lays its eggs into plant tissues and undergoes five nymphal stages till adulthood (Perdikis & Lykouressis, 1995).
In this work the period of development of the nymphal stages and mortality of *M. pygmaeus* was investigated on various host plants, in the presence and absence of prey, in an attempt of ranking for their suitabilily on the predator survival and development.

Materials and methods

Adults of M. pygmaeus collected from processing tomato, in the area of Boiotia during September 1994, were reared continously in laboratory on eggplant (cv. Bonica) infested with M. persicae. Newly emerged first instar nymphs of M. pygmaeus were always taken from the laboratory cultures to be used in the conducted experiments. Those nymphs were retained in Petri dishes. Petri dishes were 9cm in diameter bearing at their top cover a 3cm diameter ventilation hole covered by muslin. Their bottom was covered by a layer of cotton wool moistened by water. Leaves of the host plants tested, with or without aphids, were individually placed upside-down on the cotton wool.
The host plants were eggplant (cv. Bonica), cucumber (cv. Brunex), melon (cv. Galia) and broad beans. *Aphis gossypii* Glover was offered to the predator as prey on leaves of the first three host plants, as stated above, whilst on broad beans the aphid was *Aphis fabae* Scopoli. In most of the plants tested, 40 nymphs of the predator were used for each host plant; a nymph was put separately on the leaf in each Petri dish. Prey was available, as stated above, only in half of the Petri dishes. Prey was consisted of approximately 100 individuals of different instars and adults of the aphid species used, on the leaf in each Petri dish. Those individuals were taken from laboratory cultures on the respective host plants.
Petri dishes, in all experiments, were maintained in a growth cabinet at 25^0C, 65% RH and 16:8 hours L:D. In all treatments the leaf in each Petri dish was renewed every day and the nymphal stage of the predator and the mortality were, also, recorded. Data were analyzed using t-test and one-way analysis of variance (ANOVA) in which means were separated (P=0.05) using Student-Newman-Keuls test.

Results

The total developmental period of the nymphal stages of the predator was found, in all host plants tested, much shorter when aphids were provided as prey than in the cases in which they were not (Figure 1). Significant differences were found in the total period of development of

M. pygmaeus between the two treatments, presence and absence of prey, in all host plants tested (Table 1).

Significant differences in the nymphal developmental period, in the presence of prey, were found among all host plants tested ($F_{3,48}$=10.67, P<0.001). The shortest nymphal developmental period of *M. pygmaeus* was recorded on eggplant with *A. gossypii* which was slightly shorter, but not significant, than that recorded on cucumber with *A. gossypii.* A longer period of development was found on broad beans with *A. fabae* and it differed significantly from the longest one which was observed on melon with *A. gossypii.* Also, both were significantly different than those found on eggplant and cucumber (Table 2).

Significant differences in the nymphal developmental period, in the absence of prey, were found among some of the host plants tested. ($F_{3,45}$=53.11 P<0.001). The shortest period of nymphal development of the predator was observed on cucumber followed by that on eggplant but no significant difference was found between them. Longer developmental periods, without significant difference, were recorded on melon and broad beans, approximately one and a half time of those on eggplant and cucumber. However, both significantly differed from those on eggplant and cucumber (Table 2).

The mean period of each of the five nymphal stages of the predator on all treatments are presented in Table 3. Longer periods in all nymphal stages were found in the absence than in the presence of prey on all host plants studied (Figure 3). Among the five stages, the fifth was found as the longest one either when aphids were present or were absent.

The mortality of the predator on the various host plants, in the presence and absence of prey, was found as having great variability. The lowest mortality was recorded on eggplant either with or without prey, on cucumber without prey and on broad beans with prey (Figure 2). The highest mortality was observed on melon in the presence and absence of prey and particularly in the absence of prey. A moderate rate of mortality was found on cucumber in the presence of prey and on broad beans in the absence of prey.

In the cases of cucumber and melon, in the presence of prey, the high mortality was largely due to the death of the nymphs of the first and second stage. In particular, from the total number of the nymphs died, 85% and 75% were of the first and second nymphal stages on cucumber and melon, respectively. On melon, in the absence of prey, mortality was higher at the later nymphal stages (65%).

Most of the individuals of the first two nymphal stages which died, in the presence of aphids, were found with a quantity of sticky material on their tarsi. In some cases their legs had been stuck to each other or the antennae stuck on the dorsal surface of their body.

Discussion

The period of development of the nymphs of the predator on all host plants were found always shorter when prey was available. That means that aphids seem to play a favour nutritional role which accelerate the rate of nymphal development of the predator.

It was already known (Malausa, 1987) that generally *M. caliginosus* can feed on plant juices. However, in this study it was found that nymphs of *M. pygmaeus* reached adulthood on all host plants tested without prey, suggesting that this predator, also, can feed on plant juices and complete its development. Higher rates of completion of development were found on eggplant and cucumber than on melon and broad beans.

The most suitable host plant for nymphal development, either in the presence or absence of prey, was found to be eggplant followed by cucumber, broad beans and melon. In a similar

study, the developmental period of nymphs of *M. caliginosus* on melon leaves, infested by *A. gossypii* at 22^{0}C, was also found to be longer than on the most of the other host plants and preys tested (Fauvel *et al.*, 1987).

Roditakis and Legakis (1989) reffered that *M. caliginosus* was very effective in controlling aphids and whiteflies on melon. However, in this study high mortality rates of nymphs were observed on melon when prey was available whilst the same, but in a smaller scale, was observed on cucumber and this phenomenon needs further investigation.

Fauvel *et al.* (1987) referred that nymphs of *M. caliginosus* died very soon when they had to feed on tomato leaves infested by *A. fabae*. In this work, *M. pygmaeus* nymphs completed successfully their development on broad beans and in particular in the presence of *A. fabae*. This supports that *M. pygmaeus* is a polyphagous predator as it has been suggested by Alomar *et al.* 1994.

In conclusion, among the various host plants and preys tested concerning their suitability for nymphal development and survival of *M. pygmaeus*, considerable differences were found. Of those host plants, eggplant and cucumber were more suitable than the others for nymphal development, whereas eggplant was the best for the survival of the nymphal stages of the predator.

References

ALOMAR, O., CASTAÑÉ, C., GABARRA, R. & ALBAJES, R. 1990. Mirid bugs-Another strategy for IPM on mediterranean vegetable crops? *IOBC/WPRS Bulletin* **13(5) : 6-9.**

ALOMAR, O., GOULA, M. & ALBAJES, R. 1994. Mirid bugs for biological control : identification, survey in non-cultivated winter plants and colonization of tomato fields. *IOBC/WPRS Bulletin* **17(5) : 217-223.**

FAUVEL, G., MALAUSA, J.C. & KASPAR, B. 1987. Etude en laboratoire des principales characteristiques biologiques de *Macrolophus caliginosus* (Heteroptera : Miridae). *Entomophaga*, **32(5): 529-543.**

GOULA, M. & ALOMAR, O. 1994. Mirids (Heteroptera : Miridae) of interest in Integrated Pest Management on tomato crops. A guide to their recognition. *Boletin de Sanidad Vegetal Plagas* **20(1) : 131-143.**

MALAUSA, J.C., DRESCHER, J. & FRACO, E. 1987. Prospectives for the use of predacious bug *Macrolophus caliginosus* Wagner, (Heteroptera : Miridae) on glasshouse crops. *IOBC/WPRS Bulletin* **10(2) : 106-107**.

PERDIKIS, D. & LYKOURESSIS, D. 1995. Immature stages of the predator *Macrolophus pygmaeus* Rambur (Hemiptera : Miridae). Proceedings 6th National Entomological Meeting, Chania, 31 October - 3 November 1995, Crete.

PERDIKIS, D. & LYKOURESSIS, D. 1996. Aphid populations and their natural enemies on fresh market tomatoes in central Greece. *IOBC/WPRS Bulletin* **19(11) : 33-37.**

RODITAKIS, N.E. & LEGAKIS, A. 1989. *Macrolophus caliginosus* (Hemiptera : Miridae). An indigen predacious bug and perspectives for its use in an integrated pest management programme in greenhouses. 2nd International Symposium on Protected Cultivation of Vegetables in Mild Winter Climates. Iraklion, 30 October - 3 November 1989, Crete.

SAMPSON, A.C. & KING, V.J. 1996. *Macrolophus caliginosus*, field establishment and pest control effect in protected tomatoes. *IOBC/WPRS Bulletin* **19(1) : 143-146.**

Table 1. Significant differences in the total period of development of the nymphal stages of Macrolophus pygmaeus in days (X±S.E.) between the two treatments, presence and absence of prey, on each host plant.

Host plant	with prey	without prey	t	d.f.	P
Eggplant	17.11±0.19	21.05±0.80	4.556	32	<0.001
	(17)	(17)			
Cucumber	17.69±0.37	20.80±0.24	4.731	29	<0.001
	(13)	(18)			
Melon	21.12±0.97	31.66±1.16	8.481	9	<0.001
	(8)	(3)			
Broad beans	19.14±0.19	31.08±0.89	11.06	24	<0.001
	(14)	(12)			

( ) : number of replicates in each case

Table 2. Significant differences (P=0.05) in the total period of development of the nymphal stages of Macrolophus pygmaeus, in the presence and absence of prey, among various host plants.

Host plant	with prey	without prey
Eggplant	17.11±0.19 a	21.05±0.80 a
Cucumber	17.69±0.37 a	20.80±0.24 a
Melon	21.12±0.97 b	31.66±1.16 b
Broad beans	19.14±0.19 c	31.08±0.89 b

( ) : number of replicates in each case

Table 3. Period of development (X±S.E.) in days of the nymphal stages of Macrolophus pygmaeus on various host plants in the presence and absence of aphid prey.

			Nymphal stages				
Host plant	Prey	n	L1	L2	L3	L4	L5
Eggplant	*Aphis gossypii*	20	3.72±0.18	3.00±0.08	2.29±0.12	3.00±0.15	5.23±0.14
			(18)	(17)	(17)	(17)	(17)
Eggplant	-	20	3.78±0.18	3.05±0.09	3.10±0.21	4.36±0.34	6.82±0.44
			(20)	(20)	(17)	(17)	(17)
Cucumber	*Aphis gossypii*	20	3.70±0.17	2.85±0.20	3.21±0.12	2.50±0.13	5.38±0.14
			(17)	(14)	(14)	(13)	(13)
Cucumber	-	21	4.52±0.20	3.52±0.16	2.95±0.12	3.47±0.16	6.83±0.17
			(21)	(21)	(21)	(19)	(18)
Melon	*Aphis gossypii*	20	3.94±0.26	3.10±0.18	4.00±0.73	4.20±0.39	6.26±0.18
			(17)	(11)	(10)	(10)	(8)
Melon	-	20	5.59±0.43	3.87±0.21	4.28±0.48	5.25±0.75	8.66±1.45
			(17)	(15)	(7)	(4)	(3)
Broad beans	*Aphis fabae*	17	4.20±0.32	2.71±0.24	2.66±0.24	3.59±0.21	5.30±0.24
			(15)	(14)	(14)	(14)	(14)
Broad beans	-	20	4.88±0.26	4.41±0.27	6.54±0.73	7.23±0.42	7.50±0.51
			(18)	(18)	(15)	(14)	(12)

n : initial number of replicates, ( ) : number of replicates in each stage, - : absence of prey

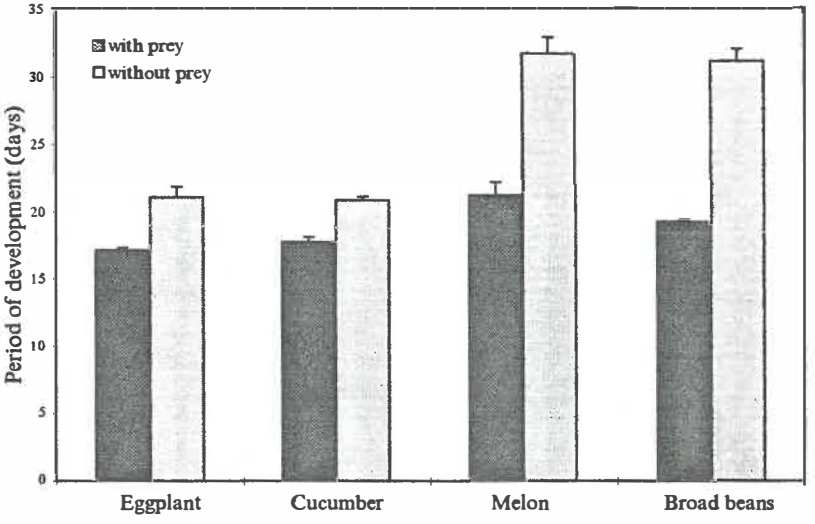


Figure 1. Total nymphal period of development in days (X±S.E.) of *Macrolophus pygmaeus* on various host plants in the presence and absence of aphid prey.

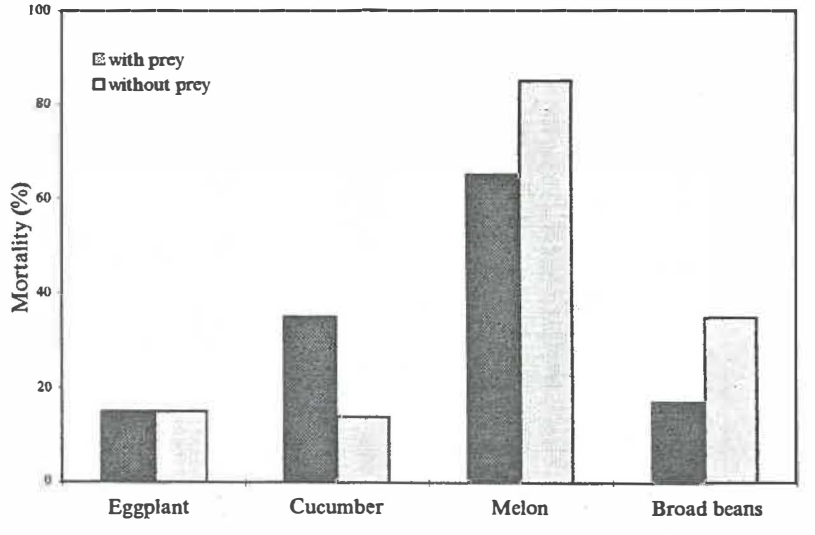


Figure 2. Total mortality of nymphal stages of *Macrolophus pygmaeus* on various host plants in the presence and absence of aphid prey.

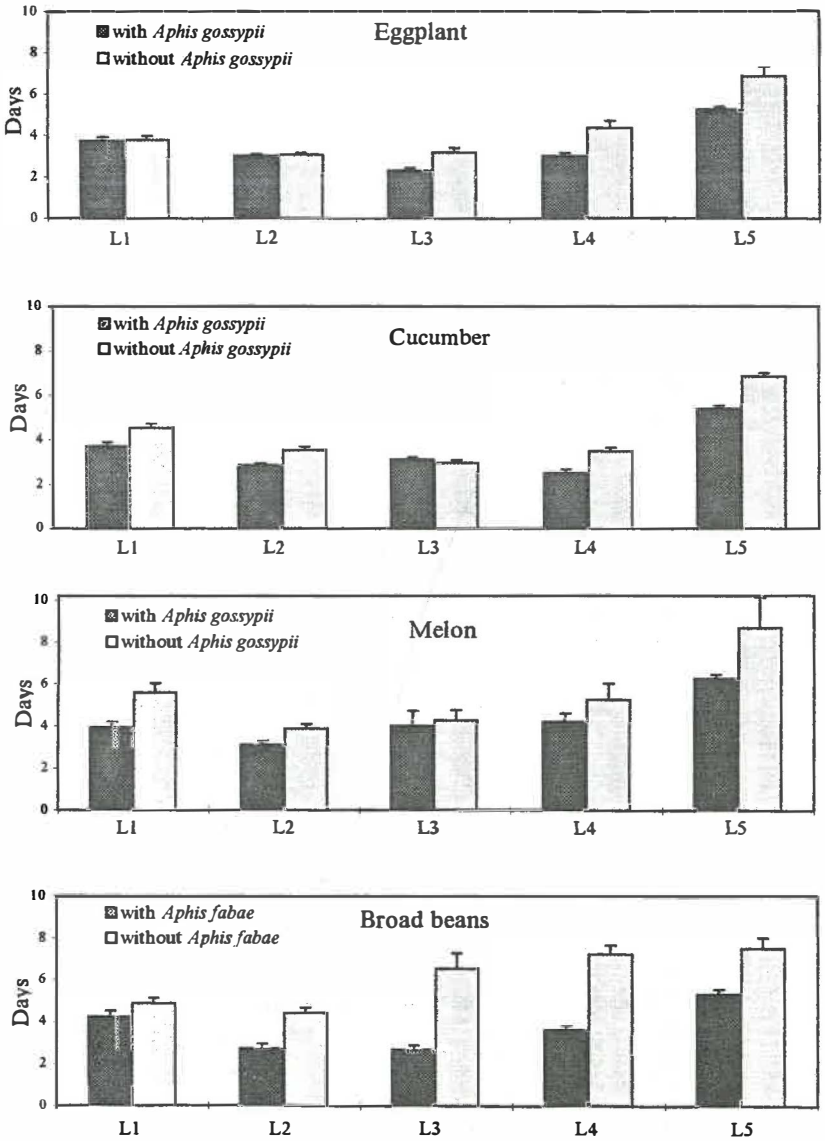


Figure 3. Period of development in days (X±S.E.) of *Macrolophus pygmaeus* nymphs on various host plants in the presence and absence of aphid prey.

SAMPLINGS OF MIRIDAE DICYPHINAE IN TOMATO CROPS OF NORTHWESTERN ITALY(*)

L. Tavella, A. Alma, C. Sargiotto
Di.Va.P.R.A. - Entomologia e Zoologia applicate all'Ambiente "Carlo Vidano"
via Leonardo da Vinci 44 - 10095 Grugliasco (TO) - Italy

Abstract - Since the reduced use of pesticides let the predators Miridae Dicyphinae colonize the IPM tomato crops, *Trialeurodes vaporariorum* ceased to cause severe damage to this vegetable in the plain of Albenga (province of Savona, northwestern Italy). Therefore, in 1996 investigations were carried out in IPM tomato crops to study the colonization and the seasonal abundance of these predators. In this area tomatoes are usually cultivated in greenhouses from winter to early summer, and in the open air in two different periods, from early May to September and from July to November. Fortnightly samplings were conducted in 11 plots (5 greenhouses, 3 spring-summer fields, 3 summer-autumn fields) from late May to September. Mirid populations were monitored beating three branches of five plants in each plot and using five yellow sticky traps localized near the checked plants. Three species were found: *Macrolophus melanotoma* (= *M. caliginosus*), *Nesidiocoris tenuis*, *Dicyphus errans*. *M. melanotoma* was the most abundant mirid reaching 75% of the specimens counted both on plants and traps; it was always present in the investigated crops. *N. tenuis* was sampled mainly in the summer-autumn fields starting from July. *D. errans* was collected in a smaller quantity than *M. melanotoma* but all over the season. Captures of mirid adults with traps in greenhouses were compared to those made in fields at the same dates without pointing out any significative difference. Inter- and intra-plant spatial distributions of mirids were analysed with statistical procedures.

Introduction

In the '80s integrated pest management (IPM) spread in tomato cultivations of northwestern Italy. The releases of auxiliaries and the consequent reduction of chemical treatments allowed the colonization of the crops by native natural enemies able to control infestations of *Trialeurodes vaporariorum* Westwood. Three species belonging to Miridae Dicyphinae were found in the investigated area: *Macrolophus caliginosus* Wagner, *Nesidiocoris tenuis* (Reuter), *Dicyphus errans* (Wolff), all characterized by a zoophytophagous behaviour (Arzone *et al.*, 1990). In the same period the presence of Miridae Dicyphinae was observed also in IPM vegetable crops of other areas of South Europe: northeastern Spain (Salamero *et al.*, 1987), southern France (Malausa, 1989), Sicily (Calabrò & Nucifora, 1993a) and Sardinia (Delrio *et al.*, 1991).

Since then, investigations have been conducted on these species to study their biology and feeding behaviour (Fauvel *et al.*, 1987; Tavella & Arzone, 1996), and to assess their efficiency in controlling pests without damaging crops (Gabarra *et al.*, 1988; Calabrò & Nucifora, 1993b). The genus *Macrolophus* has been studied also by a systematic point of view. After the taxonomic revision of Josifov (1992), this genus includes five palaearctic species, among which *M. caliginosus* and *M. pygmaeus* Rambur are still under study and at the moment considered as a complex because very difficult to separate (Goula & Alomar, 1994). Furthermore, a new synonymy was established recently by Carapezza (1995): *M. caliginosus* has to be considered a junior synonym of *M. melanotoma* (Costa) (valid name).

In 1996, six years after the first report on the predatory activity of the three mirids against whiteflies in IPM tomato crops of northwestern Italy, research was carried out in the same area

(*) Research supported by a grant of Italian M.U.R.S.T. 60%.

to check the presence of the predators and to investigate their seasonal abundance both in greenhouses and in the open air. Since the taxonomy of the genus *Macrolophus* has not been completely clarified, the specimens of this group collected in the investigated crops will be indicated in the present paper as *M. melanotoma* (= *M. caliginosus*).

Materials and methods

Plots. The research was conducted in tomato crops of the plain of Albenga (province of Savona, Liguria) during 1996. In this area tomatoes are usually grown in greenhouses from winter to early summer, and in the open air in two different periods, from early May to September and from July to November. In order to study the colonization and the seasonal abundance of the mirids, 11 IPM tomato plots were chosen: 5 greenhouses (**A**, **B**, **C**, **D**, **E**), 3 spring-summer fields (**F**, **G**, **H**), and 3 summer-autumn fields (**I**, **J**, **K**). Characteristics of the selected plots are given in table 1.

Table 1- Characteristics of the investigated tomato plots.

