IOBC / WPRS

Working Group „Integrated Control in Citrus Fruit Crops“

International Conference

on

Integrated Control in Citrus Fruit Crops

Proceedings of the meeting

at

Catania, Italy

5 – 7 November 2007

Edited by:

Ferran García-Marí

IOBC wprs Bulletin
Bulletin OILB srop **Vol. 38, 2008**

The content of the contributions is in the responsibility of the authors

The IOBC/WPRS Bulletin is published by the International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic 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 Regionale Ouest Paléarctique (OILB/SROP)

Address General Secretariat:

Dr. Philippe C. Nicot
INRA – Unité de Pathologie Végétale
Domaine St Maurice - B.P. 94
F-84143 Montfavet Cedex (France)

ISBN 978-92-9067-212-8

http://www.iobc-wprs.org

Organizing Committee

of the

International Conference on

Integrated Control in Citrus Fruit Crops

Catania, Italy

5 – 7 November, 2007

Gaetano Siscaro[1]

Lucia Zappalà[1]

Giovanna Tropea Garzia[1]

Gaetana Mazzeo[1]

Pompeo Suma[1]

Carmelo Rapisarda[1]

Agatino Russo[1]

Giuseppe Cocuzza[1]

Ernesto Raciti[2]

Filadelfo Conti[2]

Giancarlo Perrotta[2]

[1]Dipartimento di Scienze e tecnologie Fitosanitarie
Università degli Studi di Catania

[2]Regione Siciliana
Assessorato Agricoltura e Foreste
Servizi alla Sviluppo

Preface

I am proud to present the Proceedings of the meeting of the IOBC/WPRS Citrus Working Group held in Catania, Italy, in November 2007. This is the third meeting after the new period of activity of the group started in 2002 with the first meeting in Valencia, followed by Lisbon in 2005. The meeting was held at the excellent facilities provided by the Università degli Study di Catania. This third meeting experienced a considerable increase in the number of assistants and presentations. In all, there were 139 participants from 19 countries, presenting 103 communications (37 orals and 66 posters). This represents a notable increase compared to the two previous meetings of Lisbon 2005 (100 participants) and Valencia 2002 (60 participants). The number of participants per country was as follows: Italy (56), Spain (38), Morocco (5), Israel (4), Turkey (4), Portugal (3), Algeria (3), Irak (2), Iran (2), Montenegro (2), Tunisia (2), Belgium (1), Brazil (1), France (1), Ghana (1), Greece (1), Switzerland (1), United Kingdom (1) and United States (1).

The two most represented pests were the Citrus Red Scale, *Aonidiella aurantii*, with 20 communications, and the Medfly (*Ceratitis capitata*) with 17, followed by the Citrus leafminer *Phyllocnistis citrella* and the red spider mite *Tetranychus urticae,* with 7 presentations each. Citrus diseases, included in a special session on citrus diseases, showed a substantial increase compared with previous meetings, with 10 presentations. Among other topics, there was ample discussion on emerging problems of recently introduced new pests, like *Pezothrips kellyanus* and *Toxoptera citricida,* as well as on possible introductions of new diseases like the Citrus canker, transmitted by citrus the leafminer, Citrus tristeza virus, transmitted by *Toxoptera citricida,* and especially HuangLonBin (Citrus Greening), transmitted by psyllids.

Overall, the meeting was very well organized and very successful due to high number of participants and the interest of the matters presented. The objectives for next meetings would be to maintain the participation, to attract more researchers that are working in Citrus pests and diseases in the Mediterranean area, and to keep broadening the spectrum of activities of the group. The group has been traditionally a meeting place for entomologists, but has poor tradition in plant pathology. In my opinion the three main challenges for the group are to encourage cooperation within members of the WG in methodology or research projects, stimulate discussion in the meetings and at the mailing list, and to support and encourage the participation of young scientists from Southern Mediterranean countries.

The local organizer of the meeting was Gaetano Siscaro, from the Università degli Studi di Catania. He and his collaborators of the organizing committee developed an extraordinary task of organization and arrangements before and during the meeting. On behalf of all the participants I would like to express our gratitude to all of them for the very pleasant stage and for the attentions we received in Catania. I would like also to express my special thanks to the liaison officer of the group, Mohamed Besri, for his support and help in the management of the group, and for his contribution to the success of the meeting.

I look forward to see all of you at our next meeting in Agadir (Morocco), in 2010.

Valencia, April 2008

F. Garcia-Marí, Convenor

LIST OF PARTICIPANTS

Agrò, Alfonso
University of Palermo
Dept. SENFIMIZO (Entomology Section)
V.le delle Scienze, 13
90128 Palermo – (Italy)
alfonso.agro@unipa.it
Fax: + 39 091 7028826
Phone: + 39 091 7028828

Aguilar Fenollosa, Ernestina
Universitat Jaume I
Ciències Agràries i del Medi Natural
Campus del Riu Sec E
12071 Castelló de la Plana – (Spain)
aguilare@exp.uji.es
Fax: + 34 964728066
Phone: + 34 964729401

Alfaro Cañamás, Cristina
Centro de Ecologia Quimica Agrícola
Universidad Politecnica de Valencia
Cmno. de Vera s/n
46022 Valencia – (Spain)
calfaro@ceqa.upv.es
Fax: + 34 96 387 90 59
Phone: + 34 96 387 90 58

Allal-Benfekih, Leïla
University of Blida
Agronomy Laboratoire de zoologie,
route de Soumâa, BP 270
09000 Blida – (Algeria)
acrido@yahoo.fr
Fax: 00 21325431164
Phone: 00 21352319736

Ancona, Francesco
Via Pavone 39
95022 AciCatena – (Italy)
francesco.ancona@tin.it
Fax: + 39 095 767 20 04
Phone: + 39 338 90 05 028

Argov, Yael
The Israel Cohen Inst. for Biological Control
Plants Prod. and Marketing Board, Citrus Division
P.O.Box 80, 50250 Beit Dagan – (Israel)
yael@jaffa.co.il
Fax: 972 3 9683838
Phone: 972 3 9683811, line 3

Barbagallo, Sebastiano
University of Catania
Dep. of Phytosanitary Science and Technology
Via Santa Sofia, 100
95123 Catania – (Italy)
sebarbag@unict.it
Fax: + 39 095 7147285
Phone: + 39 095 7147352

Beitia, Francisco J.
I. V. I. A.
Plant Protection and Biotechnology
Ctra. Moncada-Náquera, Km. 4’5
46113 Moncada (Valencia) – (Spain)
fbeitia@ivia.es
Fax: + 34.96.342.40.01
Phone: + 34.96.3424081

Benchabane, Messaoud
Université de Blida
Agronomie BP. 270 BLIDA
09000 BLIDA – (Algeria)
mssaoudh@yahoo.fr
Fax: 213.25.433938
Phone: 213 25 433938

Besri, Mohamed Z.
Department of Plant Pathology
Institut Agronomique et Vétérinaire Hassan II
B.P. 6202 Rabat Instituts, (Morocco)
m.besri@iav.ac.ma
Tel/fax: 212 37 778364

Bonaccorsi, Alessandra
University of Catania
Dep. of Phytosanitary Science and Technology
Via Santa Sofia, 100
95123 Catania – (Italy)
abonacco@unict.it

Borras Sena, Vicente
Diputacion Provincial de Valencia Patologia Vegetal
C/ Buenos Aires n. 26
46240 Carlet – (Spain)
vicente.borras@dva.gva.es
Phone: + 33 678 704262

Boualem, Malika
Université Abdel Hamid Ibn Badiss
département d'agronomie BP. 300,
27000 Mostaganem – (Algeria)
boualemmalika@yahoo.fr
Fax: 0021345264545
Phone: 0021371682761

Boullenger, Amélie
Biological Crop Protection Ltd
Technical Advice and Sales
Occupation Road, TN25 5EN,
Wye, Ashford, Kent – (United Kingdom)
amelieb@biological-crop-protection.co.uk
Fax: + 44(0)7927 610190
Phone: + 441233813383

Bounfour, Malika
Direction de la Protection des Végétaux
Service de la Protection des Végétaux
Station Dbagh, Km 6, Avenue Hassan II,
BP 1308 40 000 Rabat (Morocco)
mbounfour@menara.ma
Phone: 00212 61097943

Boyero, Juan Ramón
IFAPA, Centro de Churriana
Laboratorio de Entomologia
Cortijo de la Cruz, s/n
29140 Málaga (Churriana) – (Spain)
juanr.boyero@juntadeandalucia.es
Phone: + 34 951036211

Cacciola, Santa Olga
University of Palermo
SENFIMIZO - Sez. Patologia e Microbiologia
Viale delle Scienze, 2
90128 Palermo – (Italy)
cacciola@unipa.it
Phone: + 39.091 7028841
Fax: +390917028882

Caleca, Virgilio
University of Palermo
Dept. SENFIMIZO (Entomology Section)
viale delle Scienze, 13
90128 Palermo – (Italy)
caleca@unipa.it
Fax: + 39.0917028882
Phone: + 39.0917028819

Campolo, Orlando
Università "Mediterranea" di Reggio Calabria
Gestione dei Sistemi Agrari e Forestali
Loc. Feo di Vito
89123 Reggio Calabria – (Italy)
orlando.campolo@unirc.it
Fax: + 39.0965339790
Phone: + 39.0965801288

Campos Rivela, José Miguel
IRTA Citrus Entomology
Carretera Balada, Km.1 43870
Amposta – (Spain)
jmiguel.campos@irta.es
Fax: + 34.977 267025
Phone: + 34.977 267026

Castañera, Pedro
CSIC, CIB Plant Biology
Ramiro de Maeztu, 9
28040 Madrid – (Spain)
castan@cib.csic.es
Fax: + 34.915360432
Phone: + 34.918373112

Catara, Antonino
University of Catania
Dep. of Phytosanitary Science and Technology
Via Santa Sofia, 100
95123 Catania – (Italy)
cataraan@unict.it
Tel/fax: + 39.0957147353

Catara, Vittoria
University of Catania
Dep. of Phytosanitary Science and Technology
Via Santa Sofia, 100
95123 Catania – (Italy)
vcatara@unict.it
Tel/fax: + 39.0957147370

Ciancio, Aurelio
CNR - Istituto per la Protezione delle Piante
via Amendola 122/D
70126 Bari – (Italy)
a.ciancio@ba.ipp.cnr.it
Fax: + 39.080 5929 230
Phone: + 39.080 5929 221

Cirvilleri, Gabriella
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100
95123 Catania – (Italy)
gcirvil@unict.it
Phone: + 39.0957147355

Cocuzza, Giuseppe
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100
95123 Catania – (Italy)
cocuzza@unict.it
Phone: + 39.0957147253

Colombo, Mario
University of Milano
Istituto di Entomologia agraria
via Celoria 2
20133 Milano (Italy)
mario.colombo@unimi.it
Fax: + 39.0250316745
Phone: + 39 02 50316748

Conti, Filadelfo
Regione Siciliana, Assessorato Agricoltura e Foreste
Dipartimento Interventi Strutturali, Servizio Fitosanitario - O.M.P. - U.O. 54
Via Sclafani 32, 95024 Acireale, Italy
fconti@regione.sicilia.it

Davino, Salvatore
University of Catania
Dep. of Phytosanitary Science and Technology
Via Santa Sofia, 100
95123 Catania – (Italy)
wdavino@unict.it
Phone: + 39.0957147360

Di Franco, Francesca
CRA - Istituto Sperimentale per l'Agrumicoltura
Biologia e Difesa
C.so Savoia n. 190
95024 Acireale (CT) – (Italy)
francesca.difranco@entecra.it
Fax: + 39.0957653113
Phone: + 39.0957653130

Di Primo, Pietro
Cerexagri Italia srl Export
Bivio Aspro Zona Industriale
95040 Piano Tavola - Belpasso (CT) – (Italy)
pietro.di-primo@cerexagri.com
pdiprimo@yahoo.it
Fax: + 39.0957131886
Phone: + 39.095 7131903

Dominguez Ruiz, Javier
Centro de Ecologia Quimica Agrícola
Universidad Politecnica de Valencia
Cmno. de Vera s/n
46022 Valencia – (Spain)
jadorui@ceqa.upv.es
Fax: + 34.963879059
Phone: + 34.963879058

D'Onghia, Anna Maria
CIHEAM/Ist. Agronomico Mediterraneo IPM
Via Ceglie 9
70010 Valenzano (BA) – (Italy)
donghia@iamb.it
Fax: + 39.0804606275/206
Phone: + 39.0804606246

Drishpoun, Yoel
Ministry of Agriculture & Rural Development,
Plant Protection Department, Extension Service
Bet-Dagan PO box 28 (Israel)
yoeldr@shaham.moag.gov.il
Fax: 972-3-9485884
Phone: 972-3-9485334

Elekcioglu, Naime Z.
Plant Protection Research Institute
Plant Protection-Entomology
Kisla Street, PK:21
01321-ADANA –Turkey
nelekcioglu@yahoo.com
Fax: 0 322 3224820
Phone: 0 322 3219581

Eltazi, Said
Domaine Abbès KABBAGE IPM
Taroudant 83 400 Sebt El Guardane – (Morocco)
saideltazi@gmail.com
Fax:212-028540618
Phone: 212- 028540401

Franco, José Carlos
Instituto Superior de Agronomia
Dep. de Protecção de Plantas e de Fitoecologia
Tapada da Ajuda 1349-017 Lisbon – (Portugal)
jsantossilva@isa.utl.pt
Fax: 213653430
Phone: 213 653226

Garcera Figueroa, MªCruz
I. V. I. A.
Plant Protection and Biotechnology
Ctra. Moncada-Náquera, Km. 4'5
46113 Moncada (Valencia) – (Spain)
cgarcera@ivia.es
Fax: 0034 963424001

Phone: 0034 963424000

Garcia-Marí, Ferran
Universitat Politècnica de Valencia
Institut Agroforestal Mediterranio
Camí de Vera 14
46022 Valencia – (Spain)
fgarciam@eaf.upv.es
Fax: 34.963 879 269
Phone: 34.963 879 250

Garzòn Luque, Eva
Universitat Politècnica de Valencia
Institut Agroforestal Mediterranio
Camí de Vera 14
46022 Valencia – (Spain)
eva_garzonluque@hotmail.com
Phone: 630637644

Gerling, Dan
Tel Aviv University
Department of Zoology
69978 Tel Aviv – (Israel)
dangr@post.tau.ac.il
Phone: +9723 6408611

Grande, Saverio
Università "Mediterranea" di Reggio Calabria
Gestione dei Sistemi Agrari e Forestali
Loc. Feo di Vito
89123 Reggio Calabria – (Italy)
saverio.grande@unirc.it
Fax: 39.0965339790
Phone:39.0965801288

Grasso, Francesco
University of Catania
Dep. of Phytosanitary Science and Technology
Via Santa Sofia, 100
95123 Catania – (Italy)
francesco.grasso@unict.it
Fax: 39.0957147277
Phone: 39.0957147368

Guillen, Marta
Tecnologias y Servicios Agrarios S.A.
Area de Servicios Agricolas
Cronista Carreres 11
46003 Valencia – (Spain)
mguille1@tragsa.es
Phone: 34 963531010

Guitian, José Maria
Tragastec crop protection
Valentin beato
28037 Madrid – (Spain)
jmgc@tragsatec.es
Fax:34917549361
Phone: 34917549355

Guncan, Alì
Ege University Faculty of Agriculture
Ege Universitesi Ziraat Fakultesi Bitki Koruma
5100 Izmir – (Turkey)
ali.guncan@ege.edu.tr
Fax:90 (232) 3744848
Phone: 0090 (535) 5879030

Haddi, Khalid
University of Catania
Dep. of Phytosanitary Science and Technology
Via Santa Sofia, 100
95123 Catania – (Italy)
kdhaddi@yahoo.fr
Phone: 39.0957147365

Hérard, Franck
USDA/ARS European Biological Control
LaboratoryCampus International de Baillarguet,
CS90013, Montferrier-sur-Lez 34988 St-Gély-du-Fesc Cedex – (France)
fherard@ars-ebcl.org
Phone: 00 33 4 99623036

Hermoso De Mendoza, Alfonso
I. V. I. A.
Protecció Vegetal i Biotecnologia
Ctra. Moncada-Náquera, Km. 4’5
46113 Moncada (Valencia) – (Spain)
ahermoso@ivia.es
Fax: 00 34 963424001
Phone: 00 34 963424083

Hernandez, Jorge
Biobest Biological Systems
Technical Support
Avda. Andalucia 66
04738 Puebla de Vicar – (Spain)
jorge.hernandez@biobest.es
Fax: 00 34 950557334
Phone: 00 34 950557333

Horta Lopes, David
Centro de Biotecnologia dos Açores
Universidade dos Açores - Departamento de Ciências Agrárias
Largo da Igreja,Terra-chã 9701-581
Terra-chã Angra Do Heroismo – (Portugal)
dlopes@notes.angra.uac.pt
Fax: 00 351295402205
Phone: 00 351295402200

Hoy, Marjorie
University of Florida
Entomology and Nematology
Bldg. 970, Natural Area Drive 32611
0602 Gainesville, FL (USA)
mahoy@ifas.ufl.edu
Fax: 352-392-0190
Phone: 352-392-1901, ext. 153