Plot	condition	surface (m^2)	plant (no.)	cultivar	planting date	displanting date
A	greenhouse	1,000	2,500	Marinda	28.12.95	10.07.96
B	greenhouse	1,150	2,875	Marinda	20.01.96	10.07.96
C	greenhouse	700	1,750	Arletta	12.03.96	31.07.96
D	greenhouse	900	2,250	Arletta	25.01.96	31.07.96
E	greenhouse	1,600	4,000	Arletta	05.03.96	12.09.96
F	open air	1,500	3,750	Arletta	05.05.96	12.08.96
G	open air	1,000	2,500	Arletta	09.05.96	15.09.96
H	open air	800	2,000	Arletta	08.05.96	15.09.96
I	open air	1,500	3,750	Lorybel	01.07.96	31.10.96
J	open air	900	2,250	Arletta	10.07.96	15.11.96
K	open air	1,500	3,750	Arletta	28.07.96	10.11.96

Samplings. Mirid populations were monitored by means of plant-tapping and yellow sticky traps. Plant-tapping consisted in beating three branches (top, middle, bottom) of five plants arranged along a diagonal in each plot. Every branch was tapped three times by hand over a white plexiglas sheet (30 × 35 cm). The mirids were collected using a pooter, placed in labelled glass tubes, and brought to the laboratory. Five yellow sticky traps of 12.5 × 20 cm (Kollant, Padova, Italy) were located near the sampled plants. They were hung vertically above the vegetation and replaced every two weeks. Surveys were carried out fortnightly from mid-May to late-September. Numbers and dates of the first and the last samplings for each plot are shown in table 2.

In the laboratory, the collected mirids were identified following the descriptions by Goula & Alomar (1994), and counted. In order to identify the species with certainty, nymphs of first and second instars sampled on plants were reared separately in glass tubes with French bean pods and *Ephestia kühniella* Zeller eggs so to reach the following instars. Traps were stored in a congelator at -20°C and then examined for mirids using a binocular microscope.

Data analysis. The number of adults captured with traps was correlated against the number of adults collected on the corresponding plant for each date and plot using Spearman correlation. In the dates, in which at least 3 greenhouses and 3 fields were surveyed, the mean number of adults captured with the five traps in each plot was compared with one-way ANOVA to test the capability of mirids to colonize protected and outdoor crops. Kruskall

Wallis analyses were performed on the mean number of mirids collected for each date in order to verify differences in the inter-plant (plant 1, 2, 3, 4, 5) and intra-plant (top, middle, bottom) spatial distributions. In case the differences were significative, a multiple comparison procedure (Student-Newman-Keuls test) and a linear regression were applied.

Table 2 - Numbers and dates of the first and the last samplings for each plot.

Plot	plant-tapping			trap exposition		
	no.	from	to	no.	from	to
A	3	27.05.96	24.06.96	4	13.05.96	08.07.96
B	3	27.05.96	24.06.96	4	13.05.96	08.07.96
C	5	27.05.96	29.07.96	5	13.05.96	29.07.96
D	5	27.05.96	29.07.96	5	13.05.96	29.07.96
E	7	27.05.96	21.08.96	8	13.05.96	12.09.96
F	5	11.06.96	06.08.96	5	27.05.96	06.08.96
G	6	11.06.96	21.08.96	7	27.05.96	12.09.96
H	5	24.06.96	21.08.96	6	11.06.96	12.09.96
I	5	29.07.96	23.09.96	5	08.07.96	23.09.96
J	4	06.08.96	23.09.96	4	29.07.96	23.09.96
K	3	21.08.96	23.09.96	3	06.08.96	23.09.96

Results

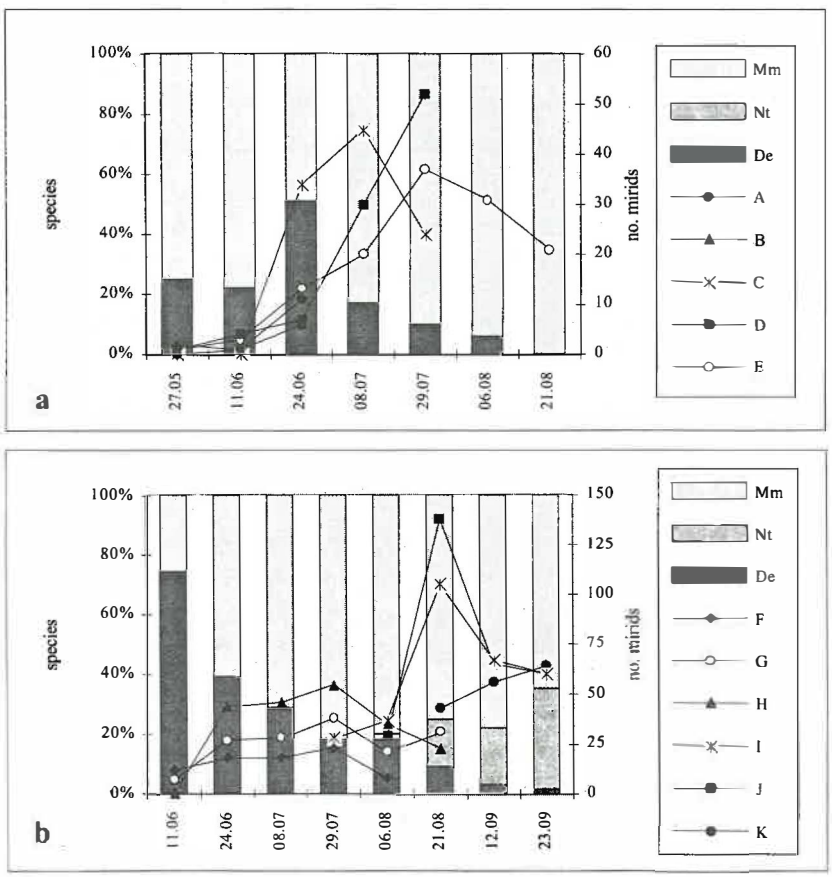
The proportion among the species and the number of mirids sampled respectively with plant-tapping and traps in each plot are shown in figures 1 and 2. Three species of Miridae Dicyphinae were collected: *M. melanotoma*, *N. tenuis*, *D. errans*. The proportion among them varied throughout the growing season. Except for the sampling of 11th June, in which *D. errans* was predominant, *M. melanotoma* was always the most abundant species, present with adults and nymphs starting from the end of May. *D. errans* was captured in a smaller quantity than *M. melanotoma* but all over the season. Its populations tended to decrease since August. The first *N. tenuis* specimens were collected on 8th June with 1 and 5 adults on plants and on traps respectively. This mirid was sampled mainly in the summer-autumn fields, where it reached the highest population density in September. The percentages of the species monitored with plant-tapping and traps in each plot are given in table 3.

Table 3 - Percentages of the species monitored with plant-tapping and traps in each plot.

Plot	plant-tapping				traps			
	total (no.)	Mm (%)	Nt (%)	De (%)	total (no.)	Mm (%)	Nt (%)	De (%)
A	12	50.0	0.0	50.0	116	36.2	0.0	63.8
B	9	66.7	0.0	33.3	158	78.5	0.0	21.5
C	103	74.8	0.0	25.2	787	71.7	0.0	28.3
D	94	87.2	0.0	12.8	625	89.6	0.2	10.2
E	126	83.3	0.0	16.7	1,300	75.8	0.0	24.2
F	79	48.1	0.0	51.9	514	37.2	0.0	62.8
G	152	59.2	0.0	40.8	1,410	52.2	0.1	47.7
H	203	90.6	0.5	8.9	1,607	86.7	0.2	13.1
I	297	63.6	25.9	10.5	2,144	66.9	26.7	6.4
J	292	92.1	4.5	3.4	1,824	93.4	3.8	2.8
K	163	58.9	41.1	0.0	1,253	82.6	16.6	0.8

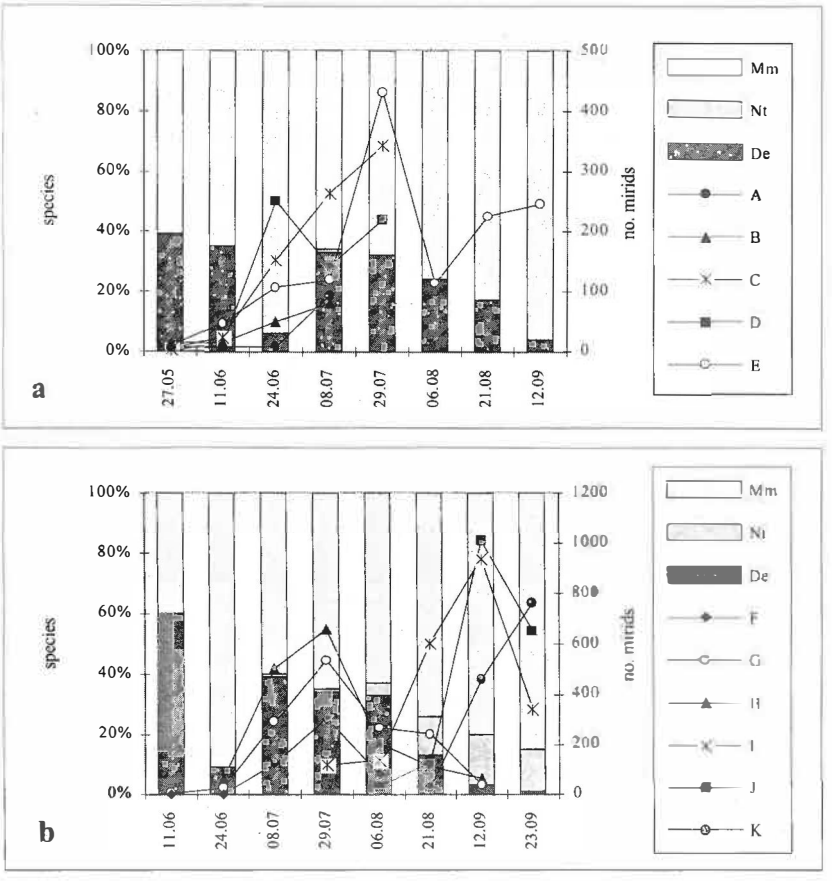
Mm: *Macrolophus melanotoma* (Costa); Nt: *Nesidiocoris tenuis* (Reuter); De: *Dicyphus errans* (Wolff)

The mirid populations tended to increase during the season: the highest number of specimens was captured with both sampling methods in September. However, towards the end of the cultivation the population dynamics showed a decreasing trend in most plots, both in greenhouses and in the open air. In every sampling date, mirid adults and nymphs were collected on plants; moreover, except for 27th May, nymphs were always more numerous than adults. Out of 1,530 specimens monitored with plant-tapping, nymphs and adults were 71% and 29% respectively; in particular the nymphs/adults ratio was 2.1 for *M. melanotoma*, 2.7 for *N. tenuis*, and 5.4 for *D. errans*. On the contrary, traps captured mainly adults: in fact, out of 11,738 specimens counted on traps, nymphs and adults were 5.3% and 94.7% respectively. The nymphs/adults ratio varied from 0.02 for *N. tenuis* to 0.2 for *D. errans*.



Mm: *Macrolophus melanotoma* (Costa); Nt: *Nesidiocoris tenuis* (Reuter); De: *Dicyphus errans* (Wolff)

Figure 1 - Population dynamics of mirids sampled with plant-tapping in greenhouses (**a**) and in fields (**b**).



Mm: *Macrolophus melanotoma* (Costa); Nt: *Nesidiocoris tenuis* (Reuter); De: *Dicyphus errans* (Wolff)

Figure 2 - Population dynamics of mirids sampled with traps in greenhouses (**a**) and in fields (**b**).

The number of mirids sampled with traps was always much higher than that collected with plant-tapping. However, the correlation between adults on traps and on plants was positive and highly significative with a Spearman correlation coefficient r = 0.518. For the samplings of 24th June, 11th and 29th July it was possible to compare the captures of adults with traps in greenhouses to those in fields; it resulted that there were not significative differences between mirid populations in indoor and outdoor crops.

Concerning the spatial distribution, statistical analyses pointed out significative differences only for the position within the plant and not for the position of the plant within the crop. Using Student-Newman-Keuls test, the mean numbers of mirids sampled with plant-tapping on the top, in the middle and on the bottom were significatively different. In relation to the sampling time, the captures of adults and nymphs on the top and in the middle showed an increasing trend: during the growing season the insects tended to colonize the highest branches (fig. 3). On the contrary, the regression between the mean number of mirids monitored on the bottom and the sampling time was not adequate.

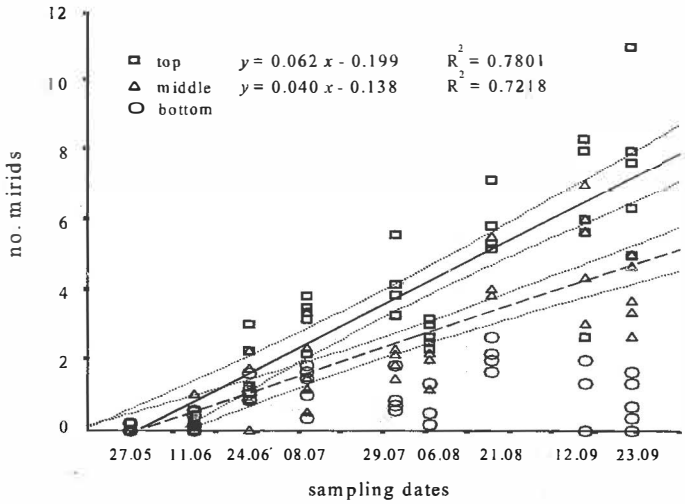


Figure 3 - Relation between mean numbers of mirids monitored with plant-tapping (top, middle, bottom) and sampling time. Regression coefficients are calculated considering first date (27th May) = 1st day and last date (23th September) = 120th day.

Discussion

The mirid species, studied as predators of *T. vaporariorum* six years ago (Arzone *et al.*, 1990), were found again and proved to live and reproduce well in IPM tomato crops since both adults and all instar nymphs were collected during the samplings. *M. melanotoma* was the most abundant throughout the growing season probably because its favourite host plant, *Inula viscosa* (Linnaeus) Aiton, is very common in the area. The predominance of this species is an undoubted advantage: in fact, although *M. melanotoma* can behave as phytophagous and complete its development on an exclusive vegetal diet (Tavella & Arzone, 1996), damage to crops due to its feeding activity has never been reported.

Injuries to vegetables can be caused by nutrition punctures of *D. errans* and above all *N. tenuis*. The performance of the latter species has been much discussed: it can prey pests but also produce damage piercing plants and injecting toxic saliva. In fact, this mirid was often recorded as a pest in several crops, among which tomato (Malausa, 1989). However, *N. tenuis* colonized the cultivations of the plain of Albenga late in the summer without compromising the harvest. On the other hand, it is a tropical species which finds its northern limit of distribution in the investigated area (Arzone *et al.*, 1990) and is not able to give rise to consistant populations.

Among the species of the genus *Dicyphus*, only *D. errans* was collected on tomato both in greenhouses and fields. It was present all over the season but not predominant, unlike what was observed on tomato of other areas of northwestern Italy (Petacchi & Rossi, 1991). *D. tamaninii*, a very efficient predator in vegetable crops of northeastern Spain (Gabarra *et al.*, 1988; Goula & Alomar, 1994), was never found in the investigated cultivations even if it is present in North Italy.

Starting from the end of May mirids succeeded in colonizing the tomato crops both in greenhouses and in the open air: they settled and reproduced immediately within the

cultivations. Populations were distributed uniformly throughout the crop; in fact mirid density was not significatively different in relation to the position of the plant in the cultivation. A relative homogeneity of population distribution was observed for mirid bugs also in tomato fields of northeastern Spain (Alomar *et al.*, 1994). Within the plant the predators preferred to settle on the highest branches which were more tender and more infested by whiteflies. Therefore, together with the use of traps, plant-tapping on the top is suggested as a convenient sampling method to check the mirid presence in the crops.

References

ALOMAR, O., GOULA, M. & ALBAJES, R., 1994. Mirid bugs for biological control: identification, survey in non-cultivated winter plants, and colonization of tomato fields. IOBC/WPRS Bull. **17** (5): 217-223.

ARZONE, A., ALMA, A. & TAVELLA, L., 1990. Ruolo dei Miridi (Rhynchota Heteroptera) nella limitazione di *Trialeurodes vaporariorum* Westw. (Rhynchota Aleyrodidae). Boll. Zool. agr. Bachic. (Ser. II) **22** (1): 43-51.

CALABRÒ, M. & NUCIFORA, A., 1993a. Presenza di Miridi zoofitofagi (Rhynchota, Heteroptera) su pomodoro e altre piante e loro utilizzabilità come ausiliari. Boll. Acc. Gioenia Sci. Nat. **26** (342): 115-131.

CALABRÒ, M. & NUCIFORA, A., 1993b. Indagini sul ruolo di tre miridi zoo-fitofagi (Rhynchota, Miridoidea) nel controllo biologico di *Trialeurodes vaporariorum*, di *Bemisia tabaci* (Rhynchota, Aleyrodoidea) e di *Myzus persicae* (Rhynchota, Aphidoidea) su pomodoro, peperone e poinsettia. Boll. Acc. Gioenia Sci. Nat. **26** (342): 335-352.

CARAPEZZA, A., 1995. The specific identities of *Macrolophus melanotoma* (A. Costa, 1853) and *Stenodema curticolle* (A. Costa, 1853) (Insecta Heteroptera, Miridae). Naturalista sicil. (S. IV) **19** (3-4): 295-298.

DELRIO, G., FLORIS, I., LENTINI, A., LUCIANO, P., ORTU, S. & PROTA, R., 1991. Osservazioni sulla lotta biologica adottata in colture protette di pomodoro in Sardegna. Atti XVI Congr. naz. it. Ent., Bari/Martina Franca, Italy, 23/28 settembre: 347-353.

FAUVEL, G., MALAUSA, J.C. & KASPAR, B., 1987. Etude en laboratoire des principales caracteristiques biologiques de *Macrolophus caliginosus* (Heteroptera: Miridae). Entomophaga **32**: 529-543.

GABARRA, R., CASTAÑE, C., BORDAS, E. & ALBAJES, R., 1988. *Dicyphus tamaninii* as a beneficial insect and pest in tomato crops in Catalonia, Spain. Entomophaga **33**: 219-228.

GOULA, M. & ALOMAR, O., 1994. Miridos (Heteroptera Miridae) de interés en el control integrado de plagas en el tomate. Guía para su identificación. Bol. San. Veg. Plagas **20**: 131-143.

JOSIFOV, M., 1992. Zur Taxonomie der paläarktischen *Macrolophus*-Arten (Insecta, Heteroptera: Miridae). Reichenbachia **29** (1): 1-4.

MALAUSA, J.C., 1989. Lutte intégrée sous serre: le punaises prédatrices Mirides dans les cultures de Solanacées du sud-est de la France. P.H.M.-Revue hort. **298**: 39-43.

PETACCHI, R. & ROSSI, E., 1991. Prime osservazioni su *Dicyphus (Dicyphus) errans* (Wolff) (Heteroptera Miridae) diffuso sul pomodoro in serre della Liguria. Boll. Zool. agr. Bachic. (Ser. II) **23** (1): 77-86.

SALAMERO, A., GABARRA, R. & ALBAJES, R., 1987. Observations on the predatory and phytophagous habits of *Dicyphus tamaninii* Wagner (Heteroptera; Miridae). IOBC/WPRS Bull. **10** (2): 165-169.

TAVELLA, L. & ARZONE, A., 1996. Development of *Macrolophus caliginosus* and *Dicyphus errans* reared on different diets (Rhynchota Miridae). Proc. XX Int. Congr. Ent., Firenze, Italy, August 25-31: 652.

VIII. Entomopathogens and molecular techniques
Entomopathogènes et techniques moléculaires

REGULATION OF A CUTICLE-DEGRADING PROTEINASE FROM THE ENTOMOPATHOGENIC FUNGUS *Verticillium lecanii* - A PATHOGEN OF THE APHID *Myzus persicae*

N.J.Bye and A.K.Charnley
School of Biology and Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK.

Summary
Isolates of the entomopathogenic fungus *Verticillium lecanii* produce several isoforms of the chymotrypsin (subtilisin) proteinase PR1. For most isolates the over-riding control of this enzyme is that of constitutive production with repression by low molecular carbon (C) and/or nitrogen (N) containing compounds. The PR1 of isolate KV 42, however, is not repressed by C or N singly though it is in combination. Enhanced chymotrypsin-like activity was found in the early stages of KV42 infection of peach-potato aphids, *Myzus persicae*, over and above that found in controls and aphids mycosed by the Vertalec isolate. The pIs of key *in vivo* proteinase isoforms match those of *in vitro* PR1-like fungal enzymes. Therefore it is suggested that PR1-like enzymes are produced in aphids during mycosis in particular for KV42 whose proteinases are less subject to catabolite repression. The results are discussed in the light of the prospect for mycoinsecticide improvement.

Introduction

Fungal pathogens of insects offer an alternative to chemicals for the control of insect pests. Our work at Bath is part of a CEC funded project aimed at improving the efficiency of mycoinsecticides against pests such as aphids and thrips on protected crops in North and South Europe. It is hoped that studies of pathogenesis will identify isolates with complementary attributes which could be combined in a mixture to produce a more potent mycoinsecticide. Since fungi penetrate insects through the external skeleton (=cuticle), it is not surprising that cuticle-degrading enzymes have been shown to be particularly important during pathogenesis. The focus of this study is the identification of isolates which can produce a key proteinase in situations which might otherwise be repressive (presence of low molecular weight metabolites) e.g. on the surface of honey dew contaminated aphids and in the haemolymph.

An extensive body of evidence has shown that a subtilisin proteinase with chymotrypsin/elastase-like specificity (PR1) produced by *Metarhizium anisopliae*, makes an important contribution to the penetration of host cuticle. We are currently investigating an analogous enzyme from the fungus *Verticillium lecanii*. All isolates produce a family of isoforms of a PR1-like enzyme when grown on insect cuticle. The present work establishes differences in regulation of PR1 enzymes between isolates and indicates that this reflects the pattern of proteinase production by these isolates during development in host aphids.

Methods

1. Materials

All chemicals and enzyme substrates, unless otherwise indicated were purchased from Sigma chemical company Ltd. Media constituents were supplied by LabM and BDH. Products for isoelectric focusing were supplied by Pharmacia Biotech. Isolates KV71 (refered to here as Vertalec) and KV42 of *Verticillium lecanii* were used. They were provided by Koppert B.V., The Netherlands.