Hurtado Ruiz, Mónica
Universitat Jaume I
Ciències Agràries i del Medi Natural
Campus del Riu Sec E
12071 Castelló de la Plana – (Spain)
mhurtado@camn.uji.es
Fax: + 34 964728216
Phone: + 34.964729404

Israely, Nimrod
Biofeed Ltd.
Nili, D.N. Modi'in
71930 Nili – (Israel)
nisraely@biofeed.co.il
Fax: 972-8-9150564
Phone 972-8-9150564

Jacas Miret, Josep Anton
Universitat Jaume I
Ciències Agràries i del Medi Natural
Campus del Riu Sec E
12071 Castelló de la Plana – (Spain)
jacas@camn.uji.es
Fax: + 34 964728216
Phone: + 34 964729401

Jucker, Costanza
University of Milano
Istituto di Entomologia agraria
via Celoria 2
20133 Milano – (Italy)
costanza.jucker@unimi.it
Fax: + 39 02 50316748
Phone: + 39 02 50316745

Karamaouna, Filitsa
Benaki Phytopathological Institute
Pesticides Control & Phytopharmacy
8 Stefanou Delta str. GR 145
61 Kifissia – (Greece)
F.Karamaouna@bpi.gr
Fax: + 30 210 8077506
Phone: + 30 210 8180332

Khalaf, Mohammed Z.
Ministry of Science&Technology
Integrated Pest Control Res. Center
P.O.Box; 765 09641 Baghdad – (Iraq)
mzkhalaf2007@yahoo.com
Phone: 07 905810042

Khlij, Anis
University of Bari
Dip. di Protezione delle Piante e Microbiologia Applicata
Via Ceglie 23
70010 Valenzano (BA) – (Italy)
anis_khl@yahoo.com
Fax::+ 39 080 4606275
Phone: + 39 0804606336

Klawi, Samira
Ministry of Science&Technology
Center for Integrated Pest Control Research
Ministry of Science & Technology
IPC Res.Ctr.,P.O. Box 765, Baghdad (Iraq)
samiraob@yahoo.com
Phone: 07 902231119

La Pergola, Alessandra
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100
95123 Catania – (Italy)
alelape@unict.it
Phone: + 39. 095 7147257

La Rosa, Rosa
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100
95123 Catania – (Italy)
larosar@unict.it
Fax: + 39.095 7147266
Phone: + 39.095 7147354

Lebdi Grissa, Kaouthar
INAT, Dept. Plant Protection
43, Avenue Charles Nicolle,
1082 Tunis – (Tunisia)
grissak@yahoo.fr
Phone: 00 216 22 682 604
Fax: 00 216 71 799 391

Liotta, Giovanni
University of Palermo
SENFIMIZO - Sez. Entomologia
Viale delle Scienze, 13
90128 Palermo – (Italy)
liottag@unipa.it
Fax: + 39 091 7028826
Phone: + 39.091 7028821

Llorens Climent, Jose Manuel
Conselleria de Agricultura, Pesca y Alimentación. Generalitat Valenciana Sección de Sanidad y Certificación Vegetal
c/ Profesor Manuel Sala, 2
03003 Alicante – (Spain)
llorens_jos@gva.es
Fax: + 34 965 93 46 88
Phone: + 34 965 93 46 28

Lo Genco, Alessandro
University of Palermo
SENFIMIZO - Sez. Entomologia
Viale delle Scienze, 13
90128 Palermo – (Italy)
alo_genco@hotmail.com
Phone: + 39.3803293055

Lo Pinto, Mirella
University of Palermo
SENFIMIZO - Sez. Entomologia
Viale delle Scienze, 13
90128 Palermo – (Italy)
lopinto@unipa.it
Fax: + 39.091 7028882
Phone: + 39 091 7028827

Longo, Santi
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100
95123 Catania – (Italy)
longosan@unict.it
Fax: + 39.095 7147286
Phone: + 39.095 7147351

Lozano-Rubio, Francisco
Biobest N.V.
Ilse Velden, 18 2260 Westerlo – (Belgium)
info@biobest.be
Fax: 0032 14257982
Phone: 0032 14257980

Lucas Espadas, Alfonso
Servicio de Sanidad Vegetal Region de Murcia
c/ Mayor, s/n 30150 La Alberca Murcia, Spain
rplana@bioiberica.com
Phone: 0034 968 366 787
Fax: 0034 968 840 049

Lucido, Paolo
University of Palermo
SENFIMIZO - Sez. Entomologia
Viale delle Scienze, 13
90128 Palermo – (Italy)
paolo.lucido@virgilio.it
Phone: + 39 333 3117588
Fax: + 39 091 7028882

Machancoses Labuiga, Ernesto José
Universidad Politecnica de Valencia
Agrosistemas Forestales del Mediterraneo
c/ Campet de Tomas n. 13 pta. 1
46220 Picasent – (Spain)
ermalab@terra.es
Phone: + 34 659 565092
Fax: + 34 96 1235114

Marin, Candido
Bioberica s.a. I-DCN II
P.I. mas Puigvert Km 680.6
08389 Barcelona – (Spain)
cmarin@bioiberica.com
Fax: + 34 937650102
Phone: + 34 937650390

Magnano di San Lio, Gaetano
Università "Mediterranea" di Reggio Calabria
Gestione dei Sistemi Agrari e Forestali
Loc. Feo di Vito
89123 Reggio Calabria – (Italy)
gmagnano@unirc.it
Fax: + 39 0965312827
Phone: + 39 0965312364

Marras, Piera Maria
Centro Regionale Agrario Sperimentale (AGRIS - Sardegna)
Loc. Bonassai – Fraz. Tottubella
07040 Olmedo (SS) – (Italy)
pmmarras@tiscali.it
Fax: + 39.79 389450
Phone: + 39.79 387309

Martínez Ferrer, María Teresa
IRTA Citrus Entomology Carretera Balada
Km.1 43870 Amposta – (Spain)
teresa.martinez@irta.es
Fax: + 34 977 26 70 25
Phone: + 34 977 267026

Maspero, Matteo
Fondazione Minoprio
Viale Raimondi, 54
20070 Vertemate Con Minoprio (MI) – (Italy)
m.maspero@fondazioneminoprio.it
Fax: + 39 031 900248
Phone: + 39 031 900224

Matamoros Valls, Enrique
Generalitat de Catalunya, Servicio de Sanidad Vegetal de las Tierras del Ebro
Ctra/ Valencia, nº 108, 43520 Roquetes
Tarragona, Spain
Phone: +34 977 441 140
Fax: +34 630 606 536
rplana@bioiberica.com

Mazih, Ahmed
Institut Agronomique & Vétérinaire Hassan II
Plant Protection
BP 18/S 80000 Agadir – (Morocco)
mazih@iavcha.ac.ma
Fax: (212) 028242243
Phone: (212) 061335351

Mazzeo, Gaetana
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100
95123 Catania – (Italy)
gamazzeo@unict.it
Fax: + 39 095 7147250
Phone: + 39 0957147356

Monzó Ferrer, César
I. V. I. A.
Protecció Vegetal i Biotecnologia
Ctra. Moncada-Náquera, Km. 4’5
46113 Moncada (Valencia) – (Spain)
cmonzo@ivia.es
Fax: + 34 96 342 40 03
Phone: + 34 96 342 41 32

Morato Ferrer, Vicente Francisco
Universidad Politecnica de Valencia
Agrosistemas Forestales del Mediterraneo
c/ Campet de Tomas n. 43 B 3° pta. 8
46220 Picasent – (Spain)
vimorfe@telefonica.net
Fax: + 34 96 1231438
Phone: + 34 659 561641

Navarro Llopis, Vicente
Centro de Ecologia Quimica Agrícola-Universidad Politecnica de Valencia
Cmno. de Vera s/n
46022 Valencia – (Spain)
vinallo@ceqa.upv.es
Fax: + 34963879059
Phone: + 34 963879058

Nobile, Giuseppe
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100
95123 Catania – (Italy)
giuseppenobile@excite.it
Phone: + 39 3338262017

Okuosojo, Abdullahi Ariyibi
Maisa Cristina De Oliveira Me, Sales
Department Rua Bom Pastor 1844 Ipiranga,
04203-002 Sao Paulo – (Brazil)
okuosojo2001@yahoo.com
Phone: +55 11 50610356
Fax: +55 11 50584929

Oliveri, Cinzia
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100
95123 Catania – (Italy)
c.oliveri@unict.it
Fax: + 39 0957147287
Phone: + 39 0957147279

Opoku, Stephen
Rural Environmetal Development Network
P.O.Box 093 KCBC Mamprobi PMB, Accra – (Ghana)
rednetghana@yahoo.com
Phone: 00233-24-2815325

Ostovan, Hadi
Fars Science & Research Branch
Islamic Azad University Entomology
Marvdasht P.O.Box:73715 181 (Iran)
ostovan2001@yahoo.com
Fax: 98 4692111
Phone: 98 4692112

Palmeri, Vincenzo
Università "Mediterranea" di Reggio Calabria
Gestione dei Sistemi Agrari e Forestali
Loc. Feo di Vito
89123 Reggio Calabria – (Italy)
vpalmeri@unirc.it
Fax: + 39 0965339790
Phone: + 39 0965801288

Pane, Antonella
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100 - 95123 Catania – (Italy)
apane@unict.it
Phone: 00 39 095 7147362
Fax: 00 39 095 7147275

Papzan, Abdolhamid
Razi University, Dept. of Extension
Agri. College, Kermanshah, Iran
h_papzan@yahoo.com
Phone: 00989181323787
Fax: 00988318323734

Pascual Ruiz, Sara
Universitat Jaume I
Ciències Agràries i del Medi Natural
Campus del Riu Sec E
12071 Castelló de la Plana – (Spain)
spascual@ivia.es
Fax: + 34 964728066
Phone: + 34964729401

Pekas, Apostolos
Universidad Politécnica de Valencia
Ecosistemas Agroforestales
Camino de Vera s/n
46022 Valencia – (Spain)
appe@doctor.upv.es
Phone: + 34 963879250

Perovic, Tatjana
Biotechnical Institut
Center for subtropical cultures
Bjelisi bb 85000 Bar, (Montenegro)
tperovic@cg.yu
Fax: 381 85311718
Phone: 381 69031635

Perrotta, Giancarlo
Assessorato Agricoltura e Foreste - Regione Siciliana Servizio 11° - Servizi allo Sviluppo
Viale Teracati, 39
96100 – Siracusa – (Italy)
giancarlo.perrotta@tin.it
Fax: 39 0931 38234
Phone: 39 0931 38234

Pinto, Andréa
ISA – DPPF
Rua Mauel Faria, 8
1º esquerdo 8125-231 Loulé – (Portugal)
andreacsbpinto@gmail.com
Phone: + 35 1965108576

Porto, Maria Elena
DOW AgroSciences
Viale Masini, 36
40126 Bologna – (Italy)
mporto@dow.com
Phone: + 39 3356522452

Raciti, Ernesto
Regione Siciliana, Assessorato Agricoltura e Foreste
Dipartimento Interventi Strutturali, Servizio Fitosanitario – O.M.P. - U.O. 54
Via Sclafani 32, 95024 Acireale, Italy
eraciti@regione.sicilia.it

Radonjić, Sanja
Biotechnical Institute
Department for plant protection
Kralja nikole bb 81000 Podgorica – (Montenegro)
sanja_radonjic@cg.yu
Fax: 382 81268432
Phone: 382 81 268 716

Ragusa Di Chiara, Salvatore
University of Palermo
SENFIMIZO - Sez. Entomologia
Viale delle Scienze, 13
90128 Palermo – (Italy)
ragusa@unipa.it
Phone: + 39.091 7028820

Raudino, Francesco
Università "Mediterranea" di Reggio Calabria
Gestione dei Sistemi Agrari e Forestali
Loc. Feo di Vito
89123 Reggio Calabria – (Italy)
franraud@tin.it
Fax: + 39 0965312827
Phone: + 39 0965312364

Rinaldi, Dario
Agrigeos s.r.l. Field Trials
Via G. Bruno, 136
95131 Catania – (Italy)
rinaldi@agrigeos.com
Fax: + 39.09 5 7461588
Phone: + 39.095 7465066

Rizzo, Roberto
University of Palermo
SENFIMIZO - Sez. Entomologia
Viale delle Scienze, 13 90128 Palermo – (Italy)
r.rizzo@unipa.it
Fax: + 39 091 7028882
Phone: + 39 091 7028819

Russo, Agatino
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100 - 95123 Catania – (Italy)
agarusso@unict.it
Fax: + 39 095 7147284
Phone: + 39 095 7147349

Russo, Marcella
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100 - 95123 Catania – (Italy)
russomar@unict.it
Phone: + 39 095 7147411

Salama, Assem
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100 - 95123 Catania – (Italy)
assemsalama@hotmail.com
Fax: 39 080 4606275
Phone:39 348 2556478

Satar, Serdar
University of Çukurova, Faculty of Agriculture
Department of Plant Protection,
01330 Adana – (Turkey)
hserhat@cu.edu.tr
Fax: 322 3386369
Phone: 322 3386699

Skillman, Stephen
Syngenta Crop Protection AG
Technical Management Insecticides
Postfach 4002 Basel – (Switzerland)
stephen.skillman@syngenta.com
Fax: + 41 61 323 6127
Phone: + 41 61 323 5866

Sierras Serra, Nuria
Bioberica s.a. I+D
CN II, P.I. "mas Puigvert" Km 680.6
08389 Barcelona – (Spain)
nsierras@bioiberica.com
Fax: + 34 937650102
Phone: + 34 937650390

Siscaro, Gaetano
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100 - 95123 Catania – (Italy)
gsiscaro@unict.it
Fax: + 39 095 7147284
Phone: + 39 0957147350

Smaili, Moulay Chrif
National Institute of Agronomic Research (INRA)
Plant Protection INRA URPV Laboratory of Entomology
14000 Kenitra – (Morocco) BP: 293
csmaili@yahoo.fr
Fax:212 37374727
Phone: 212 61704833

Sorribas, Juan
Universidad Politecnica de Valencia
Ecosistemas Agroforestales
Camino de Vera s/n
46022 Valencia – (Spain)
juasorme@etsia.upv.es
Phone: + 34 963879251

Soto, Antonia
Universidad Politecnica de Valencia
Ecosistemas Agroforestales
Camino de Vera s/n
46022 Valencia – (Spain)
asoto@eaf.upv.es
Fax: + 34 963879269
Phone: + 34 963879252

Spina, Stefania
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100 - 95123 Catania – (Italy)
sspina@unict.it
Phone: + 39 095 7147399

Suma, Pompeo
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100 - 95123 Catania – (Italy)
suma@unict.it
Fax: + 39 095 7147284
Phone: + 39 095 7147262

Tounsi, Slim
Centre of Biotechnology of Sfax
Laboratory of Biopesticides
PO Box “K” 3038, Sfax – (Tunisia)
slim.tounsi@cbs.rnrt.tn
Fax: 216 74 440 453
Phone: 216 97 589 486

Trapero, Sofia
IFAPA Centro de Churriana Ecophysiology
Cortijo de la Cruz s/n
29140 Malaga – (Spain)
sofiatrapero@yahoo.es
Fax: + 34 951036227
Phone: + 34 951036202

Tropea Garzia, Giovanna
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100 - 95123 Catania – (Italy)
gtgarzia@unict.it
Phone: + 39 095 7147359

Tsolakis, Haralabos
University of Palermo
SENFIMIZO - Sez. Entomologia
Viale delle Scienze, 13
90128 Palermo – (Italy)
tsolakis@unipa.it
Fax: + 39.091.7028882
Phone: + 39.091.7028814

Tumminelli, Riccardo
Osservatorio Malattie Piante
Interventi Strutturali
Ass. Agr. Regione Sicilia Via Sclafani, 32
95024 Acireale (CT) – (Italy)
rtumminelli@omp-acireale.org
Fax: + 39.095 7649958
Phone: + 39.095 894538

Urbaneja, Alberto
I.V.I.A. Protección Vegetal y Biotecnología
Ctra. Moncada-Náquera, Km. 4’5
46113 Moncada (Valencia) – (Spain)
aurbaneja@ivia.es
Fax: + 34 96 342 40 01
Phone: + 34 96 342 41 30

Uygun, Nedim
Çukurova University, Faculty of Agriculture,
Department of Plant Protection
01330-Balcalı, Adana – (Turkey)
nuygun@cu.edu.tr
Fax: (90)322 3386369
Phone: (90) 322 3386754

Vacas Gonzalez, Sandra
Centro de Ecologia Quimica Agrícola
Universidad Politecnica de Valencia
Cmno. de Vera s/n
46022 Valencia – (Spain)
sanvagon@ceqa.upv.es
Fax: + 34 963879059
Phone: + 34 963879058

Vela, José Miguel
IFAPA Entomología Agraria
Cortijo de la Cruz, s/n
29140 Málaga (Churriana) – (Spain)
josem.vela@juntadeandalucia.es
Fax: + 34 951 036212
Phone: + 34 951 036211

Vercher Aznar, Rosa
Instituto Agroforestal del Mediterraneo
Universitat Politècnica de Valencia Ecosistemas agroforestales
Camino de Vera 14 46022 Valencia – (Spain)
rvercher@eaf.upv.es
Fax: + 34 963 879269
Phone: + 34 963 879264

Vidal Quist, José Cristian
I. V. I. A.
Protección Vegetal y Biotecnología
Ctra. Moncada-Náquera, Km. 4’5
46113 Moncada (Valencia) – (Spain)
cvidal@ivia.es
Fax: + 34 963424001
Phone: + 34 63030633370121

Wong, Eva M.
IFAPA Entomología Agraria
Cortijo de la Cruz, s/n
29140 Málaga (Churriana) – (Spain)
mariae.wong@juntadeandalucia.es
Fax: + 34 951 036212
Phone: + 34 951 036211

Yaseen, Thaer
CIHEAM/ Istituto Agronomico Mediterraneo IPM
Via Ceglie 23,
70010 Valenzano (BA) – (Italy)
y.thaer@iamb.it
Fax: + 39 080 4606275
Phone: + 39 080 4606336

Zappalà, Lucia
University of Catania
Dep. of Phytosanitary Science and Technology
via Santa Sofia, 100 - 95123 Catania – (Italy)
lzappala@unict.it
Fax: + 39 095 7147284
Phone: + 39 095 7147258

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Classical biological control of invasive pests in Florida's citrus: the impact of Canker and Greening diseases

M.A. Hoy
Department of Entomology and Nematology, PO Box 110620, University of Florida, Gainesville, Florida, USA 32611-0620

Between 1993 and 1998, Florida’s citrus industry was invaded by the citrus leafminer (*Phyllocnistis citrella*), the brown citrus aphid (*Toxoptera citricida*), and the Asian citrus psyllid (*Diaphorina citri*). These pests either vector or encourage disease transmission and the combination of these pests and these diseases has altered IPM programs in Florida in a dramatic manner. The brown citrus aphid and Asian citrus psyllid are vectors of citrus tristeza virus and greening disease, respectively, while citrus leafminers provide openings into the leaves that favor establishment of the citrus canker bacterium. All three insects were suitable candidates for classical biological control and Dr. Ru Nguyen (Division of Plant Industry, Gainesville, Florida) and I collaborated on importing, evaluating, rearing and releasing parasitoids for each pest. Two parasitoids (*Ageniaspis citricola*, Encyrtidae and *Cirrospilus quadristriatus*, subsequently determined to be *C. ingenuus*, Eulophidae) were imported and both established in Florida, with *A. citricola* the dominant species now. Two parasitoids, *Tamarixia radiata* (Eulophidae) and *Diaphorencyrtus aligarhensis* (Encyrtidae), were imported and established for control of the Asian citrus psyllid, with *T. radiata* the most abundant. Finally, *Lipolexis scutellaris*, later designated *L. oregmae* (Aphidiidae), was imported and released with the brown citrus aphid the target, although this species also attacks melon, spirea, cowpea, and black citrus aphids. It, too, has established in Florida, as well as in Jamaica and Dominica.

Prior to discovering in 2006 that citrus greening disease, caused by *Candidatus* Liberibacter asiaticus, was well established in Florida and that Asiatic citrus canker, caused by *Xanthomonas axonopodis* pv. *citri*, could not be eradicated, arthropod pest management in Florida had focused on biological control. With each new pest invading Florida, the goal had been to find effective natural enemies to import and establish so that the effective natural enemies suppressing mites, scales, mealybugs, whiteflies and aphids were not disrupted by pesticides. Suddenly, pesticide use has increased dramatically in an attempt to reduce the spread of greening and canker diseases. Because Florida’s citrus industry is in crisis, with significant losses in production due to citrus tristeza virus, hurricanes, removal of trees during the canker eradication program and losses due to greening disease, we are in the ‘chaos stage’ of developing revised IPM guidelines. New sources of funding will be devoted to developing new IPM tools, with the ultimate goal of using host plant resistance and biological control to provide pest and disease suppression. How long this interim, and chaotic, phase in citrus IPM in Florida will last is unclear.

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IOBC/wprs Bulletin Vol. 38, 2008
pp. 2-9

The current situation of citrus pests and their control methods in Turkey

Nedim Uygun, Serdar Satar
University of Çukurova Faculty of Agriculture Department of Plant Protection 01330 Balcalı/Adana

Abstract: Citrus production areas in Turkey have risen from 40.000 ha in 1970 to around 96.000 ha in 2005 with the rate of 2.45% increase annually. Paralleling to this significant increase, abundance of indigenous pest populations and introduction of exotic pests are also increasing dramatically. In the citrus groves in Turkey, 89 pests, 34 diseases, 16 nematodes and 155 weed species have been determined until 2007. Among these species, 17 pests, 8 diseases, 1 nematode and 10 weeds are economically important and control measures should be taken. In this presentation, how the IPM tactics used for suppressing the key pests, diseases and weeds, and how to prevent the potential species reach to economic levels will be explained and discussed.

Key words: Citrus pest, control methods, Turkey

Introduction

Citrus is one of the most important crops in Turkey, and it is cultivated in Mediterranean (90.5%), Aegean (9%) and East Black Sea (0.5%) regions in the country. Production areas of citrus have risen from 40.000 ha in 1970 to around 96.000 ha in 2005 with the rate of 2.45% increase annually (Tuzcu et al., 2007).

Because of the significant increase in citrus growing area, abundance of indigenous pest populations and introduction of exotic pests increased dramatically. According to various citrus orchard survey studies in Turkey, 89 pests, 34 diseases, 16 nematodes and 155 weed species that affect citrus cultivation negatively have been determined. Among these species, 17 pests, 8 diseases, 1 nematode and 10 weeds are economically important and have to be considered as key pests. The rest of them are still in the potential pest situation. In the last 10-20 years, *Polyphagotarsonemus latus* (Banks), *Parabemisia myricae* (Kuwana), *Aleurothrixus floccosus* (Maskell), *Paraleyrodes minei* Iaccarina and *Phyllocnistis citrella* Stainton were accidentally introduced into Turkey and caused severe damage at the initial periods of introduction.

In the present paper, how the IPM tactics used for suppressing the economic pests, diseases and weeds, and how to prevent the potential species reach to economic levels will be explained and discussed.

Citrus pests, diseases and weeds species in Turkey and their control methods

This paper consist the information both our own results and results being previously published (Bodenheimer, 1951; Alkan 1953; Tuatay et al., 1972; Zoral, 1974; Soydanbay (Tunçyürek), 1976; Düzgünes, 1977; Soylu & Ürel, 1977; Soydanbay (Tunçyürek) 1978; Kansu & Uygun, 1980; Önder, 1982; Uygun and Sekeroglu, 1983; Lodos, 1984; Türkyılmaz, 1984; Sekeroglu & Cölkesen, 1989; Uygun et al., 1990; Keleş et al., 1991; Öncüer, 1991;

Yoldaş & Öncüer, 1992; Uygun et al., 1994; Uygun et al., 1995; zur Strassen, 1996; Yumruktepe et al., 1996; Uygun et al., 1996; Ulusoy & Uygun, 1996; Yılmaz, 1998; Uygun, 2001; Tuzcu et al., 2007; Önelge et al., 2007; Nas et al., 2007).

According to these publications, in citrus plantations 89 pests, 34 diseases, 16 nematodes and 155 weed species, 159 predators and parasitoids had been found in Turkey. Of course they are not all economically important. In the orchards the natural enemies able to control the main pest populations, have good natural balance with no use of broad spectrum pesticides and doing the cultural practices properly.

In the following part biological control and IPM applications are going to be summarized for economically important pests, diseases and weeds in citrus orchards in Turkey.

Citrus Pests

Mollusca

Helix aspersa Müller was one of the pests that could be found in citrus plantations. The predatory snail *Rumina decollata* (L.) was introduced in the country from California in 1986. After the predatory snail was reared and released, it was determined that *R. decollata* was adapted to the releasing area. There were no other control methods used for *H. aspersa*. Also, predatory insect *Ablattaria arenaria* Kraatz was often found on different snail species. There are other snail species like *Theba pisana* (Müller) that could be find in citrus plantations, but they usually do not damage trees.

Acarina

Ten mite species had been determined. Four of them, *Phyllocoptruta oleivora* (Ashmead), *Aceria sheldoni* (Ewing), *Panonychus citri* (McGregor) and *Polyphagotarsonemus latus* (Banks) are the most important ones. They have various natural enemies from both predator mites groups and insects. *P. citri* and other minor mite pest populations were controlled by natural enemies in the orchards where no broad spectrum pesticides were used. The main natural enemy species are *Orius minutus* (L.), *Stethorus gilvifrons* (Mulsant), *Pharoscymnus pharoides* Marseul and *Scolothrips sexmaculatus* (Pergande). *P. oleivora* is one of the key pests due to its severe damage and in some locations *A. sheldoni* needs to be controlled by acaricide applications.

Thysanoptera

There were five important thrips species determined in citrus orchards. They are *Frankliniella occidentalis* (Pergande), *Thrips meridionalis* (Priesner), *T. tabaci* Lindeman, *T. major* (Uzel) and *Pezothrips kellyanus* (Bagnall). *Frankliniella occidentalis* was the predominating thrips species in the Eastern part of the Mediterranean region. *T. major* was the predominating species in western part of the Mediterranean region. *P. kellyanus* was determined in Aegean Region of Turkey in 1995 (zur Strassen, 1996) and was also recorded in 2003 in the Mediterranean Region of Turkey (Nas et al., 2007). This thrips was accounted for 5% and 0.3% of the specimens in the Eastern and Western part of the Mediterranean Region of Turkey, respectively. Therefore more attention should be paid on *P. kellyanus*.

Homoptera

Among the pest insects, homopteran species are the most important ones, and in the citrus plantations there are 36 species known. In spite of the abundance of natural enemies, these pet species were also abundants. For example, *Aonidiella aurantii* (Maskell) has 18 natural enemy species, *Coccus pseudomagnoliarum* (Kuwana) has 21, *Saissetia olea* (Bernard) has 24, *Planococcus citri* (Risso) has 30, *Dialeurodes citri* (Ashmead) has 14 and the aphid species on citrus have 40 natural enemy species.

Aonidiella aurantii is one of the most important pests for the inland locations far from coastal border. Especially for the orchards between cotton and maize fields and near the dusty village roads the pest population can be high. *A. aurantii* has three generations per year and second and third generations could overlap each other, which cause difficulties to keep control of the pest. On the other hand, 18 parasitoids and predators were determined The most common ones are *Aphytis melinus* Debach, *Comperiella bifasciata* Howard, *Chilocorus bipustulatus* (L.) and *Cybocephalus fodori minor* E.-Y. In the orchards where no broad spectrum pesticides were used, these natural enemies are able to control the pest. Besides the protection and conservation of natural enemies, the summer oil application is recommended against the 1st and 2nd instar populations. In addition, for the new plantations due to limited mobility of *A. aurantii* the citrus seedlings should be free from the pest.

The pest species from Coccidae family are *Coccus pseudomagnoliarum* which has 21 natural enemies and *Ceroplastes floridensis* Comstock which has seven natural enemies. *Chilocorus bipustulatus*, *Exochomus quadripustulatus* (L.), *Metaphycus flavus* (Howard) and *M. helvolus* (Compere) are the main natural enemies of the *C. pseudomagnoliarum* and are able to control the pest in the orchards where no broad spectrum pesticides were used. Although *C. floridensis* has *Coccophagus scutellaris* Dalman as main parasitoid, the pest population could not be controlled efficiently. For both these pests, summer oil applications are recommended for 1st and 2nd instar populations when they reach to economic injury threshold.

Among other homopteran pests, *Icerya purchasi* Maskell has two and *Planococcus citri* has 30 natural enemies. The specific predator *Rodolia cardinalis* (Mulsant) controls *I. purchasi* and *P. citri* was controlled by a natural enemy complex, namely *Chilocorus bipustulatus, Nephus includens* Kirsh, *Exochomus quadripustulatus* and *Anagyrus pseudococci* (Girault). In orchards which are not disturbed by broad spectrum pesticides and have proper cultural practices these two species are not a problem. On other orchards, they become main pests frequently. In this case, for *I. purchasi* it is recommended that the *R. cardinalis* should be collected from the area in natural balance and brought to the orchards which have problems. For *P. citri* control, the natural enemies *Cryptolaemus montrouzieri* Mulsant and *Leptomastix dactylopii* Howard are used commercially.

In citrus plantations five whitefly species are known, *Dialeurodes citri*, *Parabemisia myricae*, *Aleurothrixus floccosus*, *Paraleyrodes minei* and *Bemisia tabaci* (Gennadius). The last two species are not economically important. There are two new species introduced in Turkish citrus ecosystems, *P. myricae* in 1983 and *A. floccosus* in 1994. Both of them have efficient parasitoids, *Eretmocerus debachi* Rose & Rosen for *P. myricae* and *Cales noacki* Howard for *A. floccosus*. These parasitoids were reared and released at the beginning of epidemy and up to now both pests are successfully suppressed by their parasitoids. *D. citri* is still an important and widespread pest. However, it has also natural enemies, 14 of them determined during various studies. Among these natural enemies, the predatory insect *Serangium parcesetosum* Siccard and the parasitoid *Encarsia armata* (Silvestri) appear as the main biological control agents. Beside the protection and conservation of the natural enemy complex, cultural practices like irrigation, pruning, fertilization and weed control to keep low humidity between the trees in the orchards are the main complementary cultural practices. Summer oil applications are also recommended beside these cultural practices.

There are five aphid species known from citrus plantations. Only two of them, *Aphis gossypii* Glover and *A. spiraecola* Patch are economically important, especially on the seedlings and young trees. They have the biggest natural enemy complex with 40 species. There were no problems in the mature orchards with no broad spectrum pesticide usage. In nurseries and on young trees, specific aphicides were recommended.

Seven species of cicadellids had been determined in citrus plantations. The most common ones are *Asymmetrasca decedens* (Paoli) and *Empoasca decipiens* (Paoli). They usually feed on weeds under the citrus trees. Usually in fall, they cause sucking damage on fruits which decrease the marketing quality of fruit. Some plant protection consultants have recommended the application of 4% lime sulphur as repellent. However, our team do not agree with this recommendation because repeated applications may cause the lime concentration to increase in the soil, affecting plant physiology and preventing activity of natural enemies of scale insects. Although at this moment there are no other alternative control methods, some management procedures are being tested at the field level, such as the proper weed control, or the plantation of weeds preferred by these cicadellid species in rows between the citrus trees to attract the pest individuals, or the pesticide application on the external tree rows around the orchard to prevent the cicadellids fly in the orchard during the migration time. The cicadellid species not only cause sucking damage but are also destructive as vectors of virus and virus like diseases to the citrus trees, like *Circulifer haematoceps* (Mulsant & Rey).

Lepidoptera

There are 12 Lepidopteran species found in citrus ecosystems in Turkey, but only three of them, *Prays citri* Milliere, *Ectomyelois ceratoniae* (Zeller) and *Phyllocnistis citrella* Stainton are economically important. *P. citri* causes damage only on lemons and *E. ceratonias* is causing damage on the navel group of oranges. *Bacillus thuringiensis* Berliner is the recommended pesticide for these two pests. In addition, the collection of fallen fruits due to *E. ceratoniae* and damaged fruits from the tree is one of the efficient control methods the decrease the pest population for coming generations. *P. citrella* was introduced into Turkey in 1994 and in a very short time, about 2-3 months, reached all the citrus growing locations except East Black Sea Region. This pest caused considerable damage in the first years after the invasion, especially in nurseries and young plantations. To control the pest damage, both biological and chemical methods, and also cultural control practices, have been studied. According to these studies, 19 natural enemies were determined; the most common ones are *Cirrospilus brevis*, *Ratzeburgiola incompleta* and *Citrostichus phyllocnistoides*. Although the overall effectiveness of natural enemies reach up to 94%, the damage on nursery plants and young trees has not declined. Therefore, the chemical control is necessary on the trees less than three years old. On mature trees, proper cultural activities such as irrigation, fertilization, pruning etc., together with prevention and conservation of natural enemies, could control the pest damage. The chemical control recommended for nurseries and young orchards consist of summer oil applications and Insect Growth Regulators.

Diptera

There were four species of Diptera determined in citrus plantations, but only *Ceratitis capitata* Wiedemann is important for the last 10-15 years. The main reason for the increase of *C. capitata* damage is that other host plants take place in or around the citrus orchards, such as apple, pear, apricot, peach, kaki, fig trees etc. The pest population increases on these fruit trees and moves to citrus in late summer or autumn. Although four natural enemy species were determined, none of them is able to control the pest. The only application recommended for this pest is bait spraying, which is acceptable in IPM strategies. The bait spraying is applied on the $1m^2$ area on the south-east side of the tree, using in combination an attractant plus spinosad or malathion. Applications are made every 10 days with successful results.

Citrus nematodes

There are 16 nematode species in citrus orchards in Turkey. *Tylenchulus semipenetrans* Cobb is the most common one. It is determined that 65% of citrus orchards in eastern Mediterranean region are infected by this nematode species at economic threshold levels. It is suggested to plant nematode free seedlings while establishing a new orchard. Cultural practices such as irrigation and fertilization are recommended to minimize the effect of nematodes in planted orchards. Also, there are some registered nematicides. Their use depends on chemicals and crop prices.