2. Transfer experiments

A 4 day old biomass of the fungus, was established at 23ºC in a liquid culture containing complete medium (supplemented Modified Czapek Dox (Oxoid). The culture was washed and starved in a basal salts medium (Cooper and Woods, 1975) for 24 hours to ensure complete catabolite de-repression. The mycelium was then transferred to basal salts deficient in one or both of carbon or nitrogen plus insect cuticle. The medium used was buffered basal salts plus trace elements (pH6) plus sucrose (1%w/v) or NH_4Cl (0.4% w/v). Ground locust cuticle, prepared by the method of Anderson (1980), was supplied at 1%(w/v).

Culture supernatant was assayed for PR1-like activity, at 16 and 36 hours following transfer,. by monitoring the release of nitroanilide (NA) from the peptide chymotrypsin substrate N-Suc-Ala-Ala-Pro-Phe-NA as described by St Leger et al (1987a) using 0.225M Tris/HCl buffer at pH8.

3. Flat-bed isoelectric focusing

Culture filtrates, 24 h post transfer into the test conditions, were prepared for iso-electric focusing (IEF) by dialysis against 1% glycine and concentration by lyophilisation. 50µg of protein was loaded into each lane of a broad range (3-10) Ampholine PAGplate (Pharmacia)- and focused for 1.5h at 30W. Gels were then either developed with Coomassie Blue or qualitatively assayed for protease activity as follows. Bands on the IEF gels were characterised identified as proteinases by their ability to degrade the gelatin of an overlayed moistened photographic film. The overlay was monitored at regular intervals and the position of bands noted as soon as they first appeared. To further characterise the bands, 1mm slices, at points where protease activity had been detected, were excised from the gel and homogenised in Tris-HCl pH8 buffer. Aliquots of the buffer were then assayed for PR1-like activity as above.

4. *In vivo* experiments

Aphids were sprayed using a Potter Tower (Burkard Ltd) with sterile distilled water or a 1 x 10^6 conidia ml^{-1} suspension of Vertalec or KV42 and incubated on leaf discs at 23ºC and 100% RH. 10 aphids from each treatment, daily up to 6d , were transferred to a well of a micro-titre plate and, immediately prior to assaying, homogenised in Tris-HCl buffer, pH8, using a multihomogeniser (Burkard Ltd). Proteinase activity was detected by measuring the release of the fluorogenic leaving group of the chymotrypsin substrate N-Suc-Ala-Ala-Pro-Phe-AMC (Calbiochem-Novabiochem AG) using a Fluoroskan plate reader over a 1 hour period.

Proteinase isoforms produced *in vivo* were identified by IEF of aphid homogenates. Five aphids from each replicate were homogenised in 20ul of Milli-Q water, particulate matter was removed and the extract focused on a broad pI range gel as described previously. The gel was overlaid with photographic film and left overnight, positions of proteases were then recorded as clearing zones on the film.

Results

PR1-like activity 16h and 36h after biomass transfer into cuticle cultures with or without additional soluble sources of carbon and/or nitrogen.

Both Vertalec and KV42 produced PR1-like activity within 16h of transfer to a cuticle-containing medium (Fig 1). A further significant increase in activity occured by 36h. For Vertalec, proteinase activity was still notable in cultures containing an additional source of nitrogen, although to a significantly lesser extent than in cultures containing just insect cuticle. When 1% sucrose was supplied to these cultures in addition to cuticle, almost total repression of PR1-like activity was observed at both time points.

Isolate KV42 at 16 and 36 h did not display any significant differences in PR1-like activity between cultures containing cuticle as the sole source of nutrients, and those containing in addition, either soluble carbon or nitrogen. However, as for Vertalec, almost total repression of PR1-like activity was observed when both soluble nitrogen and carbon were supplied to cuticle cultures at 16 and 36 h.

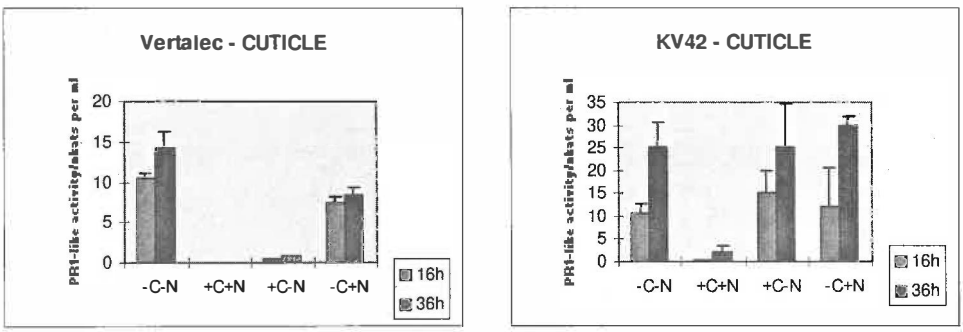


Fig.1. 4 day old fungus, grown in complete medium was starved for 24 hours before transfer to cultures containing 1% locust cuticle [-C-N], or 1% locust cuticle in the presence of additional more readily available forms of carbon and/or nitrogen: [+C+N; +C-N; -C+N]. Enzyme activity against N-Suc-Ala-Ala-Pro-Phe-pNA was recorded for each isolate at 16 and 36 h post transfer. Results in nkat NA released min^{-1} ml^{-1} are shown as the mean ± SD of 4 replicates.

Iso-electric focusing of proteinases produced by Vertalec and KV42 under different culture conditions

When grown on insect cuticle as the sole source of carbon and nitrogen Vertalec produced 4 proteinases with activity against the chymotrypsin substrate. When nitrogen was added to these cultures, one form of the enzyme, (pI 9.10) although still present, was significantly repressed. In the presence of carbon or carbon and nitrogen no proteinases with PR1-like activity were detected.

KV42 produced a very basic (≥9.47) form with comparable activity to that produced by Vertalec. However, 4 other forms of the enzyme were produced with different pI values to those of Vertalec. These forms were not repressed in the presence of carbon or nitrogen, but no PR1-like activity was observed when the two were supplied together.

Detection of proteinase activity in aphids infected with *Verticillium lecanii*

Proteinase activity against the chymotrypsin substrate was detected in homogenates from non-infected control insects over the 6d experimental period (Fig. 2). Vertalec infected insects had similar levels of proteinase activity to controls until death of the insects (between 4 and 5 days) when there was a marked increased as the fungus emerged from the host and colonised the cuticle. In contrast KV42 infected aphids contained significantly higher levels of proteinase activity than controls or insects infected with Vertalec during the development of mycosis (days 1-3).

Table 1 pI values are given for PR1-like enzymes produced by Vertalec and KV42 in cuticle cultures in the presence or absence of carbon or nitrogen, as calculated by Coomassie staining, gelatin overlay and assay against N-Suc-Ala-Ala-Pro-Phe-pNA and comparison with broad range markers.

1a Vertalec

Estimated pI			
-C-N	-C+N	+C-N	+C+N
≥ 9.47 9.10 8.83 8.62	≥ 9.47 (9.10) ** 8.83 8.62	No PR1-like activity detected in lane	No PR1-like activity detected in lane

** isoform 9.10 was slower to degrade the gelatin overlay, and exhibited a 4 fold reduction in PR1-like activity compared to the form produced in the absence of additional nitrogen.

1b KV42

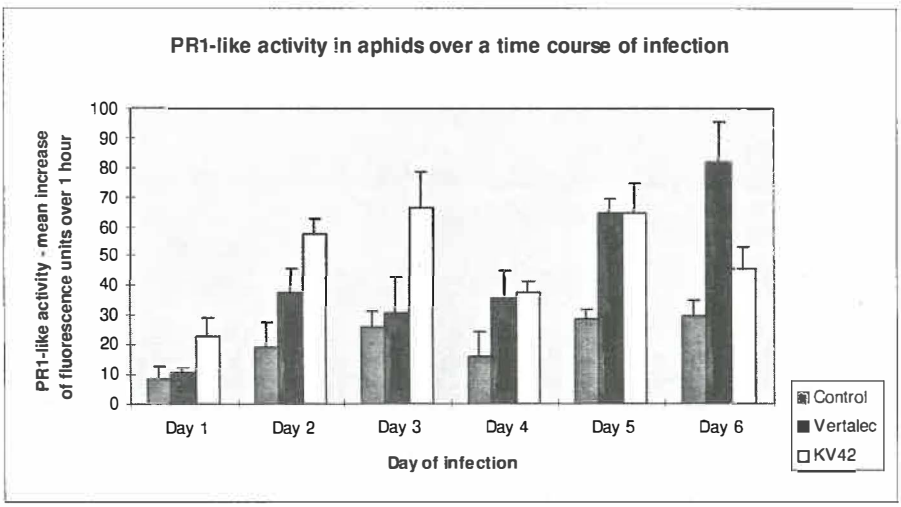
Estimated pI			
-C-N	-C+N	+C-N	+C+N
≥ 9.47 8.98 8.92 8.19 8.00	≥ 9.47 8.98 8.92 8.19 8.00	≥ 9.47 8.98 8.92 8.19 8.00	No PR1-like activity detected in lane

Detection of proteinase isoforms in extracts from infected insects

A very basic proteinase (pI 9.30) was detected in aphids of all treatments during the first 3 days of the experiment (Table 2). Its appearance in control insects indicates that it must be produced by the insect. Interestingly this isoform was not detected over the last 3 days of the experiment in aphids of any treatment. A number of other isoforms were detected only in mycosed insects. Two proteinases with pIs of 8.00 and 8.19 were found in extracts from KV42 infected insects on days 3 and 4. PR1-like enzymes with these pIs were produced by KV42 *in vitro* during growth on locust cuticle. A proteinase of pI 8.00 was also detected in Vertalec infected insects on day 4. However, no PR1-like enzyme with this pI was found in cultures of Vertalec. Additional proteolytic activity was detected at the point of loading on the IEF gel

(pI 6.8-7.5) with both isolates on day 5 and 6 viz. postmortem when the fungus was emerging from the cadavers. Finally an isoform of pI 4.2 was found on day 5 in aphids infected with KV42.

Fig 2.



Discussion

Isolates Vertalec and KV42 produce PR1-like enzyme(s) when grown on locust cuticle as the sole source of carbon and nitrogen, however they appear to display different sensitivities to low molecular weight carbon and nitrogen containing compounds. For Vertalec (and 3 other isolates of *V.lecanii* tested, data not shown) the over-riding control appears to be that of carbon repression. Nitrogen is also repressive although to a lesser extent. *M.anisopliae* has been shown also to produce PR1 under conditions of carbon and nitrogen starvation (St Leger et al 1988) though in contrast to Vertalec it is predominantly repressed by low molecular weight Nitrogen.

KV42 was the only isolate of *V.lecanii* out of those tested (data not shown) to produce on locust cuticle PR1-like enzymes that were not subject to repression by a carbon *or* nitrogen. This may be a desirable trait for a prospective biological control agent, given that the surface of aphids may be contaminated by honey dew rich in soluble carbon (sugars). All other things being equal an isolate producing a cuticle-degrading proteinase not subject to carbon catabolite repression, may be able to penetrate the host cuticle quicker and colonise the haemolymph of the host earlier. Consistent with this, enhanced proteinase activity in host insects was detected during KV42 infections earlier than in Vertalec infections.

Isoforms of PR1-like enzymes may be under different regulatory control , within and between isolates. Under conditions of nitrogen repression, one form of the enzyme produced by Vertalec on cuticle was significantly repressed whereas the others were unaffected. KV42 appeared to produce different forms of PR1-like enzyme to Vertalec and these were not

subject to catabolite repression. Two of the proteinases found in KV42 infected aphids had similar pIs to forms produced *in vitro* by this fungus. In addition Vertalec produced a proteinase (pI 8.0) *in vivo* that was not produced *in vitro* on locust cuticle though recent work (unpubl.) indicates that such a proteinase is produced when the fungus is grown *in vitro* on aphid (= host) cuticle. This may indicate host-specific induction. Preliminary results suggest that this protease may be comparable to the trypsin-like PR2 produced by *M.anisopliae*.

Table 2 Estimated pIs of proteinases in homogenates from control and fungus-infected aphids

Day of infection	Control	Vertalec	KV42
1	9.30	9.30	9.30.
2	9.30	9.30	9.30
3	9.30	9.30	9.30 8.19 8.00
4		 8.00	8.19 8.00
5		**	** 4.2
6		**	**

** protease activity detected at the point of sample loading (pI 6.85-7.35)

The long term objectives of the project are to improve mycoinsecticide performance by use of new formulations and employment of mixtures of isolates with complementary attributes. The present work suggests that the ability to produce a PR1-like enzyme in the presence of available carbon or nitrogen may be a desirable trait to consider in this programme.

References

ANDERSEN, S.O., 1988. Comparison between cuticular proteins from two locust species, *Locusta migratoria* and *Schistocerca gregaria. Insect Biochem*, **18**, (8), 751-760.

COOPER, R.M. & WOODS, R.K.S., 1975. Regulation of synthesis of cell wall degrading enzymes by *Verticillium albo-atrum. Physiol Plant Pathol* **5**, 135-156.

ST LEGER, R.J., CHARNLEY, A.K. & COOPER, R.M., 1987. Characterisation of cuticle degrading proteases produced by the entomopathogen *Metarhizium anisopliae. Arch Biochem Biophys* **253**, 221-232.

ST LEGER, R.J., DURRANDS, P.K., COOPER, R.M. & CHARNLEY, A.K., 1988. Regulation of production of proteolytic enzymes by the entomopathogenic fungus *Metarhizium anisopliae. Arch Microbiol*, **150**, 413-416.

[Part of the CEC Project entitled "Insect pathogenic fungi for environmentally friendly pest control in the glasshouse]

DISEASE DEVELOPMENT STRATEGIES OF THE INSECT PATHOGENIC FUNGI *Verticillium lecanii* AND *Metarhizium anisopliae*

J.L. Graystone and A.K. Charnley
School of Biology and Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK

Summary
Insect pathogenic fungi vary in the strategy employed to kill insects. Some grow profusely in the insect before death and thereby disrupt vital processes. Others have more limited growth, in which case toxins have been implicated in host death. Two methods for quantifying fungal growth in diseased insects have been developed in this study. These techniques will enable the identification of isolates with contrasting virulence strategies that may be usefully combined to produce a more efficient mycopesticide.

Introduction

Entomopathogenic fungi are being used as an alternative to chemical pesticides for insect control. The work at the University of Bath is part of a CEC funded project aimed at improving the performance of mycoinsecticides against pests, particularly aphids and thrips, on protected crops in North and South Europe. It is hoped that studies of pathogenesis will identify isolates of fungi, with contrasting virulence strategies, which may be combined to produce a more efficient mycopesticide

Entomopathogenic fungi invade their hosts through the external skeleton or cuticle. Once the fungus gains entrance to the haemocoel it may develop in a number of different ways. Some fungi continue filamentous development; most convert to a yeast like phase and this developmental switch is important for pathogenesis. The extent to which fungal isolates colonise insects prior to death also differs. Extensive production of blastospores and/or hyphal fragments in the haemolymph may indicate that early tissue invasion will occur. Alternatively the fungus may overcome its host after limited growth due to the production of fungal toxins.

The combination in a mycoinsecticide of a toxin producing isolate (with limited growth in the insect) and an isolate that causes death by extensive growth in the insect may improve the killing power of the preparation. In order to identify isolates with these contrasting strategies we need to be able to determine fungal growth *in vivo*. However, previous descriptions of the extent of fungal growth in infected insects prior to death have been made solely by visual inspections of the insect haemolymph. There have been no attempts to date to quantify fungal growth. This is rectified in this study where two methods have been developed to quantify fungal growth *in vivo*:

1). Quantifying the specific fungal sterol, ergosterol, in extracts from infected insects as a measure of fungal growth.

2). Using the Polymerase Chain Reaction to amplify part of the fungal nitrate reductase gene from DNA extracted from diseased insects and comparing the size of the product with that resulting from DNA extracted from known amounts of *in vitro* grown fungus.

This paper describes the application of these methods to the determination of growth of *Metarhizium anisopliae* in the tobacco hornworm *Manduca sexta* and *Verticillium lecanii* in the peach potato aphid *Myzus persicae*.

Materials and Methods

Organisms and culture conditions

Metarhizium anisopliae isolates ME1 and 703 were cultured routinely on 1/4 strength Sabouraud's dextrose agar (SDA) at 27°C. Conidial suspensions were prepared by washing with 0.04% Tween 80. *Verticillium lecanii* isolates KV54 and KV71were cultured on malt agar at 23°C. Liquid cultures of isolates of both species of fungi were grown in Modified Czapek Dox (Oxoid) supplemented with 2 gl-1 of each of casein hydrosylate, mycologoical peptone, yeast extract and malt extract. Alternatively Adamek's medium (Adamek, 1963) was used to promote blastospore production. The tobacco hornworm, *Manduca sexta*, was cultured on an artificial diet as described by Platt and Reynolds (1986). The peach potato aphid *Myzus persicae* was reared on green pepper plants in a constant temperature room at 23°C under a photoperiod of 18h light: 6h dark.

Quantifying the specific fungal sterol, ergosterol, in extracts from infected insects as a measure of fungal biomass.

Larvae of *Manduca sexta* were reared on tomato leaves to prevent interference from yeast sterols in the artificial diet. Fifth instar larvae were infected with *Metarhizium anisopliae* isolates by dipping in a conidial suspension of $1x10^7$ spores per ml. Control larvae were dipped in 0.004% Tween 80. Larvae were placed in individual Petri-dishes in a sealed desiccator containing moistened cotton wool to give 100% humidity and held at 25°C with 17 hours of light and 7 hours of dark.

The procedure used for the extraction and quantification of ergosterol was a modification of the method described by Seitz *et al* 1979. Ergosterol extraction were performed on cultures of *Metarhizium anisopliae* isolates 703 and ME1, and of *Verticillium lecanii* isolates KV54 and KV 71 that had been allowed to grow for 3, 4, 5 and 6d. All extraction's were replicated three times. Dry weights of harvested fungus were determined prior to extraction. Calibration graphs were drawn of biomass in mg against ergosterol content. Ergosterol extraction's were performed over a time course on larvae of *Manduca sexta* that had been infected with the isolates of Metarhizium anisopliae 703 and ME. Ergosterol was quantified *via* High Performance Liquid Chromatography.

Using Polymerase Chain Reaction to amplify part of a fungal gene (nitrate reductase) from DNA extracted from diseased insects

DNA extractions were perfomed on liquid cultures of ME1 and 703, control insects and on ME1 and 703 infected insects. The polymerase chain reaction (PCR) was carried out in an MJ Research programmable thermal controller. A typical reaction mixture consisted of 20mM Tris HCL (pH8.4); 50mM KCL; 1.5mM MgCl2; 1µl each of dCTP, dGTP,dTTP and dATP; 0.75µm of each primer; 1.25 units of Taq polymerase (Bioline) in a 50µl volume and overlaid with mineral oil. The DNA primers were designed from the sequence of the ME1 nitrate reductase gene (Bailey, Reynolds, Charnley and Clarkson, unpubl). The reaction parameters were as follows; initial denaturation for 2min at 94ºC followed by 35 cycles of 94ºC for 1.30 min, 48ºC for 1.30min and 72ºC for 1.30 min and a final five min extension at 72ºC. Reactions were also carried out using DNA extracted from known amounts of fungal biomass. Internal standards were employed to monitor the efficiency of the reactions (Bortolin and Christopoulos (1995). PCR products were separated on a 1.5% agarose gel containing ethidium bromide. Image analysis was used to quantify band size and intensity. Fungal biomass

in infected insects was estimated by comparing samples from mycosed insects with those that resulted from DNA extracted from known amounts of *in vitro* cultured fungus.

Results

Quantifying the specific fungal sterol, ergosterol, in extracts from infected insects as a measure of fungal biomass.

Ergosterol in spiked extracts from control insect consistently eluted from the column at around 26 minutes. There was no corresponding peak at 26 minutes in uninfected control insects that had not been spiked with ergosterol. In insects that had been infected with ME1 or 703 a peak at 26 minutes was seen in insects that had been infected for four, five, and seven days. Death occurred on day five after infection. All experiments were replicated three times.

Fast atom bombardment mass spectra was performed on the putative ergosterol fraction from *in vitro* (liquid cultures) and *in vivo* (infected insects). These had similar masses and fragmentation patterns to authentic ergosterol standards. The quantity of ergosterol extracted from mycosed insects was converted to mg of fungal biomass with the use of calibration graphs prepared from *in vitro* cultures. Ergosterol content of blastospores and mycelia was different and varied with culture age (results not shown). Thus calculations took into account the number of days after inoculation and the ratio of blastospores to hyphal fragments per µl of haemolymph of each isolate. The results (mean ± SD) are shown in Fig 1. Fungal growth in insects infected with isolate 703 was significantly greater than those infected with ME1, up to the time of death (5d). Post-mortem (6d) there was no significant difference in biomass between the isolates.

Use of quantitative Polymerase Chain Reaction to determine fungal biomass in infected insects

Initial experiments established that DNA of sufficient purity for direct PCR amplification using primers from the *M.anisopliae* nitrate reductase gene could be extracted from mycosed larvae of *Manduca sexta*. Furthermore amplifiable DNA was present in infected insects from days 1-6 after inoculation. No PCR product was achieved with any non-inoculated, control insects. A correspondence between increase in the amount of PCR product and time after inoculation was observed. Furthermore more product was detected in reactions involving DNA extracted from 703 infected insects than ME1 infected insects. This was confirmed and a quantitative estimate of fungal biomass in infected insects achieved by image analysis of PCR products run on agarose gels (data not shown) (see methods).

Discussion

The use of ergosterol as a measure of fungal biomass has allowed quantification of fungal growth in insects infected with the two isolates. The data support the microscope observations that significantly more fungal growth occured prior to host death in insects infected with 703 than ME1. Interestingly 1d after death ME1 cadavers had a similar amount of fungal biomass to 703. An important point here is that a similar inoculum was used for both isolates which do not differ significantly in virulence (unpubl). ME1 produces large amounts of the cyclic peptide toxins destruxins *in vitro* and *in vivo* (Samuels *et* al, 1988) whereas 703 does not seem to produce destruxins (Kershaw, Reynolds and Charnley, unpubl). Thus the former seems to rely substantially on toxins to cause death of the host while the latter brings about death indirectly through extensive growth in the host haemolymph.