Citrus diseases

Fungi and bacteria

There are 11 fungal and bacterial diseases which are seen in nearly all regions where citrus orchard are grown in Turkey. These are *Phoma tracheiphila* (Petri) Kanc. et Ghik., *Phytophthora citrophthora* Leonian, *Alternaria alternata* f.sp. *citri* Solel, *Colletotrichum gloeosporioides* (Penz) Sacc., *Botrytis cinerea* Pers. ex Fr., *Alternaria citri* Ell and Pierce, *Penicillium italicum* Wehmer, *P. digitatum* Sacc., *Phomopsis citri* Fawc., *Diplodia natalensis* P. Evans and *Pseudomonas syringae* van Hall'dır. In this part, important fungal and bacterial diseases and control methods of these diseases will be explained.

Phoma tracheiphila mostly causes damages in Kutdiken (local lemon varieties). Besides that, this disease causes damage in interdonato and other citrus varieties. Since this disease enters the trees via damaged parts, trees should be protected against damaging. For example, after freezing and heal damage which causes damage to trees, a protecting fungicide should be applied. Pruning should be done at temperatures over 30^{o}C and pruning wastes should be removed out of the orchard and burned.

Phytophthora citrophthora caused the most damages to lemons, followed by grapefruits. Orange and mandarin are relatively more resistant to this disease. However, this disease can be seen in all citrus varieties. The most suitable method for controlling this disease is to avoid extreme watering and to apply drip irrigation. It is also important to graft high on the tree trunk to reduce the probability that pathogen spores come in contact with the graft part through rain. Moreover, to apply bordex mixture at the trunk and main branches of trees is useful as protecting measure. It is a classical method to clean infected trunks and branches mechanically and then to disinfected with potassium permanganate. It is also suggested to apply chemicals with fosetyl-Al as active ingredient to green part of trees in the shooting season.

Alternaria alternata f. sp. *citri* was first seen in Turkey in 1992 in Mineola Tangelo. Apart from Mineola, this disease is potentially dangerous to Robinson, Marisol, Fortune and Nova mandarin. To hinder extreme shooting, nitrogen fertilizer and water should be reduced and relative huminity in citrus orchards should be avoided as cultural control methods of this disease. For chemical control, registered fungicides like iprodine and triazole should be used when shoot are 5-10 cm long. CuOH should be used as protecting fungicide.

Virus and virus like diseases

More than 15 virus and virus-like diseases were detected in Turkish citrus groves, including psorosis complex, stubborn (*Spiroplasma citri* Saglio et al.), cachexia, exocortis, infectious variegation, concave gum, impeietratura, cristacortis, satsuma dwarf, gummy bark, woody gall, tristeza, rumple, citrus chlorotic dwarf and yellow vein clearing.

Stubborn is an important disease in oranges of the navel group, mandarins and grapefruits. It is reported that 50% of the navel grup of oranges in the Mediterranean region of Turkey are infected with this disease (Yılmaz, 1998). The disease is especially common in young citrus orchards. This disease is basically transmitted by species of Cicadellidae, *Circulifer haematoceps*. As control measures, weeds should be removed from the orchard and control of insect vectors should be carried out.

Satsuma dwarf virus was fist reported in 1973 in Turkey (Yılmaz, 1998). This disease is transmitted by inoculation and mechanically. It is known that this disease is common in the Aegean region at an amount of 2% and in the Mediterranean region at an amount of 31.6% in Satsuma mandarin (Yılmaz, 1998).

Citrus chlorotic dwarf virus was detected in lemon, Tangelo mandarin, and oranges in the eastern Mediterranean region. It occurred as epidemic disease especially in Kutdiken lemon and Minneola tangelo. This disease is transmitted from one citrus plant to another by the whitefly *Parabemisia myricae*. Control measures against the insect vector are applied to control the disease.

Yellow vein clearing virus was first seen in 2000 in Cukurova region of Turkey. Symptoms of this disease can be seen in Kutdiken, Interdonate lemon varieties and sour orange. These symptoms can be seen especially in spring and fall on young leaves of shoots. It is reported that this disease is transmitted to some citrus varieties through graft inoculation (Önelge et al., 2007).

There are mild isolate of tristeza virus in Turkey. This disease has been detected in eastern Mediterranean region at an amount of 0.5% in Navel oranges and at an amount of 0.04% in Satsuma mandarin. It is known that *Aphis gossypii* transmitted this disease about 21% under laboratory condition but it has not been reported that this disease is transmitted by vectors in nature. If the main vector of this disease, *Toxoptera citricida* (Kirkaldy), enters Turkey, the situation can change.

The most important control method against virus and virus like diseases are the use of material that is free of disease and research in both state institutes and in the private is carried out sector on this field in Turkey. Also, to use proper cultural practices and control methods for insect vectors is suggested.

Citrus weeds

There are 155 weed species in citrus orchards in Turkey. Of these, 10 species are the most common. These are *Sorghum halepense* (L.), *Cynodon dactylon* (L.), *Convolvulus arvensis* L. *Cyperus rotundus* L., *Portulaca oleracea* L., *Digitaria sanguinalis* (L.), *Mercurialis annua* L., *Sonchus oleraceus* L., *Setaria verticillata* (L.) and *Paspalum paspalodes* (Michx.). Although there are a lot of methods of weed control in citrus orchards, the most common ones are ploughing, mulching and applying registered herbicides.

Conclusion

In conclusion, Turkish citrus fauna is very rich due to natural enemy species. With biological control by conservation and augmentation of these species, proper cultural practices and, when necessary, specific fungicide, herbicide, acaricide, insecticide and summer oil applications, the main pests, diseases and weeds can be controlled succesfully.

Unfortunately, most of the citrus growers, especially the small ones, are not keen on application of IPM programs. There are several reasons for this phenomena; most of the citrus farmers are not educated enough to realize the importance of IPM strategies, these farmers think that the reliance on pesticides are the easiest and safety way of controlling pests. In addition, technical staff from the extension services is not well organized to provide information to the farmers.

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IOBC/wprs Bulletin Vol. 38, 2008
pp. 10-16

Current situation of Citrus pests and the control methods in use in Morocco

Ahmed Mazih
Institut Agronomique et Vétérinaire Hassan II, Department of Plant Protection, B.P. 18/S, Agadir – Morocco. Email: mazih@iavcha.ac.ma

Abstract: With 80 000 ha, Morocco produces annually 1.2 to 1.5 million metric tons of Citrus fruits, from which 55% are exported as fresh fruits. The main areas of production are the Souss, Gharb, Moulouya, Tadla, and Haouz covering more than 80% of the total plantations. Dominant varieties are Clementine mandarin, Valencia and Navel orange. More than thirty phytophagous arthropods and snail species are present. However only four are considered as key pests: Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera, Tephritidae.), California red scale, *Aonidiella aurantii* Maskell (Homoptera, Diaspididae), mites manly Citrus red mite, *Panonychus citri* McGregor (Acarina, Tetranychidae)., and Citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera, Gracilariidae). Up to now, the pest management in Moroccan orchards still heavily relies on chemical control. However, the implementation of ecological methods is slowly taking place. Some have already been developed by research, and could be made ready for use in practice, in order to meet the new requirements of the market regarding fruit quality, environment, health, and the good agricultural practices (Eurepgap, Nature choice). Thus, biological control is in progress and alternative methods to chemical control such SIT are underway. *Phytophthora* is the most important fungal disease encountered in Moroccan citrus orchards. In general, foliar applications of potassium phosphonates are used, when necessary. Virus, bacteria, and nematodes do not have any significant economic importance. More than 200 weeds species were recorded in citrus orchards; however, the most economically important are *Cynodon dactylon*, *Convolvulus* spp, *Cyperus rotundus*, *Chenopodium album*, and *Solanum* spp. Both cultural and chemical treatments are used to control these weeds.

Introduction

Moroccan citrus plantings cover ca 80,000 ha, producing 1.2 to 1.5 million tons of Citrus fruits annually, from which 55% are exported as fresh fruits. The main areas of production are the Souss, Gharb, Moulouya, Tadla, and Haouz covering more than 80% of the total plantations. Clementine mandarin, Valencia and Navel orange varieties are dominants. More than 90% of the groves are on sour orange rootstock, which is tolerant to calcareous soil conditions and resistant to *Phytophtora* gummosis, but sensitive to Tristeza. Thus the new plantations are grafted on other rootstocks such as ‘Troyer’ and ‘Carrizo’ citranges, *Citrus macrophylla* and Citrus volkameriana (El-otmani & Zouhri, 2004).

The main constraints which growers have to deal with are the scarcity of water after several years of drought, and marketing due to the high competition in the international market. However, pest management is also a big challenge to be alleviated, in order to meet market requirements, environment preservation, attenuation of losses, and reduction of cost of production.

Pest Management

Diseases and Nematodes

Although several Citrus diseases have been reported during last 50 years in citrus orchards in Morocco (Afellah et al. 2001), losses due to virus, bacteria and nematode infestations do not have significant economic importance. *Phytophthora* is the most important fungal disease encountered. In general, foliar applications of potassium phosphonates are used, when necessary.

Weeds

A total of 403 weed species were recorded in all citrus growing areas. They belong to 51 botanical families. *Asteraceae, Poaceae, Fabaceae, Brassicaceae, Apiaceae, Lamiaceae, Boraginaceae* and *Caryophyllaceae* were the predominant families. Among the most important species, 17 are perennial weeds (Table 1) (Bouhache and Taleb, pers com.)

Table 1. Major weed species in Citrus orchards

Cynodon dactylon (L.) Pers.	*Rubia perigrina* L.
Cyperus rotundus L.	*Rubus ulmifolius* Schott.
Oxalis pes-caprae L.	*Bryonia dioica*.Jacq.
Cardaria draba (L.) Desv.,	*Solanum elaeagnifolium* Cav.
Paspalum paspalodes (Michx) Scribn.	*Arisarum simorrhinum* Durieu
Paspalum dilatatum Poiret	*Asparagus acutifolius* L.
Sorghum halepense Pers.	*Piptatherum miliaceum* (L.) Cosson.
Convolvulus arvensis L.	*Amaranthus deflexus* L.
Convolvulus althaeoides L.,	

In addition, a parasitic weed (*Cuscuta monogyna* Vahl) exists in Souss, Haouz and Moulouya regions. However, the most economically important are *Cynodon dactylon*, *Convolvulus* spp, *Cyperus rotundus*, *Chenopodium album*, and *Solanum* spp. (Bouhach and Taleb, pers. comm.). Both chemical treatments (Glyphosate 5-12l/ha, Paraquat. 1-2l/ha, Fluazifop-Butyl 4-5l/ha) and cultural practices (soil cultivation) are used to control these weeds.

Insects, Mites and Snails

More than thirty phytophagous arthropods and snail species have been reported on citrus in Morocco (Table 2). Homoptera represent the largest number of pest species. These include armored scales, soft scales, white flies, aphids, mealybug and leafhopper. The remaining, are represented by fruit fly, Lepidoptera and mites species.

The key pests around which control strategies pivot are Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera, Tephritidae), California red scale, *Aonidiella aurantii* (Maskell) (Homoptera, Diaspididae), mites mainly Citrus red mite, *Panonychus citri* McGregor (Acarina, Tetranychidae), and Citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera, Gracilariidae). The recent outbreak of some secondary pests (e.g. *Icerya*

Table 2. Phytophagous arthropods pests of citrus in morocco and their natural enemies
(Snails: *Helix pisana;* Occasional: Desert locust (*Schistocerca gregaria*: Orthoptera Acrididae))

Order	Family	Scientific name	Parasitoids	Predators
Hemiptera	Diaspididae	*Aonidiella aurantii* (Maskell) *Aspidiotus nerii* Bouché *Chrysomphalus dictyospermi* (Morgan) *Parlatoria zizyphi* (Lucas) *Parlatoria pergandii* Comstock *Lepidosaphes beckii* (Newman) *Lepidosaphes gloverii* (Packard)	*Aphytis melinus* *Aphytis lignanensis* *Comperiella bifaciata* *Aphytis lepidosaphes*	*Chilocorus bipustulatus* *Rhizobius lophantae*
	Coccidae	*Saissetia oleae* (Bernard) *Coccus hesperidum* (Linné) *Ceroplastes sinensis* (Del Guercio).	*Metaphycus helvolus* *Metaphycus flavus* *Metaphycus lounsbury* *Coccophagus cowperi* *Scutellista nigra*	*Chilocorus bipustulatus*
	Pseudococcidae	*Planococcus citri* (Risso) *Pseudococcus adonidum* (Linné)	*Leptomastix dactylopii*	*Cryptoleameus montrouzieri*
	Margarodidae	*Icerya purchasi* (Maskell)		*Novius* (*Rodolia*) *cardinalis*
	Aphididae	*Aphis spiraecola* (Patch) *Toxoptera aurantii* (Boyer) *Myzus persicae* (Sulzer) *Aphis fabae* Scopoli *Aphis gossypii*	*Aphidius colemani* *Aphidius matricariae* *Diaeretiella rapae* *Ephedrus plagiator* *Lysiphlebus fabarum* *Praon volucre*	*Chrysoperla carnea* *Harmonia* sp..
	Aleyrodidae	*Parabemisia myricae* (Kuwana) *Aleurothrixus floccosus* (Maskell) *Dialeurodes citri* (Ashmead) *Aleurodicus dispersus* Russell *Paraleyrodes minei* Luccarino	*Encarsia transvena* *Eretmocerus debachi* *Cales noacki*	Coccinellidae*:* *Clitostethus arcuatus* *Pharoscymnus anchorago* *Harmonia* sp. Chrysopidae*:* *Chrysoperla carnea* *Chrysopa* sp.
	Cicadellidae	*Empoasca* sp.		
Diptera	Tephritidae	*Ceratitis capitata* (Wiedmann)	*Opius concolor*	
Lepidoprera	Gracilariidae	*Phyllocnistis citrella* (Stainton)	*Pnigalio* sp. *Cirrospilus pictus* *Ageniaspis citricola* *Cirrospilus ingenuus* *Quadrastichus* sp. *Semielacher petiolatus* *Citrostichus phyllocnistoides*	
	Tortricidae Yponomeutidae Pyralidae	*Cacoecia pronubana* (Huebner) *Prays citri* (Millière) *Myeloïs ceratoniae* (Zeller)		
Coleoptera	Bostrychidae	*Xylomedes coronate*		
Arachnida Acari	Tetranychidea Eriophyidae Tarsonemidae Tenuipalpidae	*Panonychus citris* (McGregor) *Tetranychus cinnabarinus* (Boisd.) *Tetranychus urticae* (Koch.) *Aceria sheldoni* (Ewing) *Hemitarsonemus latus* (Banks) *Brevipalpus phoenicis* (Geijskes)		*Euseius stipulatus* *Euseius rubini* *Stethorus* sp.

purchasi) could be interpreted as the consequence of toxicity of pesticides, used against key pests, to predatory Vedalia beetle *Rodolia cardinalis*. That necessitated some interventions against this pest, so far maintained under biological control.

Common Insect Pest Control Measures: Scale Insects

California red scale

Aonidiella aurantii is an important economic pest of Moroccan citrus mainly due to cosmetic damage it causes to the fruit, resulting in downgrading or rejection at the packinghouse.

Chemical control

Organophosphorous insecticides and mineral oils are applied, alone or mixed, for armored scales control. Application once year, in late spring-early summer, is the common control measure against the scale, however some years of heavy infestation a second application is required in autumn. The most common pesticides used in Moroccan citrus orchards are shown in Table 3.

Table 3. Major pesticides used in Citrus in Morocco.

Pest	Pesticide
Medfly	Malathion (+Protein hydrolysate)
	Spinosad
	Pyrethroids: Deltamethrin Lambda – cyhalothrin
Leafminer Aphids	Imidacloprid Acetamiprid
Leafminer Mites	Abamectin
California red scale	Methidation
	Chloropyrifos
	Pyriproxyfen
California red scale Mites	Mineral oil (Sunspray, Citrole, Safe-T-side)

Biological control

Predators and parasitoids associated with scale insects (Table 2) are very effective where no or less disruptive pesticides are used (Chouibani et al. 1997). Several insectaries for mass rearing of *Aphytis melinus* are built by growers, and are now operational, in most of the citrus growing areas. Field releases are integrated in the scale control strategy. Currently the production reached several millions wasps. Others insectaries are under construction.

Cottony cushion scale

Severe tree damage, decreasing tree vigor, and defoliation were observed during last two years in some orchards. We observed an absence or low presence of predatory Vidalia beetle, and high activity of ants. All attempts using pesticides to control the scale failed. The only solution that gives a good result consisted of washing trees to remove the scales, followed by introduction of Vidalia from other orchards.

Other scales

Outbreaks of secondary pests, so far maintained under biological control, occur in some orchards, and start to become of big concern. Examples include *Lepidosaphes beckii* (Purple scale) and *Parlatoria pergandii* (Chaff Scale) in the inland orchards in Gharb region (NW) and in Berkane (NE); and *Coccus hesperidum* (brown Soft Scale) in the Souss (SW). This is probably due to the disruptive pesticides used mainly against the key pests mentioned above which lead to the suppression of their natural enemies.

Mediterranean Fruit Fly

Among key pests infesting citrus, *C. capitata* is certainly the most serious, with both direct and indirect economic impacts. Average fruit infestation can reach 10 – 20% or more during years of high infestation, or when the treatment was not correctly applied. It requires close observation during the fruit maturation period, and several insecticide sprays. In addition, as it is quarantine pest for some of the more important fruit importing countries, such as Japan and the USA, export of fruit to these countries requires cold treatment, which raises costs.

Chemical control

It is conducted by the individual farmer in full cover spray (rarely) with a wide range of different pesticides, alone, or often in localized treatment (1 row out of 3 or 4) using a mixture of pesticide and protein hydrolysate bait. Recently, the ready to use poisoned bait “Spinosad-Success appât” is applied during harvesting period, because of its low persistence (Tab. 3). Almost all treatments are applied at ground level.

Sprays are timed by monitoring for presence of the fly by the mean of traps (MaghrebMed trap baited with capsule of trimedlure and DDVP). The threshold generally admitted is 3 to 5 males/Trap/Day. However, in small farms, sprays are done once every 8 to 10 days, in late summer and autumn for the early varieties, and by the end of winter to spring, for the late varieties, according to the region.

Chemosterilization

Autosterilization in the field with the active ingredient lufenuron® gel (Insect Growth Regulator, chitin synthesis inhibitor) (Navarro-Llopis et al., 2004) was experimented in small and medium size field trials (1-5ha) in Clementine and Valencia orange orchard, using the new ‘Adress devices’ (20-25/ha). The efficacy of this method was compared with sprays treatments (Mazih et al.2007). Our finding allowed us to consider results of this method as at least equivalent to those of chemical treatment. Large-scale field trials of 15 ha are currently under way.

Mass trapping

We experimented the "attract and kill" technology" with M3 bait station and we obtained a very good results, since no sprays were done in the block where M3 was set, while several sprays were necessary in the control block (El Tazi et al 2007).

Biological control

The impact of the natural enemy *Opius concolor*, the only parasitoid recorded, seems to be very low, and the role of predators is not documented.

Sterile insect technique

Two years ago, the Citrus grower association, with the support of the Ministry of Agriculture decided to implement an area with the phytosanitary status of “Low-Pest Prevalence Area” (LPPA) to control de Medfly using IPM-Area Wide with the Sterile Insect technology.

A pilot zone of 3000 ha was chosen in the Eastern part of the Souss Valley, where citrus groves are surrounded by a physical barrier (Atlas Mountains) and areas with few sites with Medfly hosts.

Citrus leafminer

Chemical control

The pest which frightened growers in late 1990's at the beginning of its invasion to the Moroccan groves has now a tendency to be accepted as minor pest on bearing trees. However, it still is a big concern in nurseries, and in newly and grafted trees. To protect the main flushes from mid spring to autumn, for trees less than 5 years old, stem painting technique (1-3cc of Imidacloprid) is generally used.

Biological control

Five exotic parasitoids *Ageniaspis citricola*, *Cirrospilus ingenuus*, *Quadrastichus* sp. *Semielacher petiolatus*, and *Citrostichus phyllocnistoides*, were introduced, reared and released in the field from 1995 to 2000 (El Ouard et al. 2002; Rizqi et al. 2003). But only the last two species are now established the most areas, and become dominant. The two native parasitoids, *Pnigalio* sp. and *C. pictus*, are now of insignificant importance. Predators, such as spiders and chrysopa are also considered as very effective.

Mites

Among all mite species, the Citrus Red Mite, *Panonychus citri* is considered as a major pest. Proliferations of mites are in close relationship the level activity of predaceous mites (*Euseius* spp.) and coccinellid *Stethorus*. Unfortunately, the chemical products used *against* Medfly and Citrus leafminer, mainly, are highly toxic to phytoseiids.

Conclusion

Pest management in Moroccan orchards still heavily relies on chemical control, although treatment schedules differ with the insect pest and region. However, important changes are taking place and progress has been made during the last decade in terms of pest control strategy. There is much consensus that the adoption of IPM is unavoidable because of market pressures (requirements of the international market, particularly with respect to pesticide residues, phytosanitary regulations, and criteria of buyers). Most citrus growers try to export as much of their crop as possible because of the far greater returns obtained on the export market compared to domestic market. Citrus which cannot be exported because of quality problems, is sent to the domestic market.

Certification of orchards as well as packinghouses within ISO, HACCP, EUREPGAP or Nature Choice procedures is underway in most of farms to fulfill market requirements.

Implementation of IPM in Moroccan citrus orchards is in transition, and more and more growers are willing to adopt this approach. All of those reluctant are now convinced of the benefit of IPM, especially after the occurrence of the Citrus leafminer in 1994. The pest generated a huge panic and necessitated several sprays, using a great variety of pesticides, very costly with unclear results. For the first time, there was a large consensus among growers that chemical control is not a sustainable option. So, the introduction of several exotic parasitoids and its success to control Citrus leafminer, had lead to a much wider understanding and appreciation of the role of beneficial insects in exercising control over crop pests. Other parasitoids were introduced to control California red scale from the north to the south of the country (i.e. *Compriella bifaciata*).

In addition, beside biological control against California red scale using *Aphytis melinus*, and alternative methods to the traditional chemical control, such as mineral oils, other techniques are in progress. The very promising and huge SIT program against Medfly is a good example.

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California Red Scale

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IOBC/wprs Bulletin Vol. 38, 2008
pp. 19-24

Population dynamics of *Aonidiella aurantii* (Homoptera: Diaspididae) on citrus nursery trees in North and Eastern Sicily in the period 1997 - 2006

Filadelfo Conti, Roberta Fisicaro
Regione Siciliana, Servizio Fitosanitario Regionale – U.O. 54, Via Sclafani, 34 – 95024 Acireale, Italy

Abstract: During the period 1997-2006, according to previous experiences carried out in Sicily on mature citrus groves, male catches of *Aonidiella aurantii* (Mask.) were recorded in nursery citrus trees located in North and East growing area of the island. Data were obtained in five representative sites where two-three years old *Citrus* spp trees were cultivated. Pheromone yellow sticky traps were hanged at the canopy level of young trees and were changed weekly. *A. aurantii* male captures were counted under stereomicroscope in laboratory. The pheromones were changed monthly. Climatic data were recorded with mechanical or electronic meteorological station located in each sites for determining Degree-Day (DD) accumulation, using the lower development threshold of 12 °C, defined as thermal constant (K). Male captures showed four flights per year. In several years a partial 5th male flight was observed as well. The 1st flight (over-wintered generation) showed frequently a well-defined progressive peak, but when the winter temperatures were not very cool a multi-cohorts 1st flight was recorded. The following flights (1st, 2nd and 3rd generation) showed two or, rarely, three cohorts not clearly attributable to a generation, due to overlapping stages. From data collected in all sites in ten different years the mean K for total development of four flights (over-wintered, 1st, 2nd and 3rd generation) was 609 DD (± 70), in coincidence with data obtained previously for mature citrus groves in Sicily. In nursery condition due to mild climate it was possible to calculate a mean K for total development of five flights, including a partial 4th generation, with a value of 556 DD (± 47). In the different years of the observation period, the peaks of the 1st flight were recorded in a range from April to mid-May; peaks of 2nd flight were recorded from mid-June to mid-July; peaks of 3rd flight were recorded from end of July to end of August; peaks of 4th flight were recorded from end of August to mid-October; when it occurred, a 5th flight was recorder from mid-October to mid-December. On these bases, male captures peaks and Degree-Day accumulation can help for determining the optimal spray timing for *A. aurantii* control in nursery cultivation.

Key words: monitoring, pheromones, Degree-Days accumulation, thermal constant

Introduction

California Red Scale (CRS), *Aonidiella aurantii* (Maskell) is currently considered to be one of the most important pests of the Sicilian citrus industry and it is present in all Southern Italy citrus districts with a preference for inland citrus orchards (Battaglia & Viggiani, 1982; Leocata, 1992; Liotta, 1970; Longo *et al.*, 1995) and in nursery cultivation (Conti *et al.*, 2003). It can be responsible for both a loss in tree vitality and the downgrading of infested fruits. CRS is traditionally controlled with organophosphates insecticides (chlorpyrifos mainly) and mineral oil (Areddia *et al.*, 2000).

Pheromone traps have been used commercially in several countries to help in the management of this pest, by indicating in terms of males captures when an insecticide treatment is necessary (Moreno & Kennet, 1985; Grout *et al.*, 1989).

In Sicily on mature lemon groves, located in the North-eastern area of the island, according to direct observation on twigs, four generations per year were recorded and the 4th generation over-wintered with all stages except for adult winged males (Inserra, 1969). In recent years, on mature orange groves the results of trapping of male red scales for three years showed that in general four flights occurred in April and May (over-wintered generation), June and July (1st generation), August (2nd generation), September and October (3rd generation) with the 3rd generation over-wintered (Tumminelli *et al.*, 1997).

For interpreting the males peaks on CRS traps a physiological time (insect time), using daily maximum and minimum temperatures, was used in the form of Degree-Days (DD) accumulated above a lower developmental threshold of 12°C, with a thermal constant (K) of about 600 DD in °C (Kennet and Hoffman, 1985). A confirmation of this theory was obtained in Sicily with the average K, for the four main flights, of 602.7 DD between peaks (Tumminelli *et al.*, 1997) on mature citrus groves, and a K of 552 DD in nursery condition in a preliminary survey (Tumminelli *et al.*, 2003). On these bases the monitoring of CRS males was intensified in the North and Eastern Sicily citrus nursery cultivation for evaluating the number of generation of *A. aurantii*, and the DD accumulation between peaks (K), observing if any differences could be demonstrated relatively to mature orchards.

Material and methods

Data were obtained from five representative nurseries located in North and East Sicily during the period 1997-2006, for a total of 18 sites monitored. The nurseries were selected out of several sites where severe infestations of *Aonidiella aurantii* (California Red Scale) were frequently recorded prior to the beginning of the experimental project. Various citrus trees two-tree years old, prevalently *Citrus limon* (L.) Burmann f., *C. aurantium* L., *C. clementine* Hort., *C. sinensis* (L.) Osbeck, *C. madurensis* Loureiro, *C. myrtifolia* Raf., *Fortunella margarita* (Lour) Swingle, *F. japonica* (Thumb) Swingle and various hybrids were cultivated in 8-10 litres plastic pots. Trees were sprayed for various pests according to the grower's pest management strategy.

The flight phenology of CRS males was monitored whit pheromones (Isagro Italia) and yellow polyvinyl sticky cards sized 12.8 cm x 7.7 cm. Each trap was placed approximately in the middle of the nursery and was hanged at the canopy level of young trees. The sticky traps were changed and counted weekly under stereomicroscopy laboratory, using a 20% sample template when catches exceeded 200 males per card, whereas below this density we counted males on the entire card. The pheromones were changed every month (Peherson *et al*., 1991). For interpreting the population flight peaks on CRS traps a physiological time (insect time), using daily maximum and minimum temperatures, was used in the form of degree-days (DD) in centigrade accumulated daily above a lower developmental threshold of 12°C (TL); an upper developmental threshold of 38°C was also used for the calculation of degree-day accumulation, because probably when temperature exceeds 38-40°C the development of the insect is interrupted. For the calculation of the daily DD the single sine method [(T max - T min/2) - TL], with a cut off between both thresholds, was utilized (Snyder, 1985). Daily maximum and minimum temperatures were recorded with mechanical or electronic meteorological stations located in each site. Flights peaks were defined graphically and these graphics were utilized for determining the accumulation of DD between flight peaks that represent the thermal constant (K) for the development of each generation. Usually the DD value for the apex of each peak was recorded but if two close peaks appeared, related to two cohorts, the DD value at the mid-point between the two peaks was used. The average accumulation of DD between all peaks was calculated as well.

Results and discussion

Male captures showed four flights per year (Fig. 1). In several years a partial 5th male flight was observed as well (Fig. 2).

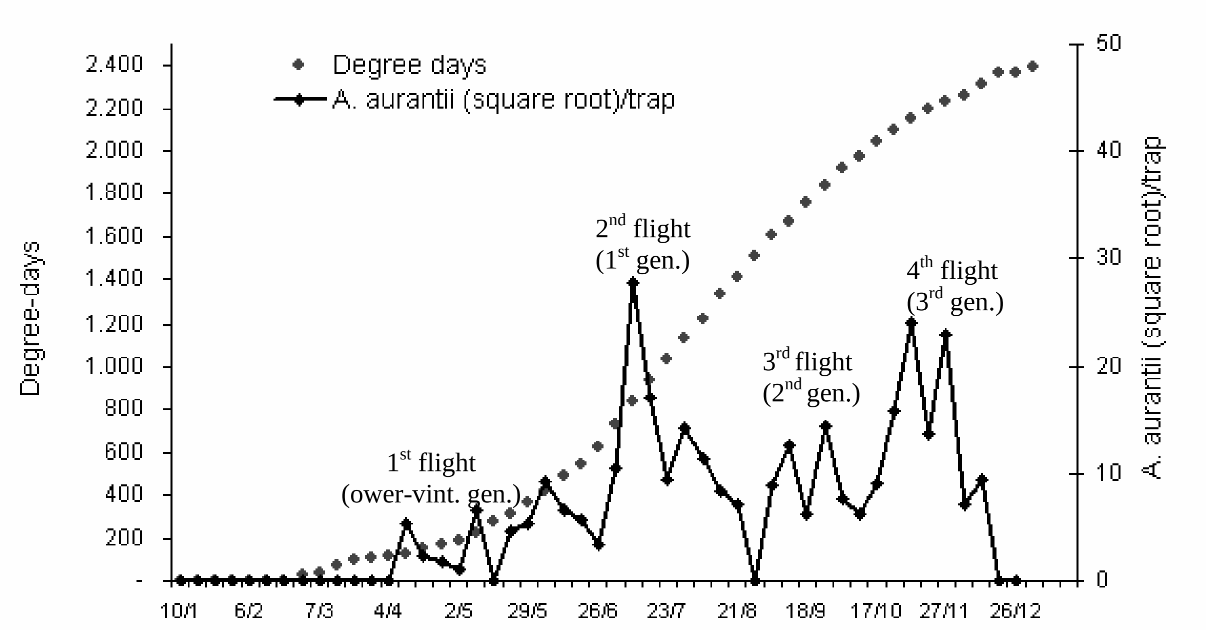

Fig. 1 Seasonal flight of California Red Scale male on citrus nursery trees in 2002 in Northern Sicily

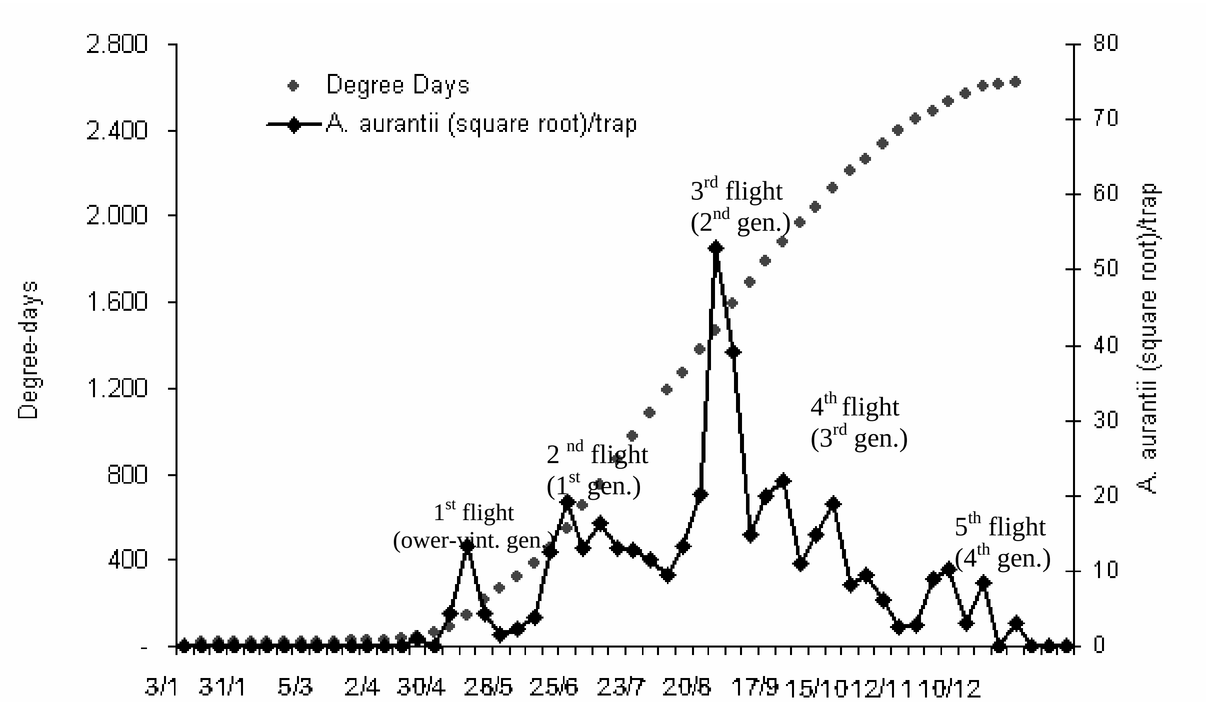

Fig. 2 Seasonal flight of California Red Scale male on citrus nursery trees in 2003 in Northern Sicily

The 1st flight (over-wintered generation) showed frequently a well-defined progressive peak, but when the winter temperature were not very cool a multi-cohorts 1st flight with relative peaks was recorded (Fig. 1). The following flights (1st, 2nd and 3rd generation) showed two or, rarely, three cohorts not clearly attributable to a generation, due to the overlapping of different stages (Fig. 1 and Fig. 2).