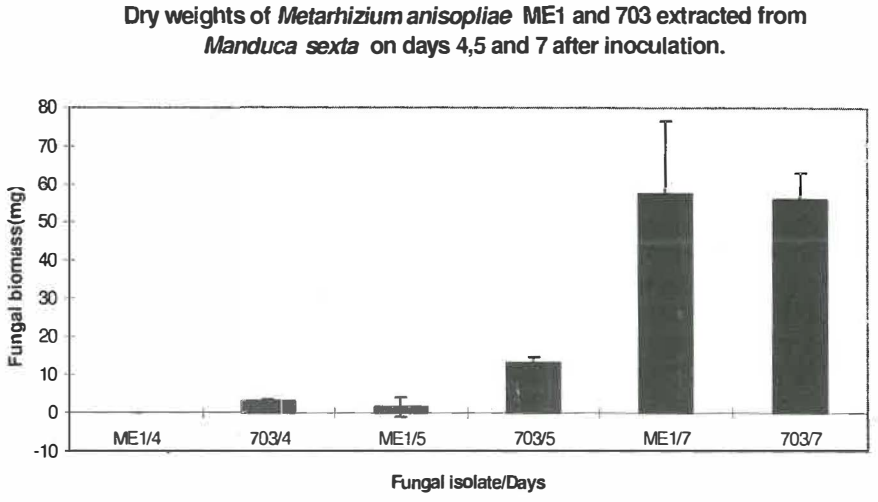


Fig1:- Shows an estimate of fungal biomass in larvae of the tobacco hornworm *Manduca sexta* infected with *M.anisopliae* isolate ME1 or 703 on days 4, 5 and 7 post inoculation.

The effects of age and growth form on ergosterol content mean that estimates of fungal biomass in infected insects can only be approximate; it is not possible to calculate the likely extent of the error. This method could be applied to other insect-fungus interactions, though lack of sensitivity would preclude use with small insects like aphids. The PCR technique, however, was much more sensitive than ergosterol quantification. Preliminary experiments showed that the primers based on the *M.anisopliae* nitrate reductase gene would amplify a fragment of the right size from *V.lecanii* DNA (data not shown). Thus it is possible that this technique could be used to quantify fungal growth in aphids. However, the relationship between biomass and DNA content, like ergosterol, may well depend on the growth form of the fungus. Thus this method also may provide only a relative estimate of fungal growth in insects.

References

ADAMEK, L. 1963. Submerged cultivation of the fungus *Metarhizium anisopliae* (Metsch). *Fol Microbiologia* **10**: 255-257

BORTIN, S. and CHRISTOPOULOS, T.K. 1975. Quantitative polymerase chain reaction (PCR) with a recombinant DNA internal standard and time-resolved fluorometry. *Clinical Chemistry* **41**: 43

PLATT, N. and REYNOLDS, S.E. 1986. The pharmacology of the heart of a caterpillar, the tobacco hornworm, *Manduca sexta*. *Journal of Insect Physiology* **32**: 221-230

SAMUELS, R.I., CHARNLEY, A.K. and REYNOLDS, S.E. 1988. The role of destruxins in the pathogenicity of 3 strains of *Metarhizium anisopliae* for the tobacco hornworm *Manduca sexta*. *Mycopathologia* **104**: 51-58.

SEITZ, L.M., SAUER, D.B., BURROUGHS, R., MOHR, H.E. & HUBBORD, J. 1979. Ergosterol as a measure of fungal growth. *Phtopathology* **69**: 1202-1203.

ST. LEGER, R.J, COOPER, R.M, & CHARNLEY, A.K. 1988. The effect of melanization of *Manduca sexta* cuticle on growth and infection by *Metarhizium anisopliae*. *Journal of Invertebrate Pathology* **52**: 459-470.

ST.LEGER, R.J., GOETTEL, M., ROBERTS, D.W., & STAPLES, R.C. 1991. Prepenetration events during infection of host cuticle by *Metarhizium anisopliae*. *Journal of Invertebrate Pathology* **58**: 168-179.

[Part of the CEC Project entitled "Insect pathogenic fungi for environmentally friendly pest control in the glasshouse]

MOLECULAR TYPING OF *Verticillium lecanii* ISOLATES BASED ON MITOCHONDRIAL DNA POLYMORPHISMS

Vassilis Kouvelis and Milton A. Typas
Division of Genetics and Biotechnology, Department of Biology, University of Athens, Panepistemiopolis, Athens 15701, Greece

Abstract

Mitochondrial genomes (mtDNA), because of their rapid rate of sequence divergence have become ingreasingly popular in both population and evolutionary biology studies of a diverse range of organisms, including fungi. Thirty isolates of the entomopathogenic fungus *V.lecanii* from various hosts and geographic origins were analysed for mtDNA polymorphisms. The analysis of mtDNA was based on two different approaches: (a) Restriction Fragment Length Polymorphisms (RFLPs) and (b) PCR amplification of mitochondrial genes. In RFLPs digestion of total genomic DNA was performed with restriction endonucleases which recognize four bases containing only GC. Primers used for PCRing were designed based on conserved regions detected when all available mtDNA genome data from filamentous fungi were compared.

Digestion of total genomic DNA with endonucleases *Cfo*I, *Hae*III and *Hpa*I and subsequent analysis of the mtDNA bands following hybridisation with the total mtDNA probe allowed the classification of the *V.lecanii* strains into 17 different groups. No correlation between grouping and insect host or location could be distinguished.

PCR amplification led to the detection of one band products for any of ND1, ND3, ND5 (NADHs), COIII (cytochrome oxidase), MS (srRNA) and ATPase6, but of several bands for ML3/4, ML7/8 (lrRNA region). The amplified gene products for ND1, ND3, ND5, COIII and MS were 550, 225, 670, 430 and 630 bp respectively. As the majority of the isolates used gave bands of the same size, for each gene tested, (with the exception of 1-5 isolates) and only *Alu*I from a variety of restriction tetra-cutter endonucleases used helped to differentiate amplification products of MS and ND5, the above functional regions of *V.lecanii* are presumed to be relatively conserved. On the contrary, the ATPase6 and large rRNA PCR gene products exhibited a greater isolate variability. The primers used for the former allowed the detection of amplification products of different sizes, varying from 450 bp to 2,200 bp. Similarly, the primer pairs ML3/4 and ML7/8 used for the latter resulted in several bands (1-6) for each isolate which could allow the differentiation of isolates. Thus, by combining results from ATPase6 and large rRNA region PCR amplification products a good classification of the isolates into 21 distinctly different groups could be achieved.

Therefore, it can be easily concluded that there is an important intra-species variation in *V.lecanii* isolates and that mtDNA differences based on PCR product analyses can be exploited for the detection of individual strains and the molecular fingerprinting of biological control agents.

Introduction

Mitochondrial genomes (mtDNA), because of their rapid rate of sequence divergence have become ingreasingly popular in both population and evolutionary biology studies of a diverse range of organisms, including fungi (Bendich 1996). Evolution based on point mutations in animal and fungal mtDNA proceeds 10-100 times faster than in the nuclear DNA (Brown *et al*, 1982 ; Taylor 1986). In this context, comparison of mtDNA provides interesting information to detect genetic variations that can be exploited for strain identification. Although mtDNA

RFLPs have been used to estimate relationship between species and for inter- and intra- species isolate detection (Garber & Yoder 1984 ; Carter *et al.*, 1990 ; Sekiguchi *et al.*, 1990), data on the molecular organization and RFLPs of the mtDNA from Deuteromycetes are scarce (Hegedus & Khachatourians 1993). We present here clear and convincing evidence that RFLPs of mtDNA and PCR amplification based on mitochondrial genes intra-species variation in *V.lecanii* isolates can be accurately estimated and the molecular fingerprinting of individual strains of this biological control agent can be achieved.

Digestion of total genomic DNA from seven *Verticillium* species, including *V.lecanii,* with *Hae*III has previously given distinctive patterns on ethidium bromide gels. Through DNA hybridisations, most of the bands were proven to be of mtDNA origin and the patterns allowed each species to be distinguished (Typas *et al.*, 1992). In the present work, 30 isolates of the entomopathogenic fungus *V.lecanii* (kindly provided by Dr.P.Bridge, International Mycological Institute, UK., Dr.W.Ravensberg, Koppert BV, The Netherlands, Dr.E.Beeling, Alsmeer, The Netherlands, Dr.L.Rovesti, Bologna, Italy, and Dr.K.A.Charnley, Bath University, UK) isolated from different hosts and geographic origins were examined. MtDNA from *V.lecanii* strain C-42 was isolated by CsCl centrifugation and labelled with digoxigenin as previously described (Typas *et al.*, 1992). Endonucleases *Cfo*I, *Hae*III and *Hpa*I were used to digest total extracted DNA from all these strains and the resulting bands were hybridised with the total mtDNA probe. The *Hae*III restriction/hybridisation pattern taken alone could differentiate the 30 *V.lecanii* isolates into 17 different groups (Table 1). Similarly, *Cfo*I and *Hpa*II could also differentiate the isolates into 14 and 15 groups respectively. Thus, when results of the restriction patterns from these three endonucleases were combined it became evident that an excellent differentiation of the isolates could be achieved as the 30 isolates could be placed into 23 distinct groups (Table 1).

Functional studies of mtDNA from different fungi lead to the conclusion that most fungal species contain a number of genes common for all mtDNAs, i.e. the ribosomal RNA genes coding for the small (SrRNA) and the large (LrRNA) subunits, the NADH dehydrogenases subunits 1-6 (ND1-6), the cytochrome oxidase subunits 1-3 (COI-III), the ATPase subunits 6,8 (ATPase6-8) ; the apocytochrome b (cyt b) and the genes for more than 15 tRNA species (Scazzochio *et al.*, 1983). By comparison of all the so far published sequences from fungal mtDNA genomes, primers were designed based on presumed conserved regions which would amplify the corresponding genes. For the SrRNA and LrRNA, the primer pairs MS1/2, ML3/4 and ML7/8 designed by White *et al.* (1990) were used. PCR amplified products were obtained for most of the primer pairs designed, but amplification conditions varied greatly from product to product. The only primers which failed to give products under any conditions used were those of ND4 and cytb. This may be due to the fact that only 3 and 4 sequences, from phylogenetically very distant fungal species, were available (published or unpublished) from which to design primers.

The primer pairs designed for the NADH degydrogenase genes ND1, ND3 and ND5 gave single amplification products of approx. 550, 225 and 670 bp respectively, for almost all isolates. The exceptions for ND1 were isolates KV42 (two bands of 800 and 1600 bp), IMI 317425 (a single 1,600 bp band) and IMI 255033 (an additional 800 bp band). Isolates VLGr VLGerb and Mycotal, also gave an additional band of 1,100 for ND3, whereas isolates IMI 338015 (a single 200 bp product), Ba (an additional product of 400 bp) and IMI 255033, IMI 338014, IMI 79606 (an additional product of 200 bp) gave different results for the ND5 primers.The amplification primers for the small ribosomal RNA subunit gave a single product of approx. 630 bp, with the exception of isolate IMI 338015, which showed a slightly larger amplification product of 680 bp. The primers for ATPase 6 showed the maximum variability in

PCR amplification product sizes. Most isolates gave a single 450 bp product. However, isolates KV56, VLGr, VLGerb gave a single 750 bp band, isolates IMI 338015 and 115-25

Table 1: Classification of the 30 *V.lecanii* isolates according : (a) to their mtDNA pattern after digestion with *Hae*III, *Hpa*II and *Cfo*I restriction enzymes, (b) to PCR products for specific mtDNA genes (NADH dehydrogenase subunits 1,3,5 ; ATPase subunit 6 ; cytochrome oxidase subunit 3 ; small rRNA and large rRNA).

Isolates	*Hae*III	Combination of *Hae*III, *Hpa*II, *Cfo*I	ND1, ND3, ND5, ATPase6, COIII, and SrRNA PCR results	Combination of mtDNA LrRNA (ML3/4-ML7/8) PCR results
C-42	1	1	1	1
115-25				2
KV54				3
Ba		2	2	4
CUMF1		3	1	5
Mycotal	2	4	3	6
318-70B		5		7
KV53			1	8
KV56			4	9
VLGr	3	6	5	10
VLGerb				11
KV 145		7	1	12
IMI 282532		8		13
Vertalec	4	9		14
KV22				15
IMI 317425	5	10		16
IMI 331550			6	17
IMI 79606	6	11	1	18
IMI 321293		12		19
KV42	7	13	7	20
KV46	8	14	1	21
KV63	9	15		22
Tv	10	16		23
IMI 338014	11	17		24
IMI 338015	12	18	8	25
IMI 255033	13	19	9	26
IMI 331593	14	20	1	27
IMI 21197	15	21		28
IMI 268316	16	22		13
IMI 115197	17	23		29

single 1,500 and 2,300 bp products respectively and isolates Ba, KV22, KV145, CUMF1 and Mycotal produced additional bands of 750, 900, 900, 2,300 and 2,300 bp respectively. Finally, cytochrome oxidase COIII primers resulted in a 430 bp product for all isolates, with the exception of KV42, KV56, IMI 255033, IMI 317425 and IMI 331550 which all gave a 1,600 bp amplification product. These size differences may be of interest here as introns in mtDNA sequences have been found before for other fungi (Michel *et al.*, 1982 ; Cummings *et al.,* 1990) and the existence of such elements may account for the observed size differences (under investigation at present). When all different patterns from PCR amplification of ND1, ND3, ND5, MS, COIII and ATPase 6 gene products were combined together, the maximum differentiation achieved was the classification of the 30 isolates into 9 distinct groups.

Attempts have been made to detect polymorphisms within the single gene products of the above PCR products by digesting the amplification products with tetra-cutter enzymes such as *Alu*I, *Cfo*I, *Hae*III, *Hpa*II, *Sau*3A and *Rsa*I. Unfortunately, only *Alu*I could marginally help to differentiate some of the isolates, and this only for the MS and ND5 products. As the approach is also time consuming it was abandoned from further experiments. Nevertheless, our results indicate that the above functional regions may be relatively conserved in *V.lecanii.*

The ML3/4 and ML7/8 primer pairs invariably resulted in several bands (1-7), varying from 450 to 2,200 bp, for each isolate. The results were reproducible under the conditions established. A classification example for the 30 *V.lecanii* isolates is given as a phylogenetic UPGMA dendrogram based on the ML3/4 pair of primers in Fig.1. The amplification product picture from ML7/8 pair of primers was also very similar. Thus, if the results from ML3/4 and ML7/8 amplification primer pairs were combined, an excellent distinguishing pattern was obtained as the 30 isolates were classified in 29 distinct groups (only isolates IMI 282532 and IMI 2683116 had identical patterns). This is obviously a much more fruitful method than the best so far integrated taxonomic approach used for the genus of *Verticillium* by Jun et al., 1991) based on morphological, physiological and biochemical characters. Therefore, it can be safely concluded that there is an important intra-species variation in *V.lecanii* isolates and that mtDNA differences based on either RFLPs or PCR product analyses can be exploited for the detection of individual strains and the molecular fingerprinting of other biological control fungal species.

References

BENDICH, A.J., 1996. Structural analysis of mitochondrial DNA molecules from fungi and plants using moving pictures and pulsed-field gel electrophoresis. J.Mol.Biol. **255 :** 564-588.

BROWN, W., PRAGER, E., WANG, A. & WILSON, A.C., 1982. Mitochondrial DNA sequences of primates : tempo and mode of evolution. J.Mol.Evol. **18 :** 225-239.

CARTER, D.A., ARCHER, S.A.A., BUCK, K.W., SHAW, D.S. & SHATTOCK, R.C., 1990. Restriction fragment length polymorphisms of mitochondrial DNA of *Phytophthora infestans*. Mycol.Res. **94 :** 1123-1128.

CUMMINGS, D.J., MACNALLY, L.K., DOMENIC, J.M. & MATSURA, E.T. 1990. The complete DNA sequence of the mitochondrial genome of *Podospora anserina*. Curr.Genet. **17** : 375-402.

GARBER, R.C & YODER, O.C., 1984. Mitochondrial DNA of the filamentous ascomycete *Cochliobolus heterostrophus.* Characterization of the mitochondrial chromosome and population genetics of a restriction enzyme polymorphism. Current Genetics **8 :** 621-628.

HEGEDUS, D.D. & KHACHATOURIANS, G.G., 1993. Identification of molecular variants in mitochondrial DNAs of members of the genera *Beauvaria*, *Verticillium*, *Paecilomyces, Tolypocladium* and *Metarhizium*. Appl.Environ.Microbiol. **59 :** 4283-4288.

JUN, Y., BRIDGE, P.D. & EVANS, H.C. 1991. An integrated approach to the taxonomy of the genus *Verticillium*. J.Gen.Microbiol. **137 :** 1437-1444.

MICHEL, F., JACQUIER, A. & DUJON, B. 1982. Comparison of fungal mitochondrial introns reveals extensive homologies in RNA secondary structure. Biochimie, **64 :** 867-881.

SCAZZOCHIO, C., BROWN, T.A., WARING, R.B., RAY, J.A. & DAVIES, R.W., 1983. Organisation of the Aspergillus nidulans mitochondrial genome. In Schweyen, R.J., Wolf, K. & Kaudewitz, F. (eds), Mitochondria 1983, Walter de Gruyter, Berlin, pp.303-312.

SEKIGUCHI, J., OHSAKI, T., YAMAMOTO, H., KOICHI, K. & SHIDA, T., 1990. Characterization and comparison of mitochondrial DNAs and rRNAs from *Penicillium utricae* and *P.chrysogenum*. J.Gen.Microbiol. **136 :** 535-543.

TAYLOR, J.W., 1986. Fungal evolutionary biology and mitochondrial DNA. Exp.Mycol. **10 :** 259-269.

TYPAS, M.A., GRIFFEN, A.M., BAINBRIDGE, B.W. & HEALE, J.B., 1992. Restriction fragment length polymorphism in mitochondrial DNA and ribosomal RNA gene complexes as an aid to the characterization of spieces and sub-species populations in the genus *Verticillium*. FEMS Microbiol.Lett. **95 :** 157-162.

WHITE, T.J., BRUNS, T., LEE, S. & TAYLOR, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds), PCR Protocols, Academic Press, San Diego, pp.315-322.

[EU Project entitled «Insect Pathogenic Fungi for Environmentally Friendly Pest Control in the Greenhouse» (AIR94-1352)]

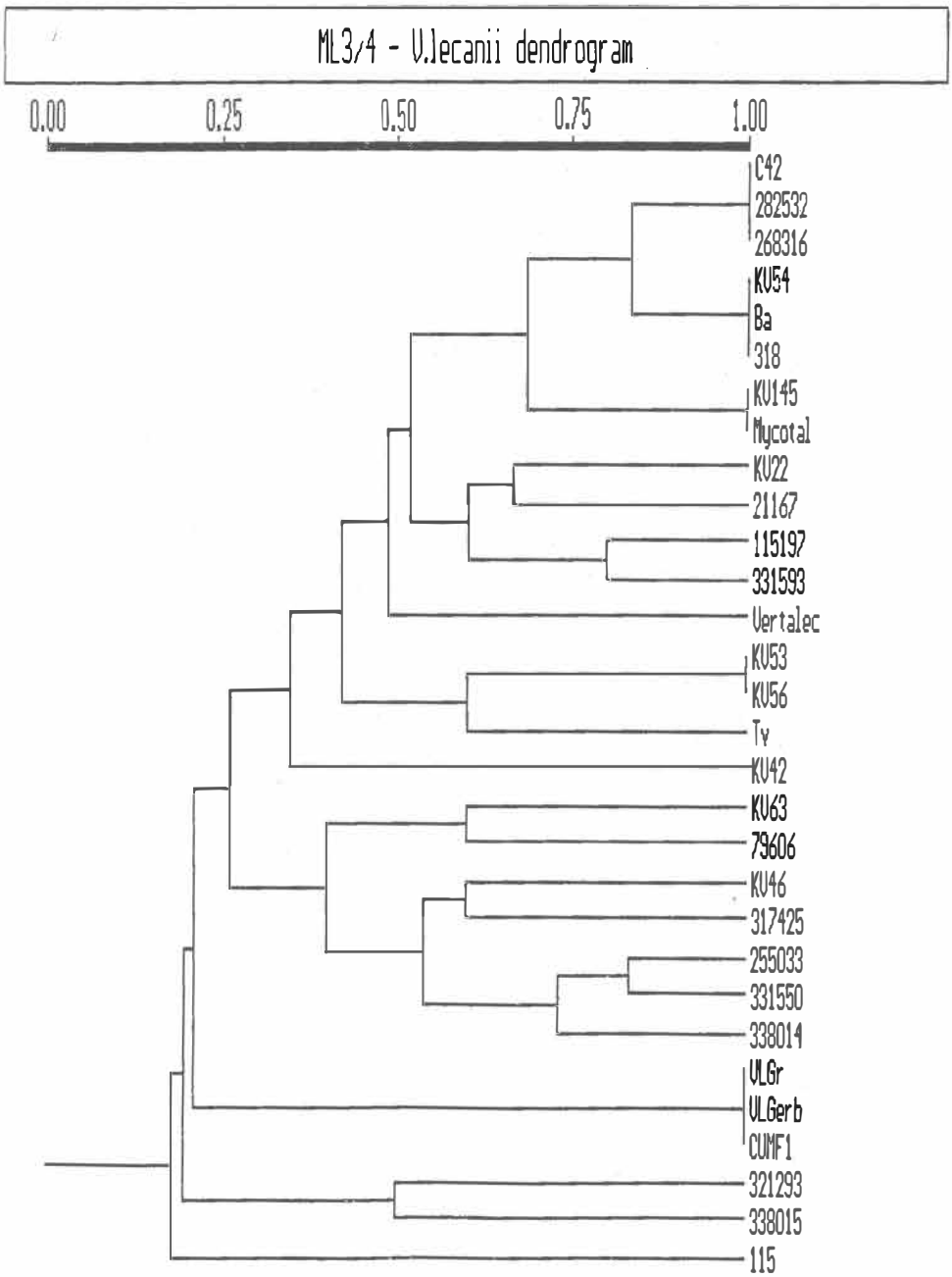


Fig. 1 : UPGMA dendrogram of *V.lecanii* isolates based on PCR amplification products obtained with ML3/4 primers. (The similarity matrix was calculated based on Jaccard coefficient).

MOLECULAR GENETIC AND INTEGRATED CONTROL: A UNIVERSAL GENOMIC DNA MICROEXTRACTION METHOD FOR PCR, RAPD, RESTRICTION AND SOUTHERN ANALYSIS.

A. Reyes, R. Linacero & M.D. Ochando.
Departamento de Genética. Facultad de Ciencias Biológicas. Universidad Complutense. 28040 Madrid. Spain.

Summary

For a long time, molecular genetic and integrated control approaches were focused on completely different topics of research. Nevertheless, in the last years, integrated control programs have begun to use an ever increasing number of molecular techniques based on DNA technology. Fundamental aspects of these programs, such as characterization and identification of species, biotypes or host races, the knowledge of the structure and dynamics of pest species populations (gene flow, colonization ...), the diagnosis of viral diseases in protected crops, the detection of resistence, etc., can be achieved by means of DNA analysis.

Thus, we have developed a new microextraction method of genomic DNA suitable for plant (monocotyledonous, dicotyledonous and lichens) and animal (invertebrate and vertebrate) species. The main improvements of this methods are: it requires small amounts of starting material, and so, it is useful for small pest insects and for screening transformed plants at early stages; it is fast, and it yields high quantities of DNA, comparable to other large scale methods.

DNA extracted according to this methodology has been proved to be of high quality and suitable to be used in species, varieties, host races and individual identification, both in plant and animal species, by means of RAPDs (Random Amplified Polymorphic DNAs). Analysis of specific genes by means of PCR (Polymerase Chain Reaction) have also been proved successfully, as well as the RFLP (Restriction Fragment Length Polymorphism) and Southern analysis of single copy genes.

Summarizing, we can say that the method here presented can be of great utility for the genetic analysis of a great number of species subject to integrated control programs, and by means of different DNA based approaches.

Introduction

Since the origin of agriculture, the man have always searched the way to improve their cultures, both modifying the species they were interested on and protecting them from the attack of agricultural pest species.

The fight against agricultural pests began with the use of chemical compounds soon after the development of the chemical industries. The use of those compounds became increasingly wide spread and some of them were thought to solve all agricultural problems. Nevertheless, major problems associated with the injudicious use of insecticides began to be reported since the 70's. Human pesticide poisoning, development of insect resistance and persistence in the environment, were illustrated, specially in developing countries (Smith & Calvert, 1976). Thus, the conciliation between pesticide practices and human and environmental health lead to what it has been called Integrated Pest Management (IPM).

In this way, in addition to the research and use of more specific compounds, appeared the insecticide synergists. These synergists were natural or synthetic chemicals that increased the lethality and effectiveness of the insecticides by an enzyme-inhibiting mechanism that restored the susceptibility of insects to the insecticide, and subsequently, considered by

themselves as nontoxic. First synergists began to be used about 50 years ago and nowadays they play an important role in the IPM programs (for a review see Bernard & Philogene, 1993).

Later on, two new relatively sustainable and environmental friendly techniques were developed as pest control strategies: the biological pest control and the sterile insect release method (SIRM). Biological control of insects was defined as the method that causes a persistent, strong reduction in the pest population following the introduction of a natural enemy, either predator or parasitoid (Beddington *et al.*, 1978). Despite the fact that theoretical considerations are known since years, only recently biological control has become more practical, as a deep knowledge of the pest species, as well as the biological control agent, and ecological considerations are required for a successful program (Kitto, 1983; Landry *et al.*, 1993).

The sterile insect released method is based on the alterations in the sex ratio in natural populations using sterile males (Robinson, 1983). As huge amounts of sterile males must be released every day to assure the effectiveness of the program, efforts and rearing costs are extremely high. Thus, different genetic sexing methods have been developed using conditional lethal genes, null alleles for ADH (alcohol dehydrogenase), mutants for XDH (xanthine dehydrogenase), etc., that only allow the development of males (Saul, 1987).

Molecular genetic advances since early 80's made available new techniques that were quickly used in pest control programs (Cockburn *et al.*, 1984). Thus, molecular genetic studies made possible to describe, characterise and clone genes encoding insecticidal proteins from different species such as *Bacillus thuringiensis* (Fujimoto *et al.*, 1993), the spider *Plectreurys tristis* (Quistad & Skinner, 1994) or plants (Gibson & Somerville, 1993). Once cloned, those genes can be introduced into plants by means of bacterial or viral vectors, direct uptake by protoplasts or mechanical introduction on metal particles or other materials (Joshi & Joshi, 1991; Boulter, 1993; Day, 1996). So, to obtain genetically engineering crop plants which exhibit insect resistance is becoming almost a routine for many crops (Perlak *et al.*, 1990; Fujimoto *et al.*, 1993; Day, 1996).

Simultaneously, molecular genetic approaches have also made possible a better knowledge of both crop plants and their pest species, which is of great importance for IPM programs. Molecular identification of different plant cultivars and pest species, biotypes, host races or populations is possible nowadays by means of DNA fingerprinting, RFLP (Restriction Fragment Length Polymorphism) or RAPDs (Random Amplified Polymorphic DNAs). The knowledge of the genetic structure and dynamics of pest species are also fundamental for designing control programs in which chemicals or biological agents are used, and can be achieved by DNA based methodologies (for revisions see Hadrys *et al.*, 1992; Caetano-Anolles, 1994; Smith & Williams, 1994; Welsh & McCleland, 1994)

In all these approaches, the first step is the isolation of genomic DNA from the biological material. Therefore, different protocols have been previously reported for animal or plant material. However, most of them require a great amount of material, are designed only for animal or plant, or more often for specific species or tissues (for a review see Cheung *et al.*, 1993). The ideal method for DNA extraction should be one that would require small amounts of starting material, applicable to a great variety of organisms, simple, relatively cheap and suitable for many different applications currently used, such as PCR (Polymerase Chain Reaction), both specific and RAPD, and restriction endonuclease digestions. Here, we report a simple and fast method that yields large amounts of DNA useful for PCR and RFLP

and that can be used either for plant (monocotyledonous, dicotyledonous and lichens) and animal (invertebrate and vertebrate) sources of DNA.

Materials and Methods

We have used the following samples for genomic DNA microextraction: different plant species: monocots as *Zea mays*, *Saccharum officinarum* and *Secale cereale*; the dicots *Spinacea olearacea*, *Arabidopsis thaliana*, *Glycine max*, *Sinapidendrum frutescens*; and the lichens *Evernia prunastri*, *Xanthoria parietina* and *Cladonia verticillaris*. Among animal species, we have tested for both, invertebrates such as *Drosophila melanogaster*, *D. subobscura*, *Ceratitis capitata* and *Dacus -Bactrocera- oleae,* and vertebrates: gonadal tissue of *Barbus bocagei*, liver of *Barbus sclateri*, and brain and liver from *Rattus norvegicus*.

Our rapid microextraction protocol is as follows: samples of about 2-10 mg are collected into a 1.5 ml tubes (a whole invertebrate, a portion of animal tissue or a leaf disc), homogenated by physical grinding using an electric motor-driven pestle or a glass rod, in 600 µl of extraction buffer containing 0.2M sucrose, 50 mM EDTA, 0.5% SDS and 100 mM Tris-HCl pH 8.5. The presence of sucrose increases the consistency of the medium and the low ionic strength avoids "salting out" of macromolecules associated to DNA, leading to a higher efficiency in the extraction of genomic DNA. After incubation at 65°C for 30 min, 120 µl of 3M potassium acetate pH 5.2 are added, mixed well by inverting the tubes a few times, to avoid DNA breakage, and kept at -20°C for 10 min. Then, the tubes are spun at 13 000xg for 20 min, and the supernatant is transferred to a new tube. If a low yield is expected, the addition of RNA (e.g. yeast tRNA) as a carrier could aid the following precipitation step. One volume of isopropanol is added and the tubes are kept at -20°C for 15-60 min (depending on the amount of material at the starting point). At this point, samples can be maintained at -20°C for months. Afterwards, they are spun at 13 000xg for 20 min, washed with 70% ethanol r.t., air-dried briefly and resuspended in TE (10 mM Tris-HCl pH 8 - 1 mM EDTA). If needed, after isopropanol precipitation, the supernatant can follow a RNAse and phenol:chloroform treatment.

Amplifications for specific PCR were carried out in 25µl containing: 100 ng of template DNA, 10 mM Tris-HCl pH 9.0, 50 mM KCl, 0.1% Triton X-100, 2mM $MgCl_2$, 200 µM dNTP, 2 µM each 21 base forward and reverse primers (unpublished data) and 1.25 u Taq polymerase (Promega, Madison, USA); and overlaid with 50 µl mineral oil. The thermal profile for PCR was 94°C for initial denaturation for 5 min, then 40 cycles of 94°C for 1 min, 55°C for 2 min and 72°C for 2 min, and finally 72°C for 6 min. When RAPD were achieved, PCR reaction mixtures were the same as above but using 25 ng as template DNA, 5 picomoles of primer and 0.4 u Dynazyme (Finnzymes Inc., Finland) as polymerase. The thermal profile for PCR was 94°C for 6 min, 45 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min, and a final step of 72°C for 6 min. In both cases, PCR were performed in a MJ Research (Watertown, USA) thermocycler (PTC-100). PCR products were separated by electrophoresis in 1.7% agarose 1xTris-acetate gels stained with ethidium bromide and visualized under UV light.

On the other hand, detection of single copy gene Emb564 (Williams & Tsang, 1991) in *Zea mays* was done. Southern transfers were achieved onto Hybond N membranes (Amersham) and hybridisations were carried out at 42°C in a 50% formamide solution. Filters were rinsed for 10 min at room temperature in 2XSSC plus 0.1% SDS and then 30 min at 68°C in 0.1XSSC plus 0.1% SDS. Detection of probe DNA-target DNA hybrids were achieved according to the luminescent manufacturers indications (Boehringer Mannheim).

Results

This method yields about 1-1.2 μg of high quality DNA per mg of sampling material in species with higher DNA content as most plants and vertebrates, and about 0.4-0.8 μg/mg in species such as *A. thaliana* and small invertebrates. The highest molecular weight DNA extracted by this method is ≥ 23 Kb and $OD_{260\ 280} \cong 1.7$.

Single gene PCR amplifications for large and small subunits of RubisCo are shown in Figure 1. As it can be observed, we have obtained the same results for both subunits using either our method of extraction or a more time consuming one.

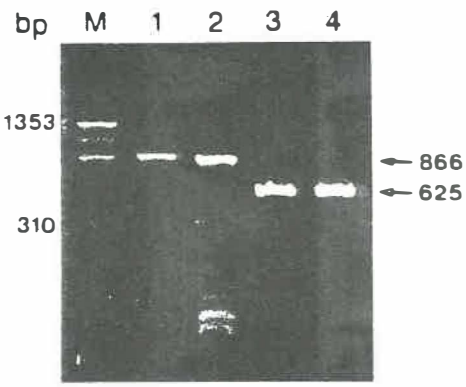


Figure 1. Single gene PCR amplification products of the large and small subunits of RuBisCo from *Spinacea olearacea* (1, 2) and *Arabidopsis thaliana* (2, 4) respectively. In lanes 1, 3 DNA was extracted according to our method while for lanes 2, 4 DNA was extracted according to Dellaporta et al. (1983). Phage ϕX174 DNA digested with *Hae* III was used as molecular marker (M).

The results of RAPDs on different animal and plant species with the primer OPB-02 (Figure 2A) have shown that different species can be identify using even a single primer (without take into consideration intraspecific variability). Thus, we have found that for each species a typical profile is observed. Moreover, when different species of the same genus are taken into consideration, such as *Drosophila melanogaster* and *D. subobscura* or *Barbus bocagei* and *B. sclateri* (Figure 2A, lanes 1 - 2 and 7 - 8, respectively) a clear species specific banding pattern is obtained. In addition, when different tissues of the same individual are analysed, as in the case of liver and brain of *Rattus norvegicus* (Figure 2A, lanes 5 - 6) the same results are observed.

The comparison between the RAPD profiles from *Zea mays* using DNAs extracted according to our method and a more time consuming maxiprep is presented in Figure 2B. As it is shown, for each of the five primers used here no differences have been detected in the amplification products.

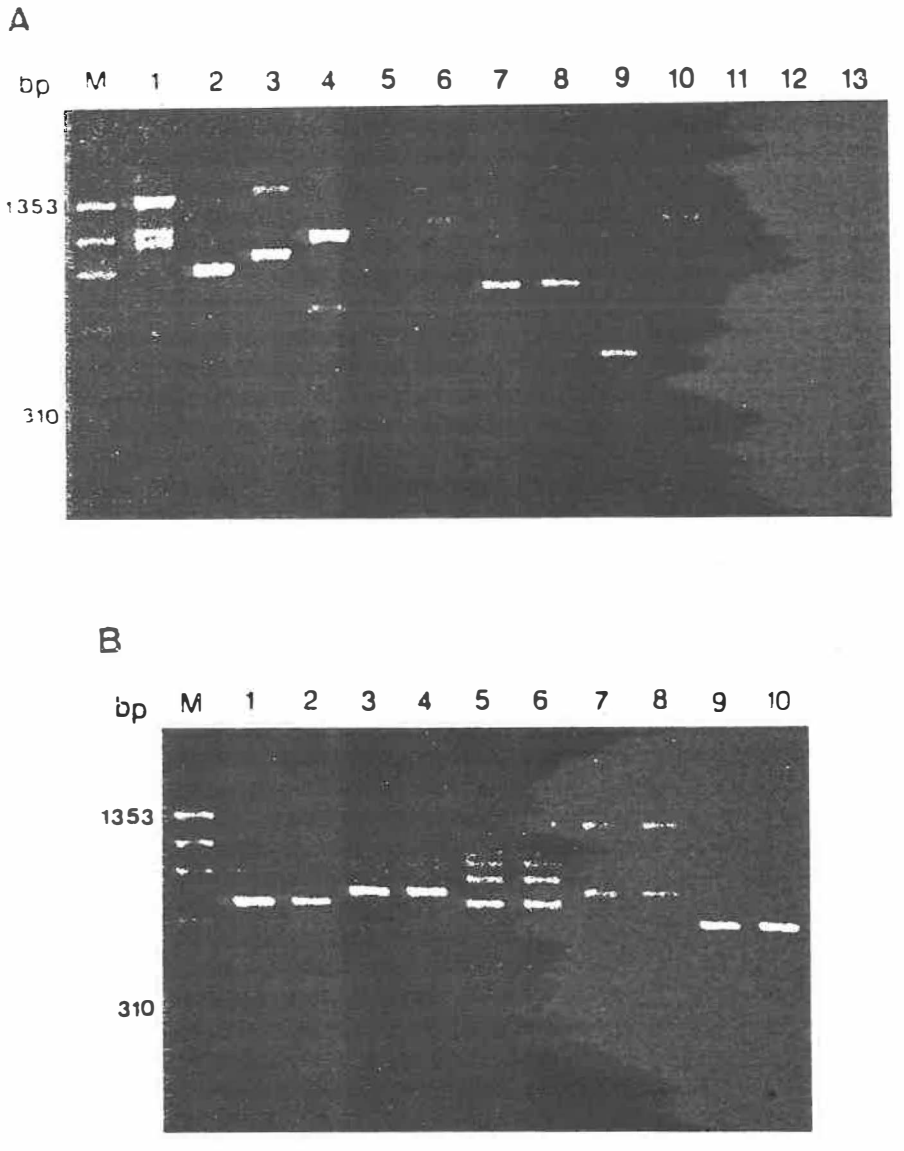


Figure 2. A) RAPD PCR products from DNA of different species isolated by our method and using the primer OPB-02 (Operon, Alameda, USA); 1: *D. melanogaster*; 2: *D. subobscura*; 3: *C. capitata*; 4: *D. oleae*; 5: *R. norvegicus* (brain); 6: *R. norvegicus* (liver); 7: *B. bocagei*; 8: *B. sclateri*; 9: *G. max*; 10: *A. thaliana*; 11: *S. frutescens*; 12: *S. cereale*; 13: *S. officinarum*. **B)** RAPD PCR from *Zea mays* extracts of DNA by our method (odd numbers, 1, 3, 5, 7, 9) and as Dellaporta et al. one (even numbers, 2, 4, 6, 8, 10) using different 10-mer primers: OPB-02 (1, 2); OPF-12 (3,4); OPF-13 (5, 6); OPF-17 (7, 8) and OPF-19 (9, 10). In both cases phage ϕX174 DNA digested with *Hae* III was used as molecular marker (M).

DNA from *Z. mays* obtained with our method has been revealed to be suitable for RFLP analysis, yielding clear restriction fragments, comparable to those obtained with DNAs from maxiprep methods (Figure 3, first four lanes). The high quality of these restriction fragments was corroborated by means of the detection of a single gene, Emb564 (Figure 3, four last lanes). Again, identical results are reported when we have used our method of extraction or another more time consuming one.

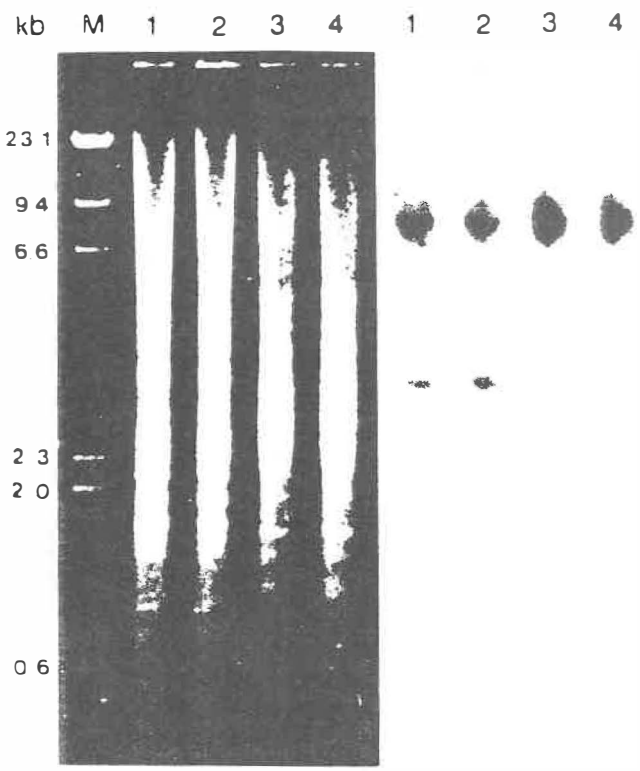


Figure 3. Detection of single copy gene in *Zea mays* by using Southern hybridisation of digoxigenin-labelled Emb564 to *Eco*R I (1, 2) and *Bam*H I (3,4) digested genomic DNA extracted by our method (1, 3) and by Dellaporta et al. (2, 4). Phage lambda DNA digested with *Hin*d III was used as molecular marker (M).

Discussion

A universal genomic microextraction method has been presented in this study. The extracted DNA has been shown to be of comparable quality to others more time consuming ones and suitable for many different molecular approaches currently used in molecular genetic analysis: specific PCR, RAPDs, RFLPs and Southern analysis.

Our DNA extraction process has several advantages when compared with other widely used protocols. It is less time-consuming than many different methods (Murray *et al.*, 1980; Dellaporta *et al.*, 1983; Rogers *et al.*, 1988; Doyle & Doyle, 1990; Pich & Schubert, 1993), and yielding identical results (Figures 1, 2B, 3). It can be used for many different plant and animal species, avoiding the need of multiple extraction buffers and protocols, while other microextraction protocols are designed specifically either for plants (Benito *et al.*, 1993; Guidet, 1994; Williams & Ronald, 1994; Stelner *et al.*, 1995) or animals (Chapco *et al.*, 1992; Cenis *et al.*, 1993). The results are highly reproducible, even when DNA extraction is carried out from different tissues of the same individual (Fig. 2B, lanes 5 and 6), while other microextraction methods have been shown to yield different RAPD profiles when DNA is obtained from different tissues (Benito *et al.*, 1993; Cheung *et al.*, 1993). Several types of analysis such as specific PCR, RAPD, restriction and Southern can be carried out, being this a great improvement, as all of the previously reported microextraction methods (Chapco *et al.*, 1992; Benito *et al.*, 1993; Cenis *et al.*, 1993; Cheung *et al.*, 1993; Guidet, 1994; Williams & Ronald, 1994; Stelner *et al.*, 1995) have been described to be useful for PCR amplifications but never for restriction and Southern analysis.

Another important advantage is the high quantity of genomic DNA recovered with this method from small amounts of biological material, what makes it an appropriate method specially when working with small size species, when there is little sample left, or when a previous screening is desired to check transformation, genotype, etc., allowing the individual going on development. The amount of DNA recovered by this method is significantly higher than that obtained by other microextraction protocols methods which yield 0.2-0.4 μg per mg of sample (Chapco *et al.*, 1992; Benito *et al.*, 1993; Cenis *et al.*, 1993; Cheung *et al.*, 1993; Guidet, 1994; Williams & Ronald, 1994; Stelner *et al.*, 1995), and it is in the range of midipreps or large scale preparations of DNA where 0.1-1 μg of DNA per mg is obtained (Murray *et al.*, 1980; Dellaporta *et al.*, 1983; Rogers *et al.*, 1988; Doyle & Doyle, 1990; Pich & Schubert, 1993). Thus, our method add the advantage of fast and wide spectrum microextractions with the high recovering of DNA of large scale preparations of DNA and the possibility of PCR, restriction and Southern analysis.

Regarding the integrated control programs, these methodologies can be applied in many different and fundamental aspects. The characterization and identification of plant cultivars provide a valuable information for plant breeding and are undoubtedly of paramount importance in the germplasm banks (Rafalski & Tingey, 1993; Caetano-Anolles, 1994). Thus, we can see that using RAPDs, we are able to characterise different species of cereal crops (Figure 2A). In the same way, in the last years, thanks to the DNA based technology, we have observed a great advance in the characterization of many crops species and cultivars as in the case of *Triticum, Vicia, Allium, Brassica*, etc. or fruit cultivars of apple, banana, papaya, etc. (for a review see Caetano-Anolles, 1994).