In the different surveys of the observation period (ten years), the peaks of the 1st flight were recorded in a range from April to mid-May; the peaks of 2nd flight were recorded from mid-June to mid-July; the peaks of 3rd flight were recorded from end-July to end-August; the peaks of 4th flight were recorded from the end-August to end-October; when it occurred, a 5th peak was recorder from mid-October to mid-December. In several cases it was difficult to record clearly the male captures of July-August because high temperature and severe spray application for different pests occurring on young trees disturbed male flights of 1st and 2nd generation (Table 1).

Table 1. Number of yearly generation of CRS according to peaks of male captures in Sicily nursery trees (range of the period 1997-2006 in a total of 18 sites)

Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
	ower-vintered gen (1st flight)								
			1st generation (2nd flight)						
					2nd generation (3rd flight)				
						3rd generation (4th flight)			
								4th generation (5th flight)	

As reported in Table 2, the mean number of CRS male captures increased from over-wintered generation (241 ± 348) to 3rd generation (2158 ± 2578), and decreased in the 4th generation (1862 ± 2573), but a very relevant variability of data was recorded during the different monitoring seasons, as demonstrated by the very high standard deviation. The different levels of captures during the ten-year survey were probably due to different seasonal climatic patterns, to chemical spray applications and in a less extent to parasitization.

Table 2. Number of CRS males captures in flights peaks of each generation in Sicily nursery trees (average of the period 1997-2006 in a total of 18 sites).

	over-wintered generation (1st flight)	First gen. (2nd flight)	second gen. (3rd flight)	Third gen. (4th flight)	Fourth gen. (5th flight)
Avg	241	862	1.682	2.158	1.862
Std ±	348	688	2.690	2.578	2.573

During the period of observation, the DD accumulation (ten-year average) among peaks showed a thermal constant (K) of 584 DD (± 91) from over-wintered to 1st generation (1st and 2nd flight), a K of 689 (± 193) DD from 1st to 2nd generation (2nd and 3rd flight) and a K of 555 DD (± 121) from 2nd to 3rd generation (3rd and 4th flight). In several cases a 4th partial generation (5th flight) was observed with a K recorded at 430 (± 124) DD from the previous generation (Table 3). This last value was lower respect to previous data because cooler temperature reduced drastically male captures interrupting the flight. Since the 1st, 2nd and 3rd generations often overlapped, the K was not correctly calculated during the different years of observation, as described by the relevant standard deviation (Table 3). According to data collected in all 18 sites in ten different years the mean K for total development of four flights (over-wintered, 1st, 2nd and 3rd generation) was 609 DD (± 70), in coincidence with data obtained for mature citrus groves in Sicily. In nursery condition due to mild climate it was possible to calculate a mean K for total development of five flights, including a partial 4th generation, with a K value of 556 DD (± 47).

Table 3. Accumulated DD between flight peaks (thermal constant - K) of CRS males captures in Sicily nursery trees (average of the period 1997-2006 in a total of 18 sites).

	K over-wintered to 1st gen.	K – 1st to 2nd gen.	K – 2nd to 3rd gen.	K – 3nd to 4th gen.	Avg. K - over-wint. to 3rd gen.	Avg. K over-wint. to 4th gen.
Avg	584	689	555	430	609	556
Std ±	91	193	121	124	70	47

In our study we observed the phenology of CRS on citrus nursery trees, which revealed a different seasonal trend in comparison to mature citrus orchard. A 5th male flight was revealed due to a warm climate during autumn and winter in several seasons. A mean K of 430 DD for this final flight let us suspect that a partial 4th generation can develop in favourable years. The high sensitivity of the pheromones allows a clear idea of male's phenology even in coincidence with severe spray applications. The calculation of a thermal constant can help for predicting the development of the generations and can be a viable and affordable tool for spray timing decision.

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Seasonal trend of California Red Scale (*Aonidiella aurantii*) populations in eastern Spain 2005-2007

A. Castaño[1], B. Escrig[1], M. Guillén[1], O. López[1], M. Llopis[1], A.B. Martínez[1], A. Moreira[1], L. Peris[1], J.J. Pérez[1], J. Sepúlveda[1], M. Vicente[1], F. Garcia-Marí[2], J.M. Guitián[3], M.P. Baraja[4], J.M. Llorens[4], P. Moner[4], V. Dalmau[4]
[1] *Tecnologías y Servicios Agrarios, S.A., Cronista Carreres 11, 46003 Valencia, Spain*
[2] *Departamento de Ecosistemas Agroforestales, Universidad Politécnica de Valencia, Spain*
[3] *Tecnologías y Servicios Agrarios, S.A., Valentín Beato 6, 28037 Madrid, Spain*
[4] *Dirección General de Investigación y Tecnología Agroalimentaria, Conselleria de Agricultura, Pesca y Alimentación, Comunidad Valenciana, Spain*

California Red Scale, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), was first recorded as an economic pest in eastern Spain in 1985. It has spread since then throughout the Comunidad Valenciana region but to the northeast area, becoming one of the most economically important citrus pests. Ongoing information is being gathered on this pest as a part of a survey program developed in this region since 2004 to detect potential foreign pests and monitor common pests on citrus (Plan de Vigilancia Fitosanitaria de Cítricos de la Comunidad Valenciana). Data from 100-400 orchards monitored regularly along the year show similar patterns for 2005, 2006 and 2007 in overall California Red Scale populations. Initial infestation of growing fruit occurs in early June and increases steadily through the summer, reaching its maximum level in October. Four male flights are being recorded, in March-May, June-July, August-September and October-November. The proportion of immature stages of the first generation reaches its maximum at the end of May-beginning of June. Differences in these population patterns among the years are discussed.

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IOBC/wprs Bulletin Vol. 38, 2008
pp. 26-33

Parasitism levels and species of natural enemies in field populations of California red scale *Aonidiella aurantii* (Hemiptera: Diaspididae) in eastern Spain

Juan José Sorribas, Raquel Rodríguez, Eugenia Rodrigo, Ferran García-Marí
Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia.
Camino de Vera s/n, 46022 Valencia, Spain.

Abstract: During 2004-2007 we sampled natural enemies of the citrus red scale (CRS) *Aonidiella aurantii* (Maskell) in 173 different citrus orchards distributed throughout the main Spanish citrus area (Valencia, east of Spain). Twigs and fruits containing CRS populations were observed in laboratory and parasitoids were reared to adults in climatic chambers. Twenty orchards were sampled periodically estimating parasitism levels in different instars stages and on different tree substrates. Pheromone and chromatics traps with periodical renovation were placed in 100 citrus orchards to identify captured parasitoids. Parasitism is present in all citrus groves with California red scale. From the 18,006 parasitoids identified 50% belong to *Aphytis chrysomphali* (Mercet) and 49% to *Aphytis melinus* DeBach. *Encarsia perniciosi* (Tower) (1%), not previously documented in Spain, has also been observed. The proportion of *A. chrysomphali* increases from South to North and in the colder months. Parasitism levels reach up to a maximum of 78% of susceptible instars, with average levels of 19% in all the sampled orchards. Higher levels of parasitism were found between August and November. The predatory complex is constituted by *Rhyzobius lophantae* Blaisdell, *Chilocorus bipustulatus* (L.), *Lestodiplosis aonidiellae* Harris, *Chrysoperla carnea* (Stephens), *Semidalis aleyrodiformis* (Stephens) and *Hemisarcoptes coccophagus* Meyer.

Key words: *Aonidiella aurantii, Aphytis melinus, Aphytis chrysomphali, Encarsia perniciosi*.

Introduction

Aonidiella aurantii (Maskell) was found in Alzira (Valencia) in 1985 and since then it has extended all around the Valencia region (east of Spain) becoming the most important citrus pest. Valencian citriculture covers 182.000 Ha along the Mediterranean coast from 37º 50' to 40º 40' North latitude. The most effective parasitoids controlling California red scale (CRS) are the ectoparasitoids of the genus *Aphytis* (Rosen & De Bach, 1979). *Aphytis melinus* DeBach is the main parasitoid all around the word. Endoparasitoids role is considered complementary to the *Aphytis* because they parasitize different scale instars. There are many examples of positive coexistence between *Encarsia perniciosi* (Tower) with *Aphytis* (Yu et al., 1990) and *Comperiella bifasciata* Howard with *Aphytis* (Bedford & Grobler, 1998).

Susceptible scale instars for parasitism by *Aphytis* are mainly third instar (young females), second instar and males, whereas *E. perniciosi* and *C. bifasciata* can parasitize all scale instars except mature females with crawlers (Foster et al., 1995).

In Spain *A. melinus* has been reared and released since 1976 (initially for the control of *Chrysomphalus dictyospermi* (Morgan)). Previous searches for parasitism in Spain, conducted usually in a small number of citrus orchards in the centre of the Valencia region, obtained *Aphytis chrysomphali* (Mercet), a native ectoparasitoid (Rosen & De Bach, 1979), in higher proportion than the introduced *A. melinus*. Observations between 1988 and 1994 yielded almost 100% of *A. chrysomphali* (Troncho et al, 1992; Rodrigo & Garcia-Marí, 1995;

Rodrigo et al., 1996), whereas in 1999-2000 Pina et al (2006) obtained 77% of *A. chrysomphali*. On the contrary, studies in Andalucía, South of Spain, found *A. melinus* as almost the unique parasitoid (Vela et al. 2007).

Since DeBach and Sundby (1963) first described competitive displacement of *A. chrysomphali* by *A. melinus* in California, many examples of this displacement have been found in other countries. Considering the Mediterranean basin, Pelekassis (1974) in Greece indicates the presence of native *A. chrysomphali* and successful establishment of released *A. melinus*. Later on, field studies of Argyriou (1974) confirm the displacement of *A. chrysomphali* in Greece only 9 years after the introduction of *A. melinus* in 1962. In Cyprus *A. chrysomphali* was displaced by *A. melinus* in the inland areas but not in the coast, where both coexist (Orphanides, 1984). Works carried on by Siscaro et al. (1999) in Sicily (Italy) describe native *A. chrysomphali* as almost completely displaced by *A. melinus*. Similar results have been reported in Morocco and Turkey (Mazih, A. and Satar, S. respectively, pers. comm., 2007). On the other hand, Benassy (1974) refers to *Comperiella bifasciata* as the most important parasitoid in the Mediterranean coast of France. This parasitoid was previously reared and released by the Antibes (France) insectarium.

The aim of this research was to identify the species of parasitoids and their relative abundance in all geographic citrus areas from east of Spain, to study the seasonal variation of parasitism and parasitoids in the field and to determine parasitism levels on susceptible scale stages. We also wanted to know the main CRS predators in Spain.

Material and methods

The information obtained came from 173 different orchards and two sources:

- Samples of twigs with leaves or fruits from 105 orchards with high *A. aurantii* density located throughout all the citrus producing areas of the Valencia region. We selected 20 of these orchards for periodical monitoring of parasitism.
- Pheromone and chromatic traps placed in 100 citrus orchards distributed all around valencian citriculture areas.

For the first procedure, along the years 2004 to 2007 we collected 150 field samples containing *A. aurantii* (25- 45 branches about 30 cm long with leaves and 20-35 fruits when possible) that were kept in evolutionaries to collect parasitoid and adult predators on yellow sticky traps of 12 x 12 cm. Evolutionaries consisted on plastic boxes of 40x30x22 cm maintained inside climatic chambers (28ºC, 60% RH) for 30 days to allow development of all natural enemies to adults.

We selected 20 orchards to study parasitism levels trying to cover all the citrus areas in which the Valencia region is divided. Orchards were monitored 3 to 5 times per year in different seasons, determining parasitoid species and parasitism levels on the three susceptible scale stages for parasitism by *Aphytis* (2^{nd} instar, males and females). Parasitism was determined separately on twigs and on fruits by counting 50 live forms of the 3 susceptible stages scale and its parasitoids.

In the second procedure, since may 2005 till may 2006, and in collaboration with the Agriculture Government of the Valencia region (Citrus Phytosanitary Survey, PVF), we identified parasitoids and predators captured on tent pheromone traps and on 14 x 20 cm yellow sticky traps sampled every 14 days from 100 citrus orchards (Fig. 1).

Identification methods

We used binocular to find and extract parasitoids from the traps, placed them on xylene for cleaning, and digested in Nesbit liquid. *Aphytis* were identified using Rosen DeBach (1979) identification book and *Encarsia* using Viaggiani (1967) and Myartseva (2001) identification keys.

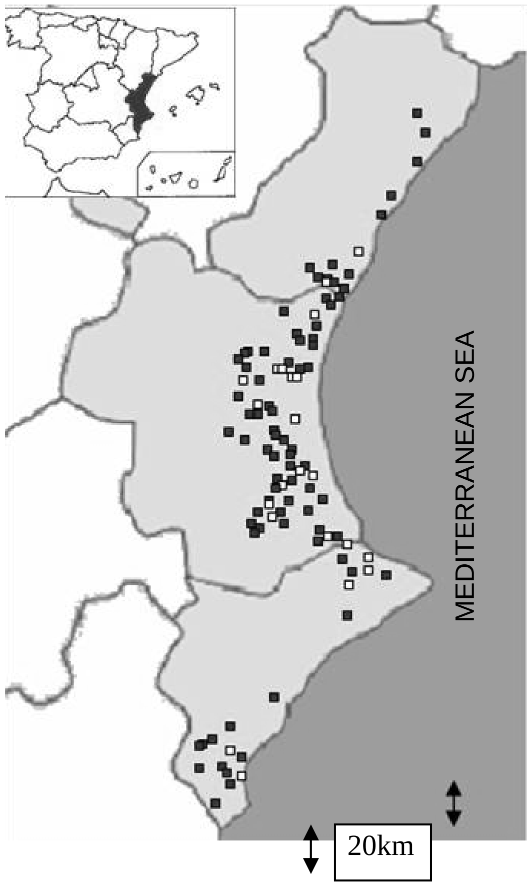

Figure 1. Valencia region and citrus groves of the Citrus Phytosanitary Survey on which traps were located (white dots are the orchards selected for parasitism).

Results

We identified 18,006 parasitoids; 49% belonged to *Aphytis melinus* and 50% to *Aphytis chrysomphali*. These ectoparasitoid species were present together in most of the citrus groves with California red scale. The presence of the endoparasitoid *Encarsia perniciosi* (Tower), not previously documented in Spain on *A. aurantii*, has been observed in a reduced humid area of Alicante province (South of the region). The 273 individuals captured represent only 1% of the total number of parasitoids but 20% of the parasitoids captured in this area. *E. perniciosi* was present in all the samples from this area reaching an average level of parasitism of 10%.

Table 2. Number of *A. melinus*, *A. chrysomphali* and *E. perniciosi* identified in evolutionaries and on field traps, in citrus orchards from eastern Spain.

No. orchards	No. samples	Sampling period	Sampling method	No. *A. melinus*	No. *A. chrysomphali*	No. *E. perniciosi*
105	233	Jan. 04 – May 07	Branches/fruits in evolutionary	6111	7417	270
100	4555	May 05 – May 06	Chromatic and pheromone traps	2684	1521	3

Comparing the two methods of sampling, the proportion of *A. chrysomphali* obtained from twigs or fruits in evolutionaries was higher (53% of the parasitoids) than those of the traps (36%). This difference can be attributed to the higher proportion of orchards sampled with traps in the south of the region, where the relative abundance of *A. chrysomphali* is much lower. *E. perniciosi* is obtained in higher numbers from the evolutionaries because the area where this species was present was sampled more intensely in evolutionaries than with traps.

In most citrus groves, both *A. melinus* and *A. chrysomphali* were present in the same sample. The group of samples where both parasitoids coexisted in similar proportion was higher than the group of samples where one of the two species predominates (Fig. 2).