A deep knowledge of the pest species is fundamental in IPM programs. Thus, DNA technology favours the identification of pest species, biotypes, host races and populations (Figure 2A). Indeed, different surveys have reported the utility of these methodologies in this

kind of analysis (Ballinger-Crabtree *et al.*, 1992; Black *et al.*, 1992; Chapco *et al.*, 1992; Cenis *et al.*, 1993; Guirao *et al.*, 1994; Reyes *et al.*, 1996). Moreover, a great deal of information about the genetic structure and dynamics of pest species populations can be achieved using those approaches, all of them key aspects for successfully integrated control programs (Ballinger-Crabtree *et al.*, 1992; Black *et al.*, 1992; Haymer *et al.*, 1992; Apostol *et al.*, 1993; Haymer & McInnis, 1994; Williams *et al.*, 1994; Baruffi *et al.*, 1995; Reyes *et al.*, 1996).

Beyond the taxonomic and identification problems, those DNA approaches have been used in crops to search new resistance genes by means of the linkage between RAPD or RFLP bands and those genes, and corroborating it via Southern (Caetano-Anolles, 1994; Smith & Williams, 1994; Welsh & McCleland, 1994). This evidence the necessity of a good method of DNA extraction suitable for those methodologies, as the one we have developed, which is suitable for RAPD, RFLP and Southern analysis (Figures 2, 3). Not only for plant species, but also for animal species, resistance genes are beginning to be identify with these approaches (Quistad & Skinner, 1994).

In those species in which insecticide resistance has been detected, molecular genetic analysis (Figures 1, 2A, 3) has been proved to be efficient in identifying the gene involved, as in the case of the mosquito gamma-aminobutyric acid receptor gene (Shotkowski *et al.*, 1996) or insect glutathione S-transferases (Fournier *et al.*, 1992).

In conclusion we can say that the new method of genomic DNA microextraction that we have developed is suitable for plant and animal species, and different DNA approaches currently used in molecular biology. The special characteristics of this method make it of great interest to those working with species of small size, or many different species or taxonomic problems or screening mutations or transformed plants at early stages, specially in the field of integrated control.

Acknowledgements

We would like to thank to J.J. Muñoz and J. Barrios for their valuable help. We are grateful to C. Callejas, M. Civantos, J.A. Cortes, O. Cubero, A. Gonzalez, and A. Torrell for providing biological material. This work was supported by grants from the DGICYT: PB89-0114 and PB93-1210.

References

APOSTOL, B.L., BLACK, W.C., MILLER, B.R., REITER, P. & BEATY, B.J., 1993. Estimation of the number of full sibling families at an ovipisition site using RAPD-PCR markers: Applications to the mosquito *Aedes aegypti*. Theor. Appl. Genet. **86**: 991-1000.

BALLINGER-CRABTREE, M.E., BLACK, W. & MILLER, B.R., 1992. Use of genetic polymorphisms detected by the random-amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) for differentiation and identification of *Aedes aegypti* subspecies and populations. Am. J. Trop. Med. Hyg. **47**: 893-901

BARUFFI, L., DAMIANI, G., GUGLIELMINO, C.R., BANDIS, C., MALACRIDA, A.R. & GASPERI, G., 1995. Polymorphism within and between populations of *Ceratitis capitata*: comparison between RAPD and multilocus enzyme electrophoresis data. Heredity **74**: 425-437.

BLACK, W.C., DUTEAU, N.M., PUTERKA, G.J., NECHOLS, J.R. & PETTORINI, J.M., 1992. Use of random amplified polymorphic DNA polymerase chain reaction (RAPD-

PCR) to detect DNA polymorphisms in aphids (Homoptera: Aphididae). Bull. Ent. Research. **82**: 151-159.

BEDDINGTON, J.R., FREE, C.A. & LAWTON, J.H., 1978. Characteristics of successful natural enemies in models of biological control of insect pest. Nature **273**: 513-519.

BENITO, C., FIGUEIRAS, A.M., ZARAGOZA, C., GALLEGO, J. & DE LA PEÑA, A., 1993. Rapid identification of Triticeae genotypes from single seeds using the polymerase chain reaction. Plant. Mol. Biol. **21**: 181-183.

BERNARD, C.B. & PHILOGENE, B.J., 1993. Insecticide synergists: role, importance, and perspectives. J. Toxicol. Environ. Health **38**: 199-223.

BOULTER, D., 1993. Insect pest control by copying nature using genetically engineering crops. Phytochemistry **34**: 1453-1466.

CAETANO-ANOLLES, G., 1994. MAAP: a versatile and universal tool for genome analysis. Plant. Mol. Biol. **25**: 1011-1026.

CENIS, J.L., PEREZ, P. & FERERES, A., 1993. Identification of various aphid (Homoptera: Aphididae) species and clones by random amplified polymorphic DNA (RAPD-PCR). Ann. Entomol. Soc. Am. **86**: 545-550

CHAPCO, W., ASHTON, N.W., MARTEL, R.K.B., ANTONISHYN, N. & CROSBY, W.L., 1992. A feasibility study of the use of random amplified polymorphic DNA in the population genetics and systematics of grasshoppers. Genome **35**: 569-574.

CHEUNG, W.Y., HUBERT, N. & LANDRY, B.S., 1993. A simple and rapid DNA microextraction method for plant, animal and insect suitable for RAPD and other PCR analyses. PCR Methods Applic. **3**: 69-70.

COCKBURN, A.F., HOWELLS, A.J. & WHITTEN, M.J., 1984. Recombinant DNA technology and genetic control of pest insects. Biotechnol. Genet. Eng. Rev. **2**: 69-99.

DAY, P.R., 1996. Genetic modification of plants: significant issues and hurdles to success. Am. J. Clin. Nutr. **63**: 651S-656S.

DELLAPORTA, S.L., WOOD, J. & HICKS, J.B., 1983. A plant DNA minipreparation: Version II. Plant. Mol. Biol. Rep. **1**: 19-21.

DOYLE, J.J. & DOYLE, J.I., 1990. Isolation of plant DNA from fresh tissue. Focus **12:** 13-15.

FOURNIER, D., BRIDE, J.M., POIRIE, M. & PLAAP, F.W., 1992. Insect glutathione S-transferases. Biochemical characteristics of the major forms from houseflies susceptibile and resistant to insecticides. J. Biol. Chem. **267**: 1840-1845.

FUJIMOTO, H., ITOH, K., YAMAMOTO, M., KYOZUMA, J. & SHINAMOTO, K., 1993. Insect resistant rice generated by introduction of a modified delta-endotoxin gene of *Bacillus thuringiensis*. Biotechnology **11:** 1151-1155.

GIBSON, S. & SOMERVILLE, C., 1993. Isolating plant genes. Trends Biotechnol. **11**: 306-313.

GUIDET, F., 1994. A powerful new technique to quick prepare hundreds of plant extracts for PCR and RAPD analyses. Nucleic Acid Res. **22**: 1772-1773

GUIRAO, P., BEITIA, F. & CENIS, J.L., 1994. Aplicación de la técnica de RAPD-PCR a la taxonomía de las moscas blancas (Homoptera:Aleyrodidae). Bol. San. Veg. Plagas **20:** 757-764.

HAYMER, D. & McINNIS, D., 1994. Resolution of populations of the Mediterranean fruit fly at the DNA level using random primer for the polymerase chain reaction. Genome **37**: 244-248.

HAYMER, D., McINNIS, D. & ARCANGELI, L., 1992. Genetic variation between strains of the Mediterranean fruit fly, *Ceratitis capitata*, detected by DNA fingerprinting. Genome **35**: 528-533.

JOSHI, R.L. & JOSHI, K., 1996. Strategies for expression of foreign genes in plants. Potential use of engineered viruses. FEBS Lett. **281**: 1-8.

KITTO, G.B., 1983. Biological control of insect plants. Isozymes Curr. Trop. Biol. Med. Res. **11**: 197-211.

LANDRY, B.S., DEXTRAZE, L. & BOIVIN, G., 1993. Random amplified polymorphic DNA markers for DNA fingerprinting and genetic variability assessment of minute parasitic wasp species (Hymenoptera: Mymanidae and Trichogrannatidae) used in biological control programs of phytophagous insects. Genome **36**: 580-587.

MURRAY, M.G. & THOMPSON, W.F., 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res. **8**: 4321-4325.

PERLAK, F.J., DEATON, R.W., ARMSTRONG, T.A., FUCHS, R.K., SIMS, S.R., GREENPLACE, J.T. & FISCHHOFF, D.A., 1990. Insect resistant cotton plants. Biotechnology **8**: 939-943.

PICH, U. & SCHUBERT, I., 1993. Midiprep method for isolating DNA from plants with a high content of polyphenolics. Nucleic Acid Res. **21**: 3328.

QUISTAD, G.B. & SKINNER, W.S. 1994. Isolations and sequencing of insecticidal peptides from the primitive spider, *Plectreurys tristis* (Simon). J. Biol. Chem. **269**:11098-11101.

RAFALSKI, J.A. & TINGEY, S.V., 1993. Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. Trends in Genet. **9**: 275-286.

REYES, A., CALLEJAS, C., RODA, P. & OCHANDO, M.D., 1996. Caracterización genética en *Ceratitis capitata* asociada a fruto hospedador. II. Análisis mediante RAPD-PCR. Bol. San. Veg. Plagas **22**: 361-371.

ROBINSON, A.S. 1983. Sex-ratio manipulation in relation to insect pest control. Annu. Rev. Genet. **17**:191-214.

ROGERS, S.O. & BENDICH, A.J., 1988. Extraction of DNA from plant tissues. Plant. Mol. Biol. Man. A**6**: 1-10.

SAUL, S.H., 1986. Biotechnology and fruit flies. In: Fruit Flies. A.P. Economopoulos (Ed.). Elsevier. Amsterdam. pp; 191-195.

SHOTKOSKI, F., MORIS, A.C., JAMES, A.A. & FRENCH-CONSTANT, R.H., 1996. Functional analysis of a mosquito gamma-aminobutyric acid receptor gene promoter. Gene **168**: 127-133.

SMITH, R.F. & CALVERT, D.J., 1976. Health-related aspects of integrated pest management. Environ. Health Perspect. **14**: 185-191.

SMITH, J.S.C. & WILLIAMS, J.G.K., 1994. Arbitrary primer mediated fingerprinting in plants: case studies in plant breeding, taxonomy and phylogeny. In: Molecular Ecology and Evolution: Approaches and Applications. B. Schierwater, B. Streit, G.P. Wagner & R. DeSalle (Eds.).Birkhauser Verlag. Basel. pp. 5-15.

STELNER, J.J., POKLEMBA, C.J., FJELLSTROM, R.G. & ELLIOT, L.F., 1995. A rapid one-tube genomic DNA extraction process for PCR and RAPD analyses. Nucleic Acid Res. **23**: 2569-2570.

WELSH, J. & McCLELAND, M., 1994. Fingerprinting using arbitrarily primed PCR: Application to genetic mapping, population biology, epidemiology, and detection of differentially expressed RNAs. In: The Polymerase Chain Reaction. K.B. Mullis, F. Ferre & R.A. Gibbs (Eds.). Birkhauser. Boston. pp. 296-303.

WILLIAMS, C.E. & RONALD, P.C., 1994. PCR template-DNA isolate quickly from monocot and dicot leaves without tissue homogeneization. Nucleic Acid Res. **22**: 1917-1918.

WILLIAMS, B. & TSANG, A., 1991. A maize gene expressed during embryogenesis is abscisic acid-inducible and highly conserved. Plant. Mol. Biol. **16**: 919-923.

WILLIAMS, C.L., GOLDSON, S.L., BAIRD, D.B. & BULLOCK, D.W., 1994. Geographical origin of an introduced pest *Listronotus bonariensis* (Kuschel), determined by RAPD analysis. Heredity **72**: 412-419.

USE OF ENTOMOPATHOGENIC FUNGI FOR PEST CONTROL IN PROTECTED CROPS IN ITALY.

L. Rovesti, G. Grazzi & R. Viccinelli

C.N.R. - Centro di Studio dei Fitofarmaci
via Filippo Re, 8 - 40126 Bologna - Italy

Summary

As part of an E.U.-funded project ("Insect pathogenic fungi for environmentally friendly pest control in the glasshouse" (AIR3 - CT94 - 1352)), a series of trials have been carried out to evaluate the feasibility of using entomopathogenic fungi to control various pests in protected crops in italy. Target species so far have included aphids (on cucumber, aubergine, sage), whiteflies (on gerbera, poinsettia, aubergine and tomato) and thrips (on gerbera and aubergine). Results indicate that mycoinsecticides could find practical application in the control of whiteflies also under the climatic and cultural conditions found in southern Europe. On the other hand, not so promising are the results against aphids and thrips, for which more work is needed before the actual potential of available mycoinsecticides can be fully evaluated.

Introduction

The use of entomopathogenic fungi (EPF) to control insect pests in protected crops has long been attempted in North-European countries, where some mycoinsecticides are already registered and commercially available. On the other hand, much less experience has been gained so far on the use of these bioinsecticides under the conditions found in Southern Europe, the main differences being both climatic and agronomical. As a matter of fact, the vast majority of greenhouses in South European countries are simple structures with very little control over the climatic conditions inside them. As a consequence, they are characterised by high temperatures and low relative humidity (RH) levels during much of the growing season. As it is generally aknowledged that relative humidity is the factor which mostly affects the efficacy of mycoinsecticides, it is important that specific expertise be gained under "southern" conditions, if the use of these biocontrol agents is to be implemented beyond its present limits. For this reason work was undertaken in Italy on this subject, and a series of trials carried out during 1995-1996, within the framework of the E.U.-funded project "Insect pathogenic fungi for environmentally friendly pest control in the glasshouse" (AIR3 - CT94 - 1352).

Materials & methods

Experimental design. Trials were carried out at commercial growers'. Except in case of the trial on sage, when plants were arranged in a separate greenhouse, trials took place in ordinary greenhouses which were run according to good agricultural practices. The experimental design used was randomised blocks, with plot size varying with crop and pest species (from 12-35 m^2 in case of: aphids on aubergine and cucumber; whiteflies on tomato, gerbera and aubergine; thrips on aubergine, and from 1-5 m^2 in case of: aphids on sage; whiteflies on poinsettia; thrips on gerbera). Details of pest species/crops are indicated in the "Results" section.

The technical assistance of Ms. Elena Chiapparino, Mr. Andrea Crudeli and Ms. Elena Ogheri is gratefully acknowledged.

Recording of climatic conditions. Climatic parameters (T°C, R.H. and solar radiation) were recorded in all trials, using a digital data logger (model Guardian 1, Silimet S.r.l., Italy). Besides average daily values, average night (20,00-08,00 hs.) and day (08,00-20,00 hs.) values were also calculated.

Equipment for application of treatments. Sprays were generally applied by means of a motorised knapsack sprayer (mod. Fox F320, Fox S.r.l., Italy), or pressure-operated handsprayer in case of small plots (poinsettia, sage). Water-sensitive paper was used to ensure that the spraying equipment would provide adequate coverage of the vegetation. In case of gerbera, this led to substitute the knapsack sprayer for a lower volume knapsack atomizer (mod. S80, KWH, Holland) after the first application. Application of *V. lecanii* in a dry form (powder) was made by diluting the required amount of spore powder with silicium oxide (1:3 - 1:9 depending on plot size) and applying the resulting mixture with a hand-operated duster.

Experimental treatments. The EPF tested included *V. lecanii*- (as Vertalec® and Mycotal®, WP formulations, provided by Koppert, The Netherlands), *Paecilomyces fumosoroseus* (granular formulation provided by Biobest, Belgium) and *Metarhizium anisopliae* (two experimental formulations, i.e. wettable powder and granular formulation, provided by Bayer AG, Germany).

For spray applications, spores were pre-soaked in water (1,5-3 hs.). Against thrips, beside foliar sprays soil applications were also made, using either non-presoaked spores from w.p. formulates or (only in case of *M. anisopliae)* a granular formulation which was incorporated into the 5 top cm of soil 3 days before the start of the trial. The addition of the oil adjuvant, Codacide (Microcide Ltd., United Kingdom), was also tested together with *V. lecanii*.

Assessments. In all trials assessments were made just prior to the first treatment application, and repeated on a weekly basis afterwards. Aphids: a given number of plants were marked beforehand, then checked weekly recording the number of living/dead/parasitised aphids. Whiteflies: at each assessment a set number of leaves were picked from the plots and the number of living/dead/parasitised larvae and pupae counted with the aid of a dissecting microscope (in case of large leaves, i.e. aubergine and gerbera, a 9 cm d. (=63,5 cm^2) disk was cut from the leaf and counted for the assessment). Thrips: turpentine extractors were constructed, in which flowers (gerbera) or small leaves (aubergine) were placed. Thrips repelled by the turpentine vapours were caught in a tube filled with 70% ethanol. Preliminary efficiency trials had shown an acceptable efficiency of these simple traps (approx. 90% recovery in case of flowers, 50-60% with leaves).

Results

Results of the greenhouse trials are summarised in graphs 1-16 in the following pages (only results for the most significant treatments are reported). The No. of insects/leaf was the criteria adopted to express the efficacy of treatments in all cases but one (trial on gerbera, No. 6, for which % larval mortality is reported). In each graph, the applications of the chemical insecticide (boldtype arrows) and of the mycoinsecticides (thinline arrows) is also indicated.

Aphids.

Control by *V. lecanii* was on the whole very poor. In one of the trials on cucumber (n. 2) the aphid population was controlled by the parasite, *Aphidius colemani,* a single introduction of which had been made by the grower a week before the start of the trial. Parasitisation rate increased very rapidly, almost completely eliminating the pest population in approximately a fortnight. None of the fungal treatments interfered with the activity of the parasite. As for the other two trials on this crop, in one of them (n. 3) even the repeated application of the

chemical (heptenophos) did not control the pest. On sage (n. 5), treatments appeared to be more effective and, despite no fungus-killed insects could be seen, the number of aphids on treated plants was consistently lower than on untreated ones (except in case of *V. lecanii* plus Codacide oil).

Whiteflies

On gerbera (n. 6-7), at least in one instance (n. 7), the whitefly population was supposedly a resistant one, and weekly treatments made by the grower using mixtures of chemicals would not achieve an acceptable control. In this trial, a double application of buprofezin was totally ineffective. In such situation 4 weekly sprays of *V. lecanii* gave the best control, preventing an increase in the number of whitefly larvae/leaf. In this specific trial the application of *P. fumosoroseus* was ineffective; however, it must be said that, due to a mistake, the use of 0.05% Tween 80 (recommended by Biobest) was omitted. The high mortality recorded in the control plots in the other trial (n. 6) was due to the overcrowding of larval population and the abundant production of honeydew.

On tomato (n. 8-9) weekly applications of the fungi remarkably reduced the number of insects/leaf, though control was not as effective as with buprofezin. The addition of Codacide oil appeared to enhance the performance of *V.lecanii* in one istance (n. 8), but not in the other. In the same trial the whitefly population was effectively eliminated after two weeks of the start of the trial by a naturally occurring predator, *Macrolophus* sp.. An assessment made by counting adults and larval stages of the predator did not show differences among the different treatments (data not shown).

At the same grower's a trial with large (1200 m^2/plot, each containing two 25 m^2 untreated areas), unreplicated plots had been set up to compare *V. lecanii* to buprofezin. Also in this case predation by *Macrolophus* did not allow to complete the trial.

On aubergine (n. 10-11) both trials were started in presence of a rather high level of infestation (much of which represented by pupae in trial n. 11). The best control, though still not satisfactory, was obtained with a double application of buprofezin. On the whole all fungal treatments reduced the pest population compared to the untreated, but none was comparable to the chemical.

On poinsettia (n. 12-14), despite the favourable climatic conditions, none of the fungi gave good control, though all treatments reduced to a lesser or greater extent the number of insects/leaf. It should be noted however that in one of the trials (n. 14) neither buprofezin nor the grower's treatment schedule (one fumigation with sulfotep plus one spray with parathion) gave better results than the fungi. In the second trial, with a somewhat lower infestation level, control by the grower (two chemical sprays) was almost complete.

Thrips

The only treatment which effectively controlled *F. occidentalis* was a double application of acrinatrine (Rufast®). However, it is important to mention that this chemical is not allowed for use in greenhouses. Of the other treatments none controlled the pest, including the largely used acephate. In both trials the pressure from the pest was very high, in spite of the fact that the populations were lowered prior to the start of the trials by chemically spraying the crops.

On aubergine (n. 16), two of the fungi (i.e. *M. anisopliae and P. fumosoroseus)* appeared to limit to some extent the development of the trhip population, compared to *V. lecanii* (alone or with the oil adjuvant). At a visual assessment carried out at the end of the trial, plants treated with *M. anisopliae* looked slightly less damaged than those from control plots or sprayed with any of the other fungi (data not shown). The constant decline in the

number of thrips/leaf recorded in the control plots might be explained with the fact that the leaves of these plants became quickly damaged, resulting not attractive to the thrips any more.

Discussion

Average daily RH values recorded in the trials against aphids on cucumber were in the range 70-80%, with night RH values of approx. 90%. This was not enough to induce sporulation of *V. lecanii* for the whole duration of the trials, while a remarkable growth of the fungus on aphid cadavers was observed following a decrease in temperature and a consequent increase in RH after the trials n. 2-3 were terminated. In aubergine a wider range of RH values was recorded; in this case sporulation was observed only after the very wide temperature range recorded in the first half of the trial (lowest temp. of approx. 10°C, highest of up to 40°C) narrowed; this despite an overall decrease in RH values. From our observations it appears therefore that more than the absolute RH values, growth and sporulation of *V. lecanii* is influenced by the combination of RH and temperature. More detailed laboratory studies are needed before any definite conclusion can be drawn on this aspect. Obviously, lack of sporulation on parasitised aphids is a drawback, limiting the horizontal spread of the mycosis to other aphids of the colony, and therefore reducing the overall effect of the treatment.

More promising appears the potential of EPF for the control of whiteflies. In this case, to achieve good results, applications should be started at an early stage of infestation, as in case of a late start eradication of the pest may be difficult even using a chemical insecticide (as observed in aubergine). In case of *V. lecanii*, the strain used in Mycotal® seems reasonably tolerant to low humidity, and acceptable results were obtained also in "dry" crops such as gerbera where the recorded values of RH were 70-80% (24 hour value) or 50-60% (daytime value). To this regard, also Ravensberg et al. (1990a) observed the lack of correlation between RH level in the greenhouse and whitefly mortality, pointing out the importance of the microclimatic conditions at the phyllosphere level. It should be noted that artificially increasing the humidity in the greenhouse, which has been suggested by some authors (e.g. Helyer et al., 1992) would be acceptable only to very few growers, if any. On poinsettia, where climatic conditions were very favourable to the fungi, the fact that only partial control was achieved may be due to either the pest species (better control was generally obtained for *T. vaporariorum* than for *B. tabaci*) or the plant species, or possibly to both.

From our trials it appears also that the performance of these fungi is not strictly dependent on the volume of water in which they are applied, as long as spores are correctly delivered to the appropriate sites on the crop. To this regard, despite the low efficiency of the duster used in our trials, the efficacy of the application of *V. lecanii* in a dry form was in some cases comparable to that obtained with the application in water. Silicium oxide may have accounted in part for mortality due to its slight insecticidal properties, but sporulated cadavers were regularly found for both whiteflies and aphids (in this latter case the sporulation, observed only at the end of trials as mentioned above, was on the whole comparable to that of the plots where the fungus was applyed by spraying).

The use of adjuvants (such as Codacide) may increase the overall efficiency of the mycoinsecticides, although our results have been inconsistent. It should be noted however that with the spraying equipment used in our trials not always the appropriate level of agitation of the tank mixture recommended by the producer (Microcide Ltd.) may have been satisfied. Nevertheless, it is worth mentioning that when this oil was added to *V. lecanii*, the rate of parasitised (=overgrown with the fungus) whiteflies was consistently higher than when using the fungus alone. In a previous experience, Helyer (1993) observed that the addition of Codacide increased significantly the effect of the treatment with *V. lecanii* on thrips. He also

noticed a direct insecticidal effect of the oil on both thrips and spider mites. It should be noted however that the application rate he used was very high and unlikely to be acceptable in practice.

In conclusion, while the currently available mycoinsecticides appear still not effective enough for practical control of aphids, they may certainly be valuable for whitefly control, especially where resistance to chemical pesticides is a problem. As for thrip control, it is difficult at this stage to fully understand the potential of these fungi, but certainly there is a lot of scope to their development in this field, especially taking into account the level of resistance shown by these pests to many (all?) of the chemicals authorized for greenhouse use. All in all, the best option for the control of all the mentioned pests seems the development of an IPM strategy which can benefit of the demonstrated harmlesness of these fungi to many beneficial insects (see e.g. Ravensberg et al., 1990b).

References

MICROCIDE Ltd. Codacide: Directions for use.

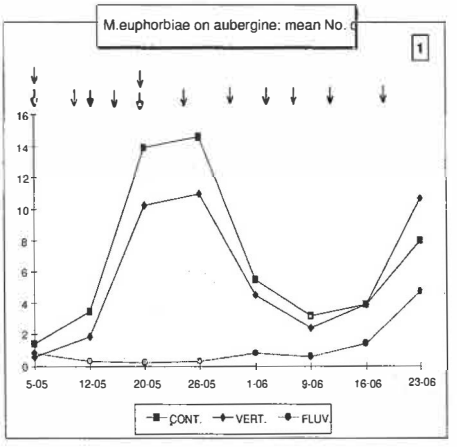
RAVENSBERG, W.J., MALAIS, M. & VAN DER SCHAAF, D.A. (1990a). Applications of Verticillium lecanii in tomatoes and cucumbers to control whitefly and thrips. SROP/WPRS Bull.**XIII/5**: 173-178.

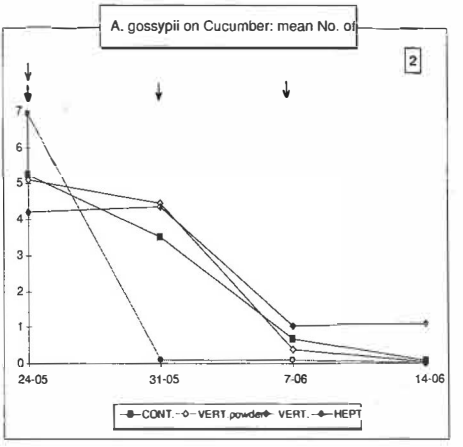
HELYER, N. (1993). IOBC/wprs Bulletin, vol. **16 (2):** 63-66.

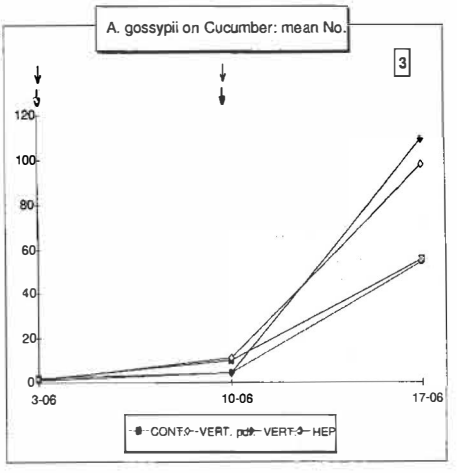
HELYER, N., GILL, G., BYWATER, A. & CHAMBERS, R. (1992). Elevated humidities for control of chrysanthemum pests with Verticillium lecanii. Pestic. Sci., **36**: 373-378.

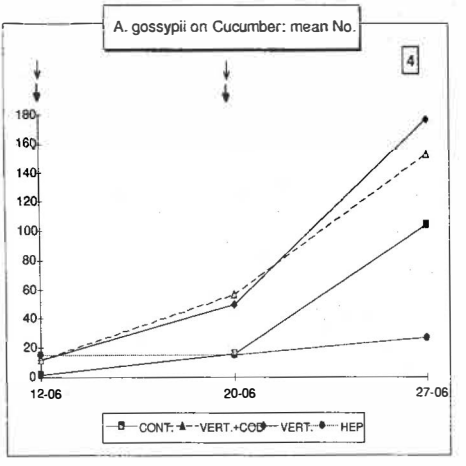
RAVENSBERG, W.J., MALAIS, M. & VAN DER SCHAAF, D.A. (1990b). Verticillium lecanii as a microbial insecticide against glasshouse whitefly. Proc. BCPC - Pests and Diseases, 265-268.

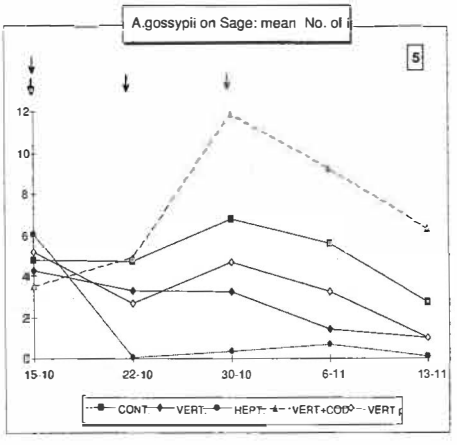
Trials on aphids







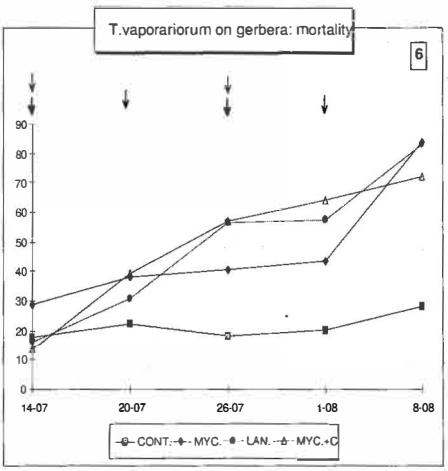


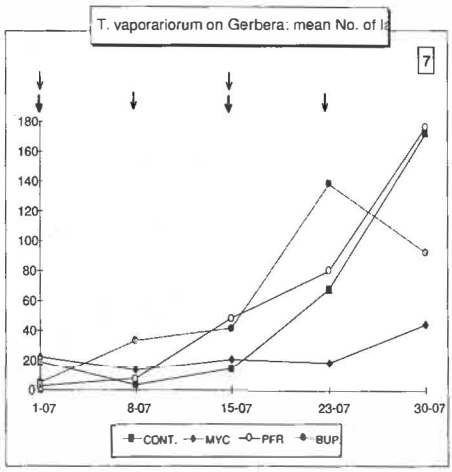


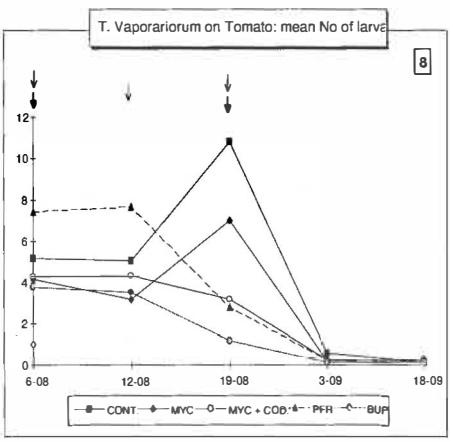
Legend:

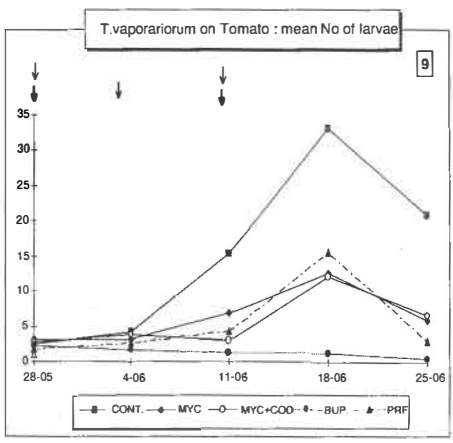
ACEPH.= Acephate
BUP.= Buprofezin
CONT. = Control
COD.= Codacide
HEPT. = Heptenophos
MYC.= V. lecanii (Mycotal)
M.a. = M. anisopliae
PFR = P. fumosoroseus
RUFAST: Acrinatrine
VERT.= V. lecanii (Vertalec)

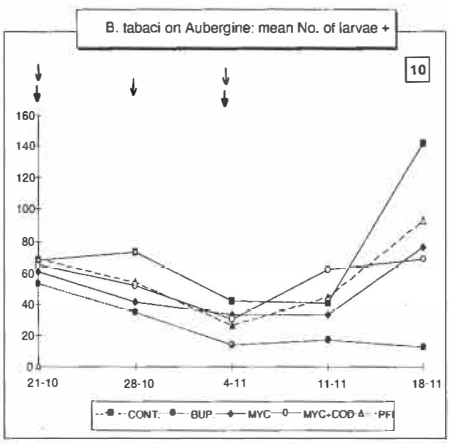
Trials on Whiteflies

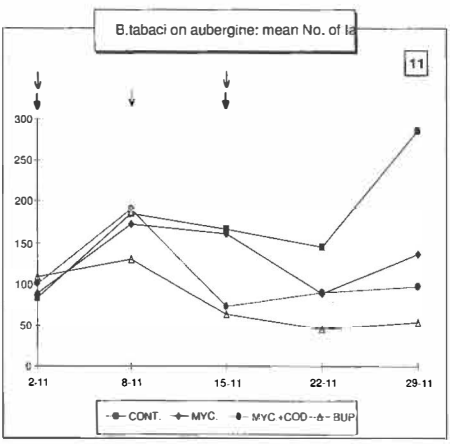


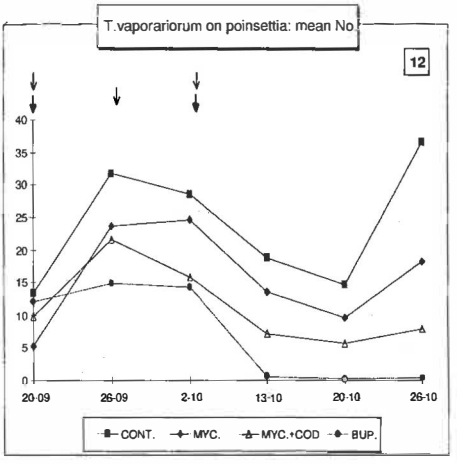


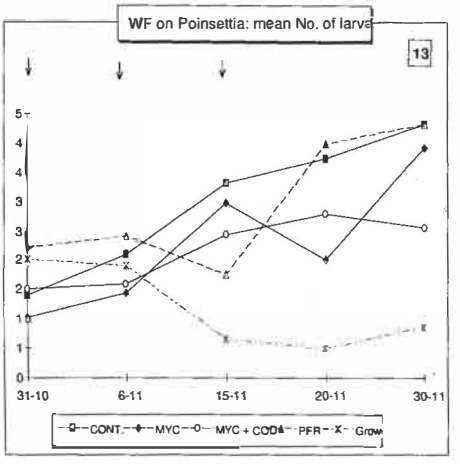


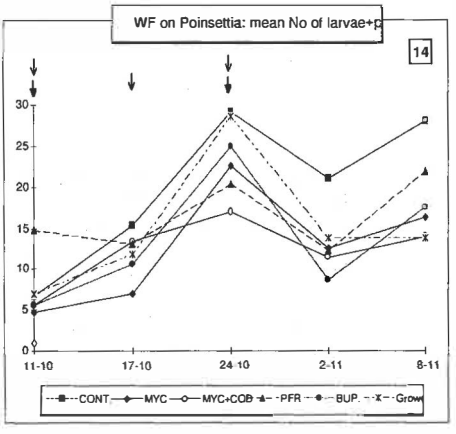




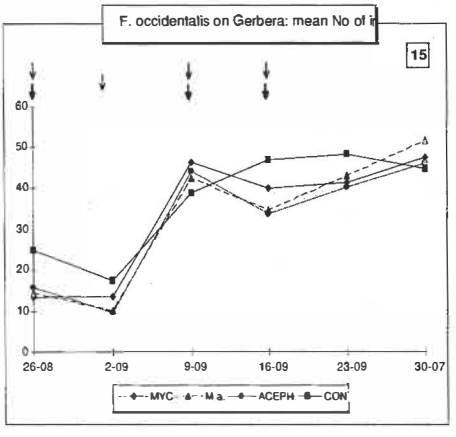


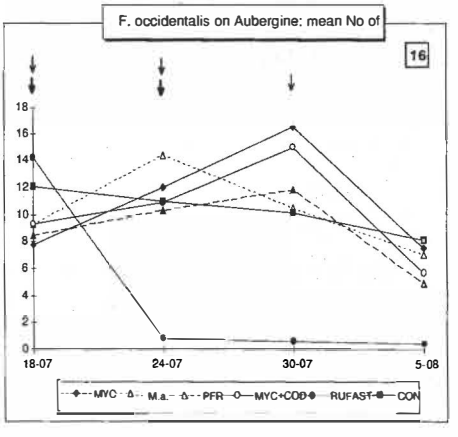






Trials on Thrips





POSTER ABSTRACTS
RESUMÉES DES POSTERS

IPM IN VEGETABLE PROTECTED CROPS IN CANARY ISLANDS

A. Carnero-Hernández; Hernández-García, M.; Torres-del-Castillo, R.; Hernández-Suárez, E.; Pérez-Padrón, F.

Departamento de Protección Vegetal. Instituto Canario de Investigaciones Agrarias.
Aptdo. 60. 38200 La Laguna, Tenerife. Islas Canarias. España.

Abstract

This article summarizes some different aspects of the Integrated Pest Management in the Canary Islands with mention of the most important pests present on tomato and sweet pepper crops.

In protected tomato crops the possibilities of biological control of noctuid eggs (*Lepidoptera: Noctuidae*) by the predator *Cyrtopeltis tenuis* (*Heteroptera: Miridae*) has been studied. The control by *C. tenuis* has been very effective. Also the action of the mirid on larvaes and adults of whiteflies *Trialeurodes vaporariorum* and *Bemisia tabaci* has been analyzed. In relation to *B. tabaci* the presence of the *B. tabaci* **strain "B"** is confirmed. Also the possibilities of biological control by the parasites of *Encarsia* genus (*Hymenoptera: Aphelinidae*) are being studied.

On sweet pepper protected crops some trials have been conducted to study the population dynamic of the main aphid pests (*Aphis gossypii* and *Myzus persicae*) and the aphid-parasitoid relationships as well as to evaluate the efficacy of *Aphidius colemani* and *Lysiblebus testaceipes* (*Hymenoptera: Aphidiidae*) as biological control agents against these aphids. A new check list of aphid predators is given.

Lastly, detailed information concerning biological control of *Frankliniella occidentalis* (*Thysanoptera: Thripidae*) by *Orius albidipennis* (*Heteroptera: Anthocoridae*) and leafminer are presented.

DEMONSTRATION GREENHOUSES OF INTEGRATED PEST MANAGEMENT ON HORTICULTURE IN REGIÃO AUTÓNOMA DA MADEIRA

A. P. Félix[1], P. J. Duarte[2] & A. Mexia[3]

[1] Direcção de Serviços de Investigação Agrícola, Divisão de Protecção Integrada, Estrada Engº Abel Vieira, 9135 Camacha - Madeira
[2] Direcção de Serviços de Produção Agrícola, Divisão de Horticultura Caminho Grande e Preces, 9300 Câmara de Lobos - Madeira
[3] Secção Autónoma de Protecção Integrada, Instituto Superior de Agronomia Tapada da Ajuda, P-1399 Lisboa Codex

Abstract
Since 1993 has been in developded the Subprogramme Control of *Trialeurodes vaporariorum* (Westwood) / POSEIMA - Madeira. As a part of this Subprogramme demonstration greenhouses were installed in four places of the Região Autónoma da Madeira with the objective to use methods and technics of integrated pest management and divulged and demonstate near farmers and technicians of the region.
We present the developed methodologies and the obtained results.

Key-words: Integrated pest management, protected crops, horticulturals, Madeira

EFFECTIVENESS OF SODIUM BICARBONATE AGAINST POWDERY MILDEW OF ROSE AND PEACH

A. M. Pennisi

Dipartimento di Agrochimica e Agrobiologia, University of Reggio Calabria, Pza S. Francesco di Sales, 2, 89061 Gallina (RC), Italy[1].

Abstract

Powdery mildew (*Sphaeroteca pannosa* (Wallr.:Fr.) Lev. var. *rosae*) is the most relevant disease of rose in plastic-house cultivations in Sicily. A distinct variety of the same fungus (*S. pannosa* (Wallr.:Fr.) Lev. var. *persicae*) causes powdery mildew of peach, the major disease of this plant in some producing areas of Italy. Multiple applications of fungicides per growing season to control mildew have resulted in the selection of fungal populations resistant to the benzimidazole and DMI group of fungicides. The risk of fungicide resistance and the public concern about the reduction of the use of pesticides on food crops induced us to test the effectiveness of sodium bicarbonate as an alternative "biocompatible" fungicide in controlling powdery mildew of rose and peach.

In 1993, a series of experiments was performed in a plastic-house with PE film covering, on six cultivars of rose (Dallas, Dora, Jagranda, Marbella, Monalisa, Omega).

A separate series of experiments was performed in an orchard on eight cultivars of peach (Glover, Hale, Settembrina, Red Haven, Fayette, Fairtime, O'Henry, Suncrest) and three of nectarine (Andromeda, Fantasia, Sbergio) for three consecutive years, 1992, 1993 and 1994.

The sodium bicarbonate at all concentrations tested significantly reduced the incidence and severity of powdery mildew infections on both rose and peach compared to the control and was not phytotoxic. On peach and nectarine the treatment with $NaHCO_3$ at concentrations of 400 g/hl, plus 1% mineral oil, applied as a foliar spray at 7-days time interval, was more effective than Myclobutanil. Conversely on rose Myclobutanil was more effective. Sodium bicarbonate, however, reduced significantly powdery mildew infections compared to the control. The results show that the bicarbonate has a preventive, but not curative effect. This being not toxic and environmentally safe, appear suitable to be used in integrated control and in anti-resistance strategies both in plastic-house and in open field.

[1] Phone: +39-965-682754, fax: +39-965-682755. E-mail: ampennisi@csiins.unirc.it

BIOLOGICAL CONTROL OF *TETRANYCHUS CINNABARINUS* BY *Phytoseiulus persimilis* UNDER SMALL GROWER HIGH TUNNEL PLASTIC GREENHOUSE CONDITIONS.

E. Sekeroglu T. Colkesen K. Karut C. Kazak
Dept. of Plant Protection, C. U. Agricultural Faculty
01330 Adana, Turkey

Abstract
This study was carried in 30 m long high tunnel plastic greenhouse with 6 growing beds. Predators were released three times with one week interval. About 10000 predators were released to entire area at each release by evenly distributing predators to 3 m intervals. Counts of prey and predators were done on 40 randomly selected leaves from each bed. Each bed was evaluated separately. Although the numbers of *Phytoseiulus persimilis* was steady and more or less similar for all six beds, population development of *Tetranychus cinnabarinus* showed some differences among the beds. The very low proportion of adults in *T. cinnabarinus* population structure in all beds indicated that there was a heavier predation pressure by *P. persimilis* on immatures of prey.

Please correspond to E. Sekeroglu
e-mail: seker@pamuk.cu.edu.tr

POSSIBILITIES OF APPLICATION OF PREPARATES ON NATURAL BASIS IN THE ENVIRONMENT SAVING PEST MANAGEMENT (IPM) OF GREENHOUSE PAPRIKA

Carnero, A.[1]; M. Hernández-García[1]; R. Torres[2]; E. Hernández-Suárez[1]; I. Kajati[3]; Z. Ilovai[4]; E. Kiss[4]; Cs. Budai[4]; I. Hatala-Zsellér[4]; Zs. Dancsházy[3].

(1) Dpto. Protección Vegetal del Instituto Canario de Investigaciones Agrarias. Apartado 60, 38200 La Laguna - Tenerife, Islas Canarias. Spain
(2) Servicio de Agricultura del Cabildo Insular de Tenerife.
(3) Budapest Fövárosi Növényegégügyi Allomás. Budapest, Hungary.
(4) Csongrád Megyej Növényegégügyi és Talajvédelmi Allomás-Biologiai Védekezési Laboratórium. Hódmezövasárhely. Hungary.

Abstract

The primary pest in paprika production under glasshouse are the aphids, thrips and moths. They differ from the other pest in that they are vector of several virus diseases. Present management principles have focused on decreasing the vector numbers, indirect methods have been used for restricting the spread of viruses.