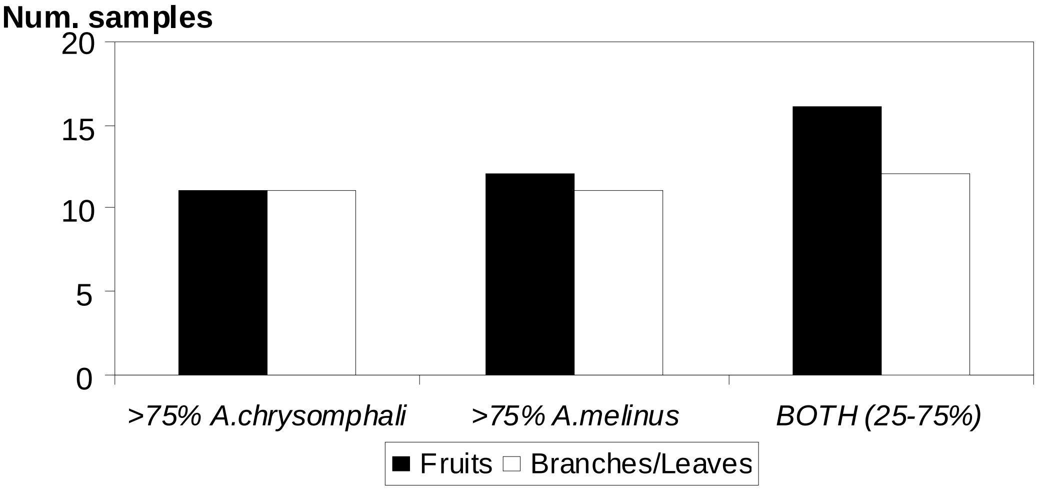

Figure 2. Number of samples of branches/leaves or fruits with California Red Scale containing different proportions of *A. chrysomphali.*

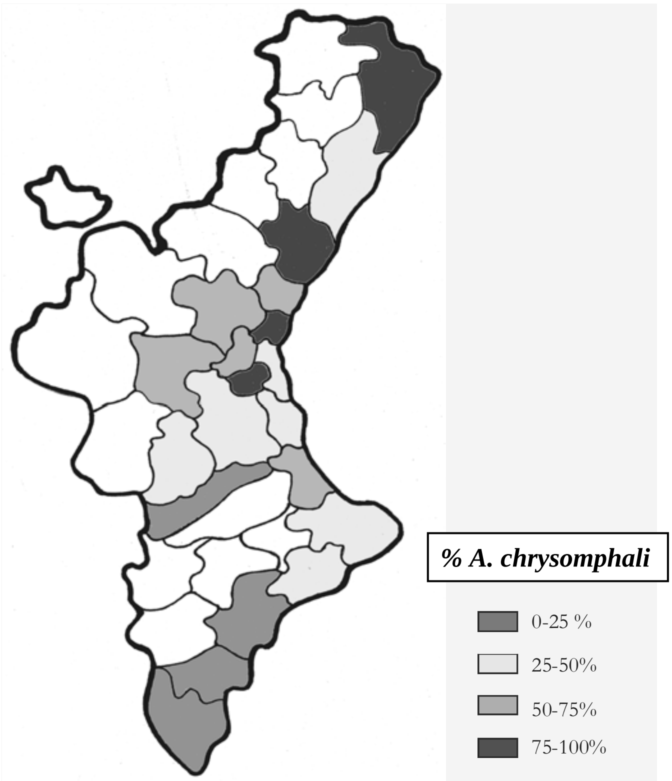

Figure 3. Distribution map of *A. chrysomphali* in relation with total number of *Aphytis* in citrus orchards of eastern Spain.

The proportion of *A. chrysomphali* increases from South to North, from less than 1% to more than 90% of the parasitoids. Thus, in the southern areas of Valencia region *A. melinus* has displaced *A. chrysomphali*, but not in the rest, where *A. chrysomphali* is present in much higher proportion, especially in northern areas (Fig. 3).

We have observed that, when both species are present in the same citrus grove, their relative abundance varies along the year, hot periods being preferred by *A. melinus* and cold periods by *A. chrysomphali* (Fig. 4). *A. chrysomphali* decreases during the warmer months of the year.

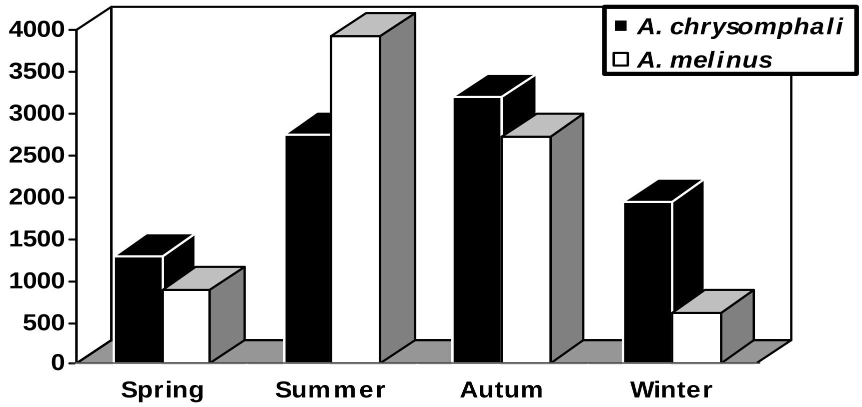

Figure 4. Seasonal distribution in the number of *A. chrysomphali* and *A. melinus*, in citrus orchards from eastern Spain.

Maximum parasitism levels by *Aphytis* reached up to 78% of the susceptible stages, with an average level in all samples of 19%. Higher levels of parasitism were found between August and November for the three scale stages susceptible to be parasitized by *Aphytis*. Young females (H1) were much preferred by *Aphytis* than males and second instar stages (Fig. 5). Higher rates of parasitism by *E. perniciosi* were observed in April, and reached up to 33% of the susceptible stages.

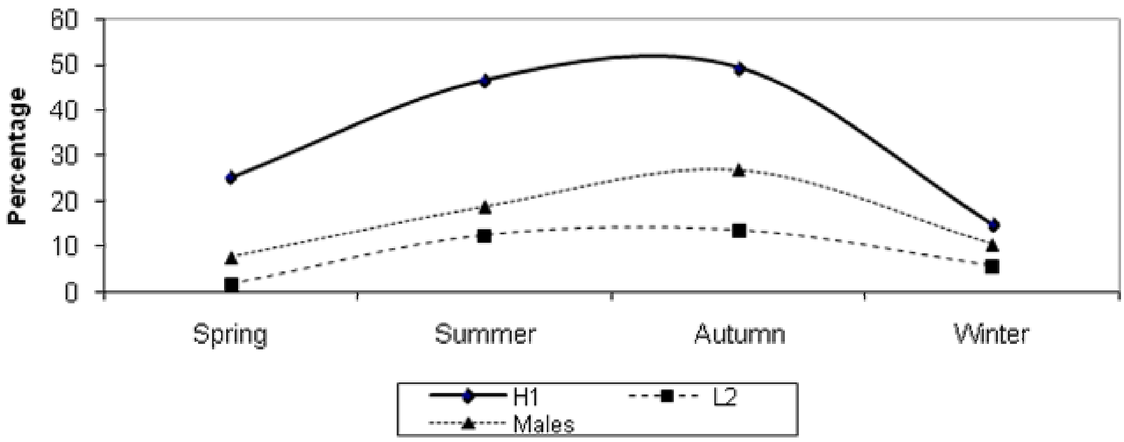

Figure 5. Seasonal evolution of parasitism levels by *Aphytis* on the three susceptible scale stages of *Aonidiella aurantii*, in citrus orchards from eastern Spain.

The predatory complex is constituted by *Rhyzobius lophantae* Blaisdell (Coleoptera: Coccinellidae), *Chilocorus bipustulatus* (L.) (Coleoptera: Coccinellidae), *Lestodiplosis aonidiellae* Harris (Diptera: Cecidomyiidae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), *Semidalis aleyrodiformis* (Stephens) (Neuroptera: Coniopterygidae) and *Hemisarcoptes coccophagus* Meyer (Astigmata: Hemisarcoptidae). The number of insects we have observed predating on *A. aurantii* or captured in evolutionaries or traps is shown in the Table 2. The most important predators are *L. aonidiellae* and *R. lophantae*. *L. aonidiellae* is a cecidomyiid that penetrates below the scale cover and usually prefers female scales. *R. lophantae* is a generalistic predator than can feed on all scale instars and is able to make a hole in the scale cover to feed on the insect below.

Table 2. Number of *Aonidiella aurantii* predatory insects found in citrus orchards from eastern Spain.

NAME	**Nº INSECTS**
Lestodiplosis aonidiellae	113
Rhyzobius lophantae	98
Semidalis aleyrodiformis	17
Chrysoperla carnea	12
Hemisarcoptes coccophagus	9
Chilocorus bipustulatus	6
Conwentzia psociformis	5

Discussion

If we compare the fast adaptation and establishment of *A. melinus* occurred in California (DeBach & Sundby, 1963), Greece (Argyriou, 1974) or Sicily (Siscaro et al. 1999) and the subsequent displacement of previously present species like *A. chrysomphali*, with the long period passed since releases of this parasitoid in Spain were carried out, we should conclude that environmental or biological factors are reducing or avoiding the spread of *A. melinus* to the North areas of the Valencia region.

One of these factors could the low winter temperatures. Our observations show that declining temperatures during autumn and winter affects more negatively *A. melinus* than *A. chrysomphali* survival. It is known from laboratory experiments that *A. chrysomphali* is more tolerant to extreme cold and less tolerant to extreme heat temperatures in all its development stages. The threshold of development is estimated as 8.5 ºC for *A. chrysomphali* and 11ºC for *A. melinus* (Abdelrahman, 1974 a, b).

Flight of *Aphytis* could be also affected by reduced temperatures during winter. This added to the lack of suitable scale stages during this period (most of them are gravid females) could cause a “bottle neck” on parasitism, reducing parasitoid populations drastically. The spring generation of the scale will develop without biological control by parasitoids. At this moment, beginning of spring, releases of *Aphytis* could be very useful to increase biological control of CRS populations.

Encarsia perniciosi is found in several citrus areas all around the word (Florida, Uruguay, Australia) as parasitoid of CRS, but it is usually constricted to humid o semitropical areas with high rainfall and warmer temperatures all over the year. This weather conditions are found in Valencia region in a particular area of Alicante province called “La Marina” but not in other areas of the region, therefore the expansion of this parasitoid in Spain could be limited by its climatic requirements.

Our observations show that the proportion of CRS parasitoid species in a particular orchard could be very different in summer or in winter. Thus, studies to determine predominant parasitoids in an orchard or crop area should be carried out all along the year and not restricted to limited periods.

In conclusion, after many years of its introduction and establishment, *A. melinus* has not displaced *A. chrysomphali* in many areas of eastern Spain as parasitoid of CRS. Mass releases of parasitoids could be very useful as a complement for naturally occurring parasitism and should be focused on spring and early summer, when naturally occurring parasitism levels are usually lower.

Acknowledgements

We thank the Citrus Phytosanitary Survey Project for the trap samples and Alejandro Tena from the Polytechnic University of Valencia for his critical review. This work was supported by the project AGL2005-07155-C03-03 from the Ministerio de Educación y Ciencia of Spain.

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Host size availability for *Aphytis* parasitoids in field populations of California red scale *Aonidiella aurantii,* in Eastern Spain citrus groves

Apostolos Pekas, Amparo Aguilar & Ferran García-Marí
Institut Agroforestal Mediterraní, Camí de Vera 14, 46022 València, Spain

Abstract: The availability of suitable for parasitism California red scale (CRS) *Aonidiella aurantii* (Maskell) sizes affects the activity and distribution of its ectoparasitoids *Aphytis melinus* (DeBach) and *A. chrysomphali (*Mercet). The seasonal trend and spatial variation in the body size of different development stages in field populations of *A. aurantii* in the Community of Valencia (eastern Spain) citrus groves have been studied trying to assess its influence on parasitism rates and species of *Aphytis* present. Different citrus orchards were periodically sampled along 2007. Body, cover and exuvia of healthy and parasitized scales from branches, leaves and fruits were measured in the laboratory. The sizes of the scale cover and of the scale body were closely correlated. The scale body size was influenced by the season of the year, plant substrate and location. *Aphytis melinus* preferred third instar scales in the size range of 0.38-0.82 mm^2 (in surface area of the scale body), whereas *A. chrysomphali* parasitized mostly 0.15-0.33 mm^2 second instar females and 0.10-0.32 mm^2 second instar males. The influence of the range of scale sizes found in the field on species of parasitoids attacking CRS is discussed.

Keywords: *Aonidiella aurantii,* parasitoids, *Aphytis,* host size, biological control

Introduction

California red scale (CRS), *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) is one of the most important citrus pests worldwide (Ebeling, 1959; Talhouk, 1975). In Spain it is present since 1911 but it was not until 1985 when the first severe damages were recorded (Quayle, 1911; Rodrigo & Garcia-Marí, 1992). Nowadays CRS is present all over the citrus growing regions of Spain; it completes three generations annually and causes considerable damage (Ripollés, 1990; García-Marí & Rodrigo, 1995).

Several programs of biological, chemical and integrated control have been developed for the control of CRS in different regions of the world (Compere, 1961; De Bach, 1974; Argyriou, 1986; Ripollés 1990; Rosen, 1995). The most important natural enemies for the biological control of the CRS are the ectoparasitoids that belong to the genus *Aphytis* Howard. They are considered more effective than the endoparasitoids and the predators (Rosen & De Bach, 1976; 1979; 1990; Rosen, 1994). In the Community of Valencia the most important ectoparasitoids of CRS are *Aphytis chrysomphali* (Mercet) and *A. melinus* (DeBach) (Troncho et al., 1992; Rodrigo et al., 1996; Pina, 2003; 2006).

The competition among *Aphytis* species is perhaps the best known case of competitive displacement in classical biological control (De Bach & Sundby, 1963). *Aphytis chrysomphali* was displaced from nearly all of its range in southern California by *A. lingnanensis,* which then was displaced over much of its range by *A. melinus* (De Bach & Sundby, 1963; Rosen & DeBach, 1979). Similar displacements of *A. chrysomphali* by *A. melinus* have been observed in other citrus growing regions as Greece, (Argyriou, 1967); Cyprus, (Orphanides, 1984); Australia; (Dahms & Smith, 1994); Argentina (De Santis & Crouzel, 1994); Sicily (Siscaro y Mazzeo, 2003). However, in eastern Spain such displacement has apparently not happened for the moment. *Aphytis melinus* was introduced and dispersed throughout the whole citrus growing region of the Valencia Community but the autochthonous *A. chrysomphali* has not been displaced until now (Troncho et al, 1992; Pina, 2003; 2006; personal observation).

One of the mechanisms by which *A. melinus* has displaced *A. lingnanensis* is that of recourse pre-emption (Reitz & Trumble, 2002) because it utilizes smaller hosts for the production of female progeny than *A. lingnanensis* (Luck et al., 1982; Luck & Podoler, 1985). Additionally, host size has been found to influence *Aphytis* sex ratio (Luck et al., 1982) and size (Opp & Luck, 1986; Yu, 1986). The size of CRS varies between plant substrate and locality (Luck & Podoler, 1985). Therefore, the examination of the scale size on different plant substrates and localities as well as the different sizes-developmental stages of the scales preferred by the *Aphytis* species are of great importance for the evaluation and improvement of biological control of CRS in Eastern Spain. In this study we analyze the relationship between the size of the body and cover of different developmental stages of CRS, we examine the spatial variation and seasonal trend in the body size in field populations from the Valencia area and we determine the host size-stage preferences of the different *Aphytis* species.

Materials and methods

The survey was conducted from February 2007 to October 2007 in fifteen citrus orchards throughout all the citrus growing region of Valencia, each orchard being sampled at least twice for the winter and summer generations of the scale.

At each sampling site twigs between one and three years old and fruits (when available) infested with CRS were collected from several trees. The sample included three substrates wood, leaves and fruits. From each substrate the scales and bodies of 20 live gravid females without crawlers, third instar female and second instar male and female were measured using a micrometer under a stereomicroscope. Stages were defined using the description of Ebeling (1959). The width x length of each scale and body was used as an index of their size (Luck & Podoler, 1985). We considered the gravid females as the maximum size that CRS can attain as at this stage the female seals the body under the cover and stops feeding (Forster et al, 1995).

From each sample, the scales of 20 parasitized third instar females and second instar males and females from each substrate (if available) were measured with the same method as described above. Then they were converted to scale body sizes using the relationship between scale cover and scale body previously established. The parasitoid pupae found were identified (Rosen & De Bach, 1979) and, when unrecognizable parasitoid stages were found (eggs, larvae and prepupae), they were transferred to crystal vials for rearing, emergence of adults and identification (Rosen & De Bach, 1979).

Results and discussion

Relationship between scale body and scale cover size

The sizes of the scale cover and of the scale body were closely correlated. The relationships established for each developmental stage were: scale body = 0.2041 x (scale cover) + 0.0797, (R^2 = 0.50, N = 381) for the second instar males, scale body = 0.1966 x (scale cover) + 0.0731, (R^2 = 0.72, N = 430) for the second instar females and scale body = 0.2017 x (scale cover) + 0.2264, (R^2 = 0.81, N = 759) for the third instar females.

Factors influencing scale body size

The insects measured on fruits were always of larger body size than those located on leaves and wood for all the developmental stages and localities examined during the period that fruits were available (from 14 of February until 13 of March of 2007). The gravid females on fruits and leaves were significantly larger than those on wood (One-way ANOVA; $F_{2,212}$ =

9.03, P = 0.0002), the third instar females were larger on fruits, of intermediate size on leaves and smaller on wood ($F_{2,221}$ = 5.53, P = 0.0046), the second instar females were significantly larger on fruits than those on leaves and wood ($F_{2,152}$ = 4.82, P = 0.0094). The second instar males followed the same pattern, with the individuals measured on fruits being significantly larger than those on leaves and wood ($F_{2,172}$ = 3.45, P = 0.0339) (Fig. 1).