A shift from the present practice would be required by using substances which: 1.prevent virus transmission, 2. are repellent and 3. are safe for the parasites.

In the frame of the intergovernmental scientific cooperation agreement between Hungary and Spain, the Hungarian experts could study some active ingredients of natural origin for this particular aim on the island of Tenerife. In addition to testing in a pilot experiment the efficiency of natural pesticides against the pest in a netthouse for organic production, we studied the side-effects on aphid parasites and predators, as well as the occurrence of viruses.

Experimental results show that the studied substances (paraffin oil, fatty acid copper salt, white oil, fatty acid potassium salt and calcium polisulfid) are adequate to stop the early rapid increase of aphids, without disturbing the establishment of *Aphidius colemani*. Almost no virus diseases have developed due to the infection free young plants.

SLUG PESTS OF GREENHOUSE ORNAMENTALS

Ewa Dankowska & *Tadeusz* Baranowski.

Department of Plant Protection, University of Agriculture,
ul.D[1]browskiego 169/171, Poznañ, Poland

Abstract

Slugs are very important and troublesome pests in control of greenhouse ornamental plants in Poland. The most harmful species are: *Deroceras laeve* (Mûll.) and *Lehmania valentiana* (Ferussac). *D. laeve* is the most widely distributed species and it usually occurs on the following plants species: Gerbera, Alstroemeria, Asplenium, Cymbidium, Spathiphyllum. *D. leave* is particularly dangerous in gerbera, crop where it can cause damages of the affected plant parts (flowers, leaves).

This presentation shows the results of the experiments on the rhabditid nematode *Phasmarhabditis hermaphrodita* as a potential biological control agent for slugs.

ÉTUDE COMPARATIVE DE LA RÉPARTITION DE LA POPULATION DE *Bemisia tabaci* GENNADIUS (HOMOPTERA: ALEYRODIDAE) SUR 8 VARIÉTÉS DE PIMENT SOUS ABRI-SERRE

Benmessaoud Boukhalfa H.

Département de Zoologie Agricole et Forestière
École Nationale Supérieure d'Agronomie, El Harrach (Alger), Algérie

Résumé
L'étude de la sensibilité variétale d'une culture vis à vis de l'insecte ravageur, Bemisia tabaci, verie selon des proportions sensibles. L'expérience pratiquée, sur 8 variétés de piments codées V1, V2, V3, V4, V5, V6, V7 et V8 a été menée sous le même abri-serre pour les différentes variétés. L'infestation naturelle a démontré que la répartition spatio-temporelle de l'insecte, se faisait d'une manière identique pour la majorité des variétés de piments utilisées.
Cependant, deux variétés semblent se dégager du lot avec un taux d'infestation de 22% et 16% pour les variétés V5 et V8, tandis que pour les autres variétés, le taux d'infestation varie de 9 à 11% de la population totale.

Introduction

Le déprédateur *Bemisia tabaci*, a été signalée pour la première fois dans la région de Biskra (Sud-est Algérien) par Benmessaoud et al. en 1989.

Cette région, de nature subdésertique et à vocation strictement phoénicicole, abrite depuis quelques années des cultures maraîchères variées, et plus particulièrement le piment qui est très appreciées dans les plats culinaires de la région.

B. tabaci, comme tous les aleurodes, passe par les 3 stades de développement (embryonnaire, larvaire et imaginale). Seules les larves néonates et les adultes sont mobiles. Actuellement ce ravageur est un facteur limitant de la production de la production de piment et constitue, malgré les traitements insecticides appliqués, un redoutable déprédateur.

Plusieurs travaux sur la résistance de différentes plantes hôtes ont été réalisées notamment par Berlinger *et al.* (1983), Barten *et al.* (1994) et Van Giessen *et al.*(1995).

Une étude préliminaire de la répartition des populations de *B. tabaci* sur 8 variétés de piment a été effectuée sous un abri-serre, dans le but de comparer la sensibilité d'une variété par rapport à une autre vis à vis de l'infestation naturelle de *B. tabaci*.

Matériel et Méthodes

L'étude expérimentale s'est réalisée sous un abri-serre de 400 m^2. Un plan de culture de 8 variétés de piments, réparties sur 3 lignes de 57 plants par variété, soit un total de 171 plants. La répartition des différentes variétés sur la parcelle s'est faite d'une manière aléatoire.

L'échantillonnage, hebdomadaire, des populations est réalisée par le dénombrement des seuls stades, visibles à l'oeil nu, soient les adultes et les larves de 4ème stade. Le choix de 3 plants par variété, pris au hasard, pour chaque observation répond au plan d'échantillonnage aléatoire.

Résultats

L'analyse des résultats obtenus montre que l'évolution des populations imaginales et larvaire (Fig.1) dégage 4 maxima de densités et un cinquième inachevé, ce qui correspondrait à 5

générations d'adultes. Le maximum est enregistré le 25 Mars, avec une densité de 4.8 adultes au dm^2.

Le même maximum est enregistré pour les larves de 4ème stade le 25 Mars avec une densité moyenne de 27.66 larves par dm^2 (Fig.1).

D'autres parts, la comparaison des populations par variété, démontre que (Fig.2), les larves de quatrième stade se retrouvent à des fortes proportions sur les variétés V5 et V8 avec respectivement 21 et 18% qui seraient les deux variétés qui offriraient le meilleur endroit pour leur développement. Les six autres variétés se partagent le reste de la population de larves de quatrième stade. Les populations adultes choisissent les mêmes variétés V5 et V8 avec des proportions assez fortes de 22 et 20% et des densités moyennes qui dépasseraient les 12 individus par dm^2.

Conclusions

Ainsi donc, le choix de l'insecte pour l'implantation de son lieu de développement est un facteur déterminant. Le végétal offre différentes possibilités de développement à l'insecte qui varient selon la variété. L'explication de ce phenomène serait peut-être d'origine chimique. Des recherches dans ce sens et la connaissance exacte de l'élément en question, nous aideraient à sélectionner la variété la plus apte à se défendre contre tous les ennemis naturels et par là réduire les utilisations des pesticides.

Références

BARTEN J.H., THOME M., STEVENS M.R., SHUSTER D.J., SCOTT J.W. & CHAMBLISS L. 1994. Evaluating the resistance in tomato to the silverleaf whitefly *Bemisia argentifolii*. Phytoparasitica 22:330-331.

BENMESSAOUD BOUKHALFA H., BENMESSAOUD K. & TALABOULMA h. 1989. Premières observations sur la présence et les densités de populations de l'aleurode des serres *Trialeurodes vaporariorum* West (Homoptera: Aleyrodidae) en Algérie. Réunion OILB/SROP. Antibes. France.10p.

BERLINGER M.J., DAHAN R. & SHEVACH-URKIN 1983. Resistance to the tobacco whitefly Bemisia tabaci in tomato related species: a quick screening method. IOBC/WPRS Bull. 7:39-40.

GIESSEN W.A. van, MOLLEMA C. & ELSEY K.D. 1995. Design and use of a simulation model to evaluate germplasm for antibiotic resistance to the greenhouse whitefly (*Trialeurodes vaporariorum*) and the sweet potato whitefly (*Bemisia tabaci*). Entomol.exp.appl. 76:271-286.

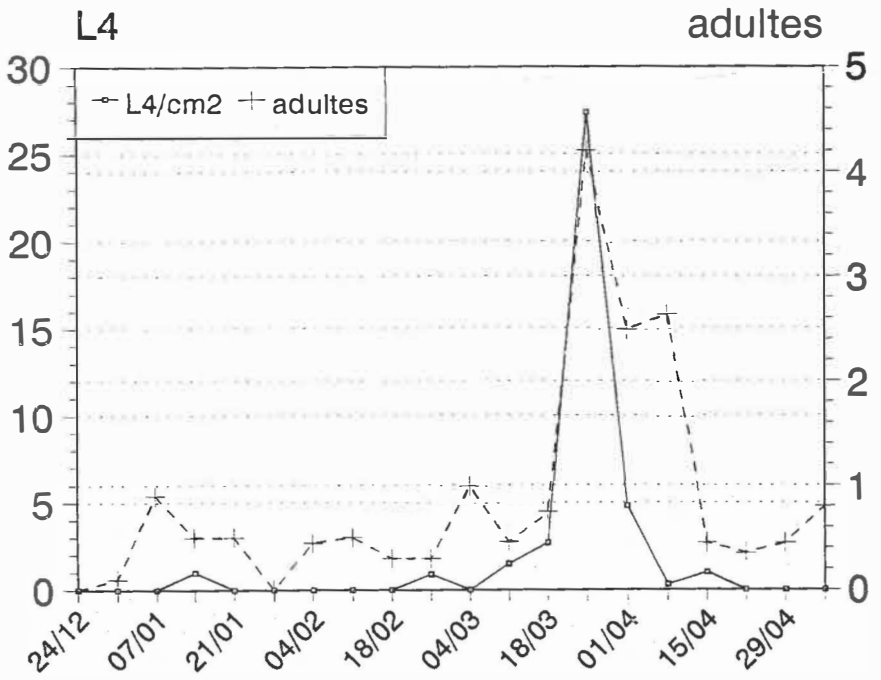


Figure 1. Évolution de la population de *Bemisia tabaci* sur piment

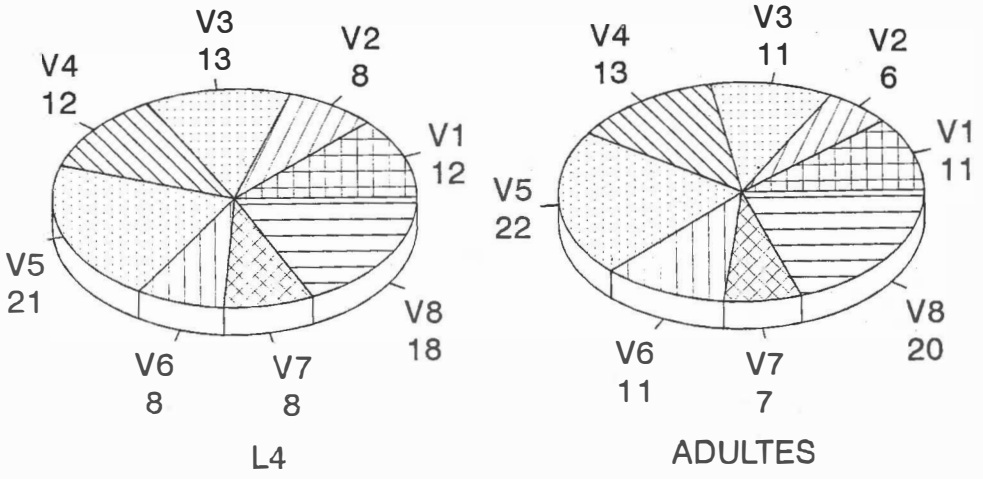


Figure 2. Pourcentages de la population larvaire et imaginale de *Bemisia tabaci* dénombres sur 8 variétés de piment

DÉTERMINATION DE LA SENSIBILITÉ VARIÉTALE DE TROIS VARIÉTÉS DE POIVRON À L'INFESTATION NATURELLE DE *Bemisia tabaci* GEN. SOUS ABRI-SERRE DANS LE SUD-EST ALGÉRIEN

Benmessaoud H.

Département de Zoologie Agricole et Forestière
École Nationale Supérieure d'Agronomie El Harrach (Alger), Algérie

Résumé
Le dispositif expérimental, suivi pour la détermination de la sensibilité variétale à l'infestation naturelle de *Bemisia tabaci*, a consisté en un échantillonnage d'un lot de 5 lignes de 114 plants chacune sous abri serre de 400 m^2 doté d'un météographe suspendu pour le contrôle de la température et l'humidité.
Les trois variétés de poivrons expérimentées ont fait l'objet d'une répartition sous forme de ligne de cultures disposées d'une manière aléatoire de façon à faciliter le libre choix de l'insecte quand au lieu de ponte et de développement.
Un total de 570 plants a fait l'objet d'un dénombrement hebdomadaire. L'échantillonnage de l'infestation de B. tabaci a permis de dégager une des trois variétés échantillonnées comme la plus apte à recevoir les fortes pullulations de l'insecte avec un taux moyen de présence de 47% pour la seule variété V3 par rapport aux deux autres.

Introduction

L'aleurode du tabac, *Bemisia tabaci* Gennadius, s'est introduite en Algérie il y a de celà quelques années. Actuellement les aleurodes *B. tabaci* et *Trialeurodes vaporariorum* West. font partie des ravageurs les plus importants des cultures légumières à travers toute l'Algérie (Benmessaoud, 1997). Les principales cultures infestées pour l'aleurode du tabac sont le poivron, le piment, la tomate, l'aubergine, la courgette, le concombre et le haricot.

Des études (réalisées sous la direction de l'auteur) sur la bioécologie de ce ravageur ont été réalisées (Khouri & Kouaci, 1992; Keroui & Sayoud, 1993; Iftene & Maskri, 1994) et se poursuivent actuellement sur le poivron dans la région de Biskra.

Matériels et méthodes

Les trois variétés de poivrons dont la nomination commerciale est codée (V1= LIPARI, V2= Italico et V3=6295) ont fait l'objet d'une répartition sous forme de lignes de cultures disposées d'une manière aléatoire de façon á faciliter le libre choix de l'insecte quand à l'endroit de ponte et de développement.

Le dispositif expérimental suivi pour la détermination de la sensibilité variétale à l'infestation naturelle de B. tabaci, a consisté en un échantillonnage d'un lot de 5 lignes de 114 plants chacune, soit un total de 570 plants. Un abri serre de 400 m^2 doté d'un météographe suspendu pour le contrôle de la température et de l'humidité relative.

L'échantillonnage hebdomadaire a consisté à dénombrer in situ les stades visibles à l'oeil nu en l'occurrence des larves de 4ème stade et les adultes, pendant 19 semaines ce qui correspondait à la durée d'installation de la culture.

Résultats

Une étude des fluctuations des populations de *B. tabaci* par plant a été réalisée (Fig. 1). Les échantillonnages ont débuté dès l'apparition des adultes dans la serre, soit le 24 Décembre

1996. Les densités moyennes observées et relevées varient de 1 à 2 adultes par plant du mois de Décembre au début du mois de Mars, ce qui correspondrait à des températures moyennes de l'ordre de 15 à 25ºC (Fig. 1). Avec l'augmentation des températures sous serres avoisinant les 25 à 30ºC avec un maximum de 40 à 45ºC. Nous notons parallèlement des densités par plant de 14 adultes le 14 Mars. Au delà de cette date, les densités moyennes d'adultes (Fig. 2) diminuent et nous dénombrons à la fin de nos observations une densité moyenne de 2 adultes par plant.

Les densités moyennes des larves de 4ème stade (Fig. 2b) accusent une évolution similaire à celles des adultes à l'exception de quelques dates où les densités sont plus importantes comme pour le 18 février avec une densité de 5 L4/plant et le 25 mars avec une densité de 97 L4 par plant échantillonné.

Le suivi des observations pendant 19 semaines nous a permis de dégager l'existence de 4 générations et une cinquième partielle (Table 1).

Générations	densité maximale	Date du maximum enregistré	période	observation
Première	1 adulte/plant	31 Décembre	24 Dec-07 Janv.	
Deuxième	1 adulte/plant	14 Janvier	07 Janv-28 Janv.	
Troisième	2 adultes/plant	04 Février	28 Jan-25 Fév.	
Quatrième	14 adultes/plant	25 Mars	25 Fev-30 Avr.	Chevauchant avec cinquième
Cinquième	2 adultes/plant	?	06 Mai-?	

Table 1. Résumé des générations enregistrées.

La cinquième génération correspond à la fin des cultures sous abris. Le nombre de générations annuelles dans les régions du Sud-est Algérien est probablement plus important. En effet, les adultes de la cinquième génération migrent sur les cultures de plein champ, notamment le melon et la pastèque, qui persistent jusqu'aux premiers jours de l'été.

Néanmoins, les températures du mois d'août étant trop importantes et pouvant atteindre les 50ºC, vont arrêter le développement de l'aleurode qui doit probablement estiver sous forme de larves de 4ème stade ou alors être éliminés par les grandes chaleurs.

Cet arrêt de développement, observé dans les régions du nord du pays, par Talaboulma (1989) et Kebir (1991) dans le cas d'une hivernation des larves de 4ème stade, pour *T. vaporariorum*.

La détermination de la sensibilité des 3 variétés de poivron a été effectuée par le dénombrement des larves de 4ème stade et des adultes.

L'analyse des résultats obtenus après dénombrement nous permet de dégager une variété (V3) comme étant la plus sensible, puisqu'elle reçoit près de 39% de la population imaginale et près de 47% de la population larvaire de 4ème stade.

Références

BENMESSAOUD.BOUKHALFA, H. 1997. Bioécologie de deux aleurodes des cultures legumières *Bemisia tabaci* et *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) sur le litoral et sub littoral Algérois. Sem. Nat. Jijel. Juin 1997, 4p. (sous presse)

IFTENE et MASKRI, 1994. Dynamique des populations de *Bemisia tabaci* Gen. sur tomate sous abri-serre et essai de lutte biologique par l'utilisation d'*Encarsia formosa* Gah. Thèse Ing. Agro. I.N.A. Alger 103 p.

KEBIR, M.H. 1990. Contribution à l'étude de l'aleurode des serres: Trialeurodes vaporariorum West (Homoptera: Aleyrodidae) à l'I.N.P.V. d'El Harrach. Thèse Ing. Agro. I.N.A. Alger 80 p.

KEROUI, W. & SAYOUD, H. 1992. Dynamique des populations de *Bemisia tabaci* Gen (Homoptera: Aleyrodidae) sur tomate sous abri-serre et essai de lutte biologique par l'utilisation d'*Encarsia formosa* (Hymenoptera: Aphelinidae). Thèse Ing. Agro. I.N.A. Alger 123 p.

KHOURI, N. & KOUACI, S. 1992. Contribution à l'étude de la bioécologie de *Bemisia tabaci* Gen (Homoptera: Aleyrodidae) sur tomate sous abri plastique. Thèse Ing. Agro. I.N.A. Alger 101 p.

TALABOULMA, H. 1989. Contribution à l'étude de la bioécologie de l'aleurode des serres *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) sur aubergines à l'I.N.P.V. de Boufarik. Thèse Ing. Agro. I.N.A. Alger 90 p.

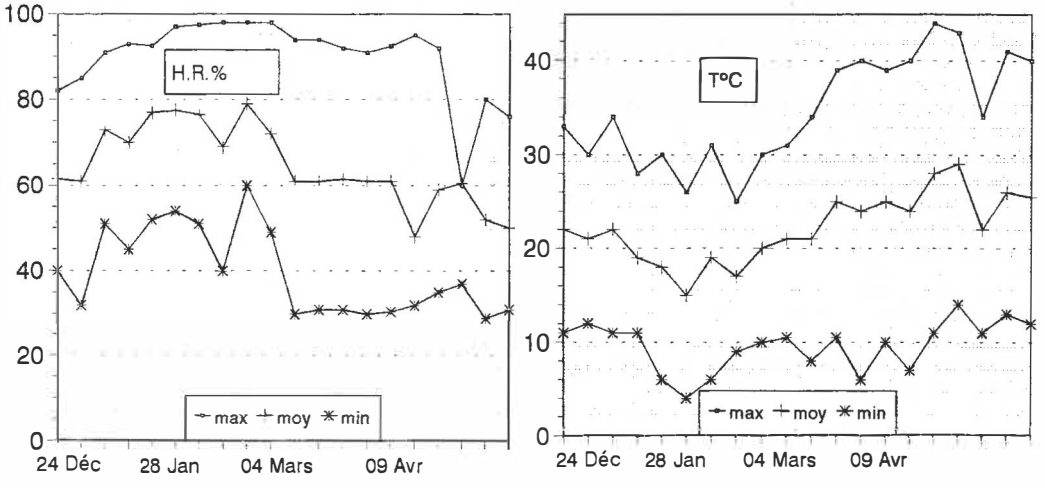


Fig. 1 Evolution de H.R. et T. sous abri serre

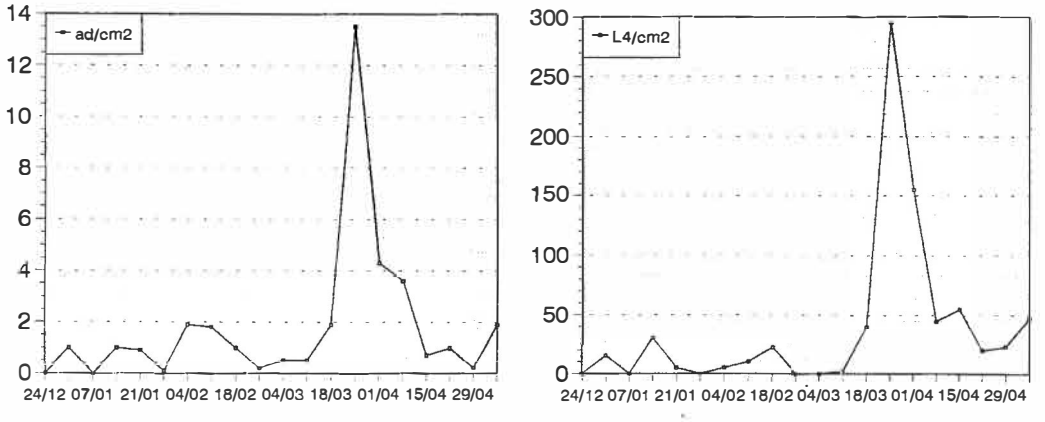


Fig.2 Evolution des populations de Bemisia tabaci sur poivrons

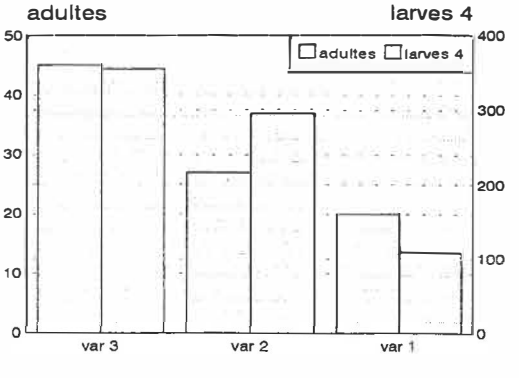


Fig.3 Répartition des populations de Bemisia tabaci par variété