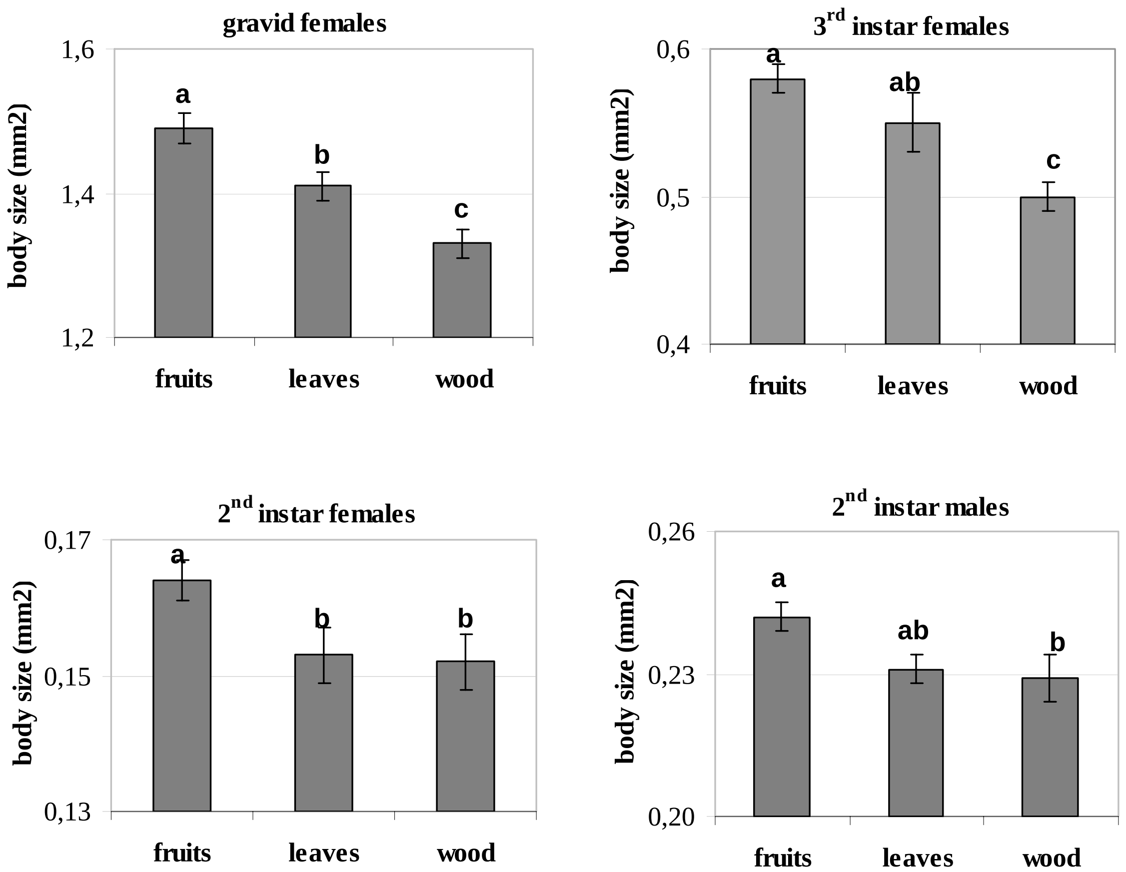

Figure 1. Body size of different CRS developmental stages and sexes on three substrates in Valencia (Eastern Spain) from February until March of 2007. Each bar represents the average and standard error of CRS body size from five citrus orchards.

The fraction of third instar female CRS suitable for the production of female *A. melinus* progeny is greater for the winter generation than for the summer and autumn generations for the three substrates examined. On fruits the 86% (N= 114) and 78% (N= 106) are suitable for the production of female *A. melinus* progeny for the winter and summer-autumn generations respectively (Fig. 2). The same pattern is observed on leaves with 83% (N= 167) for the winter generation and 69% (N= 176) for the summer-autumn generations greater than the lower threshold for the production of female *A. melinus* progeny. On wood the fractions were 75% (N= 226) and 68% (N= 344) fro winter and summer-autumn generations respectively.

Host size-stage parasitoid preferences

Each parasitoid species preferred a different host stage-size range of CRS (table 3, Fig. 3). *Aphytis chrysomphali* preferred mostly to parasitize 0.15-0.30 mm^2 (in surface area of the scale body) second instar males and only females were obtained. *Aphytis melinus* preferred 3rd instar females for the production of female progeny and 2nd instar (males and females) for the production of male progeny. The size range above which more female than male *A. melinus* are produced is that of 0.35-0.40 mm^2 (Fig. 4).

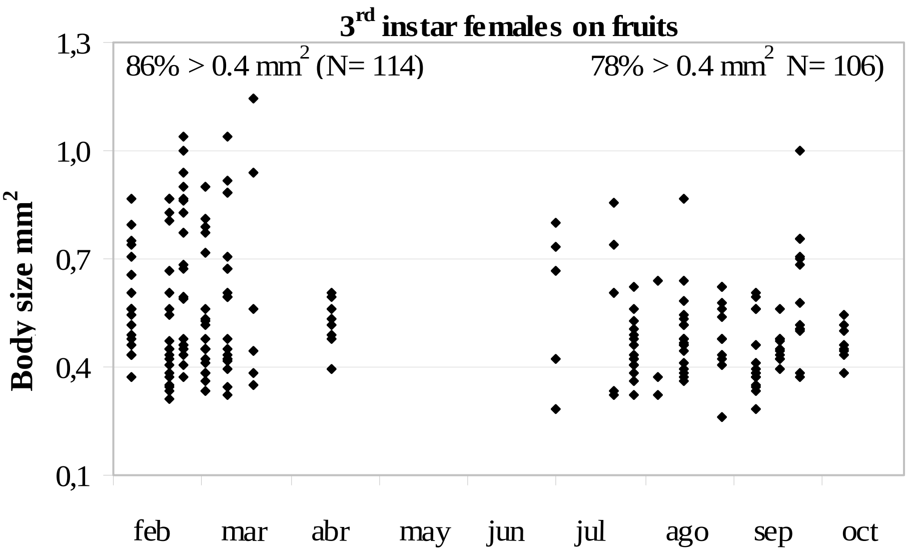

Figure 2. Body size availability and seasonal trend of CRS 3rd instar females on fruits from 15 locations in Valencia (Eastern Spain) for the winter and summer-autumn generations.

Table 3. Percent host stage preference for each *Aphytis* species at fifteen citrus orchards in the area of Valencia (Eastern Spain) from February to August 2007.

	Parasitoid species	
Host stage	*A. chrysomphali*	*A. melinus*
3rd instar female	24 %	57 %
2nd instar female	25 %	19 %
2nd instar male	50 %	23 %

Conclusion

There are important differences in the CRS body size depending on the plant substrate, location and season of the year. *Aphytis melinus* seems to be more influenced by these differences as it needs hosts of a determined size above which, it produces female progeny. Suitable hosts for the production of female *A. melinus* are present during the winter as well as the summer-autumn generations of the CRS. However, there are moments that these suitable hosts for the production of female *A. melinus* do no exist because of the seasonal variation in the population structure of the scale. Therefore *A. melinus* suffers bottlenecks in the density of suitable hosts for the production o female offspring. Host size availability in combination with the scale population structure and the influence of climate on each parasitoid species may explain the composition and seasonal fluctuations of the *Aphytis* species complex parasitizing CRS in Valencia.

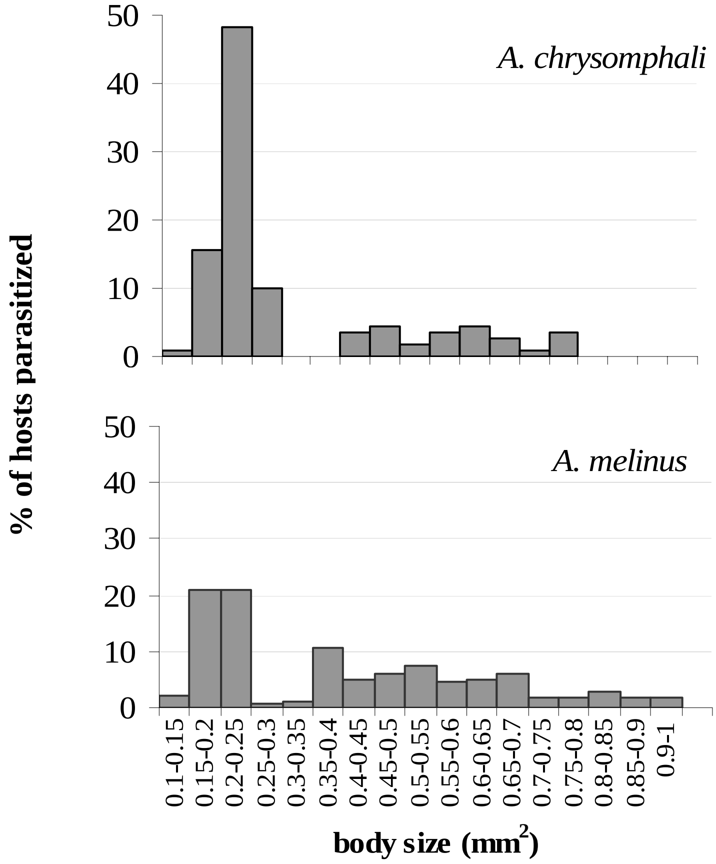

Figure 3. Distribution of CRS body sizes parasitized by *A.* gr *lingnanensis* (N= 68), *A. chrysomphali* (N= 175) and *A. melinus* (N= 210) at 15 citrus orchards in the area of Valencia (Eastern Spain) sampled between February and August 2007.

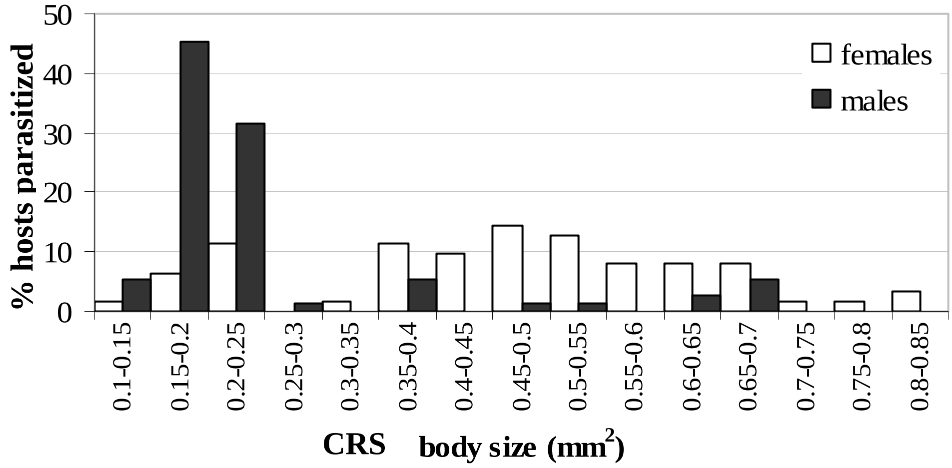

Figure 4. Distribution of CRS sizes yielding male (N = 73) or female (N = 62) *A. melinus*. Data from 15 citrus orchards in the area of Valencia (Eastern Spain) sampled between February and August 2007.

Acknowledgements

We would like to thank A. Tena for providing useful ideas and for critical review of the manuscript. Financial support was provided by the Ministerio de Educación y Ciencia project AGL2005-07155-C03-03.

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Parasitoid survey of California Red Scale (*Aonidiella aurantii*) in Citrus groves in Andalusia (South Spain)

J.M. Vela[1], M.J. Verdú[2], A. Urbaneja[2], J.R. Boyero[1]
[1] *IFAPA. Centro Churriana (Málaga). Cortijo de la Cruz s/n. 29140, Churriana (Málaga), Spain; josem.vela@juntadeandalucia.es*
[2] *Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Ctra. Montcada-Náquera km 4.5, Montcada, 46113, Valencia, Spain*

California Red Scale (CRS) is one of the most important citrus pests all around the world. In the last years this pest has considerably increased its negative effects in the citrus industry of Andalusia. This pest owns a relatively rich complex of natural enemies, such as parasitoids which in certain parts of the world are successfully used in inoculative releases to control this pest. However, no information is available about the parasitoid complex in Andalusia. For this reason, we have sampled citrus orchards in four Andalusian provinces (Huelva, Cádiz, Málaga and Almería) from March 2005 to May 2007, on a 45 days basis. Infested leaves, twigs and fruits were taken to the laboratory, where 100 scales per plant substrate were observed under microscope binocular. We have detected three Aphelinidae (Hymenoptera) species: *Aphytis melinus* DeBach, *Aphytis* sp. (group *lingnanensis*) and *Encarsia* sp. The former was the most common, being the second less abundant. *Encarsia* sp. was extremely rare. The percentage of *Aphytis* parasitism was higher on females than on males. The parasitism was higher on leaves than fruits and twigs.

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On the presence and diffusion of *Comperiella bifasciata* How. (Hymenoptera: Encyrtidae) in Southern Italy

Gaetano Siscaro[1], Francesca Di Franco[2] & Lucia Zappalà[1]
[1]*Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, via S. Sofia 100, 95123 Catania, Italy;*
[2]*CRA – Centro di ricerca per l'Agrumicoltura le Colture Mediterranee, Corso Savoia 190, 95024 Acireale (CT), Italy*

Abstract: The results of a field survey on the presence and diffusion of *Comperiella bifasciata* How. (Hymenoptera: Encyrtidae), endoparasitoid of *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), in Sicily and Calabria are presented. The aim of the survey, which started in 2003 and is still continuing, was to confirm the establishment of the encyrtid and to draw a map of its diffusion, 15 years after its first introduction. For this purpose, infested fruits (20) and twigs (4 meters, 1-2 years old) were collected in 10 groves in South Eastern Sicily (Siracusa province). Half of the sample was observed and the parasitized instars were isolated and reared until the adult parasitoids emerged. The remaining 50% was kept into emergence boxes and the obtained parasitoids were collected and identified. The presence of the parasitoid was also monitored using pheromone traps for the California Red Scale in different citrus groves. The data collected showed that the encyrtid is well adapted and has colonized a wide area, 50km, on average, far away from the first introduction site, as highlighted by the presence of the species in more than 70% of the monitored orchards. The survey will be continued and expanded in order to acquire quantitative data on the parasitic activity of the encyrtid.

Key words: *Aonidiella aurantii*, biocontrol, endoparasitoid, citrus

Introduction

The California Red Scale [*Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae)] is considered to be one of the main pests of citrus groves in the Mediterranean basin (Siscaro & Mazzeo, 2003; Franco et al., 2006; Garcia-Marí, 2006). Continuous investigations on the population dynamics of the scale as well as on its main mortality factors have been carried out over the last decades. The data up to now collected show that in Sicily the natural enemies complex is constituted by the parasitoids *Aphytis chilensis* Howard, *A. chrysomphali* (Mercet), *A. lignanensis* Compere, *A. maculicornis* (Masi), *A. melinus* DeBach, and *A. proclia* (Walker), and *Encarsia perniciosi* (Tower) (Hymenoptera: Aphelinidae) and by the predators *Chilocorus bipustulatus* (L.), *Rhyzobius lophantae* Blaisdell (Coleoptera: Coccinellidae), *Cybocephalus rufifrons* Retter (Coleoptera: Cybocephalidae) and *Lestodiplosis aonidiellae* Harris (Diptera: Cecidomyiidae) (Siscaro et al., 1999; Siscaro & Zappalà, 2005b).

Several biological control programs were contemporaneously conducted and in 1988 the parasitoid *Comperiella bifasciata* Howard was introduced in Western Sicily from Israel; further releases were carried out in 1990 and in 1994 in the Eastern part of the island as well as in Calabria (Southern Italy). Soon after these introductions, the parasitoid was only occasionally recovered (Liotta et al., 1990; Siscaro et al., 1999; Siscaro & Zappalà, 2005a).

Material and methods

The aim of the survey, which started in 2003 and is still continuing, was to confirm the establishment of the encyrtid and to draw a map of its diffusion, 15 years after its first introduction. For this purpose, infested fruits (20) and twigs (4 meters, 1-2 years old) were collected in 10 groves in South Eastern Sicily. Half of the sample was observed and the parasitized instars were isolated and reared until the adult parasitoids emerged. The remaining 50% was kept into emergence boxes and the obtained parasitoids were collected and identified.

The presence of the parasitoid was also evaluated scoring the captures on the pheromone traps used for the California Red Scale located in different citrus groves. The monitored fields were chosen among orchards subjected to different pest management strategies (organic, integrated and conventional) in order to eventually highlight differences in the levels of presence of the parasitoid due to the pest management techniques adopted.

Results and discussion

The data collected showed that the encyrtid is well adapted in Eastern Sicily and has colonized a wide area, 50km, on average, far away from the first introduction site. The species was found in more than 70% of the orchards monitored through samples collection and captures on pheromone traps. In 2004 the captures started in March almost concurrently with *A. aurantii* male first flight, then they increased reaching the highest values in August-October (Figure 1a). In 2005, the mean captures were higher than the previous year (Figure 1b) while they decreased in 2006 when no conventional orchard was monitored (Figure 1c). In 2007, the mean captures per trap (partial data) were higher in the conventional orchard than in the integrated one with the highest value (32.5 adults/trap) reached in the month of June; no organic orchard was monitored during the year.

The observations conducted in Calabria didn’t reveal any presence of the encyrtid in this region.

The survey will be continued and expanded in order to acquire quantitative data on the parasitic activity of the encyrtid. The presence of *C. bifasciata* both in organic orchards and in conventionally managed orchards is of particular interest and could be of great help in the quick diffusion of the encyrtid, already successfully started, in all citrus growing areas of Southern Italy. Besides, these data on the establishment and dispersal of *C. bifasciata*, several years after its first introduction, highlight the fact that in classical biological control programs the results should be evaluated in a long-term perspective in order to allow the host-parasitoid interaction to be fully expressed in the areas of new introduction.

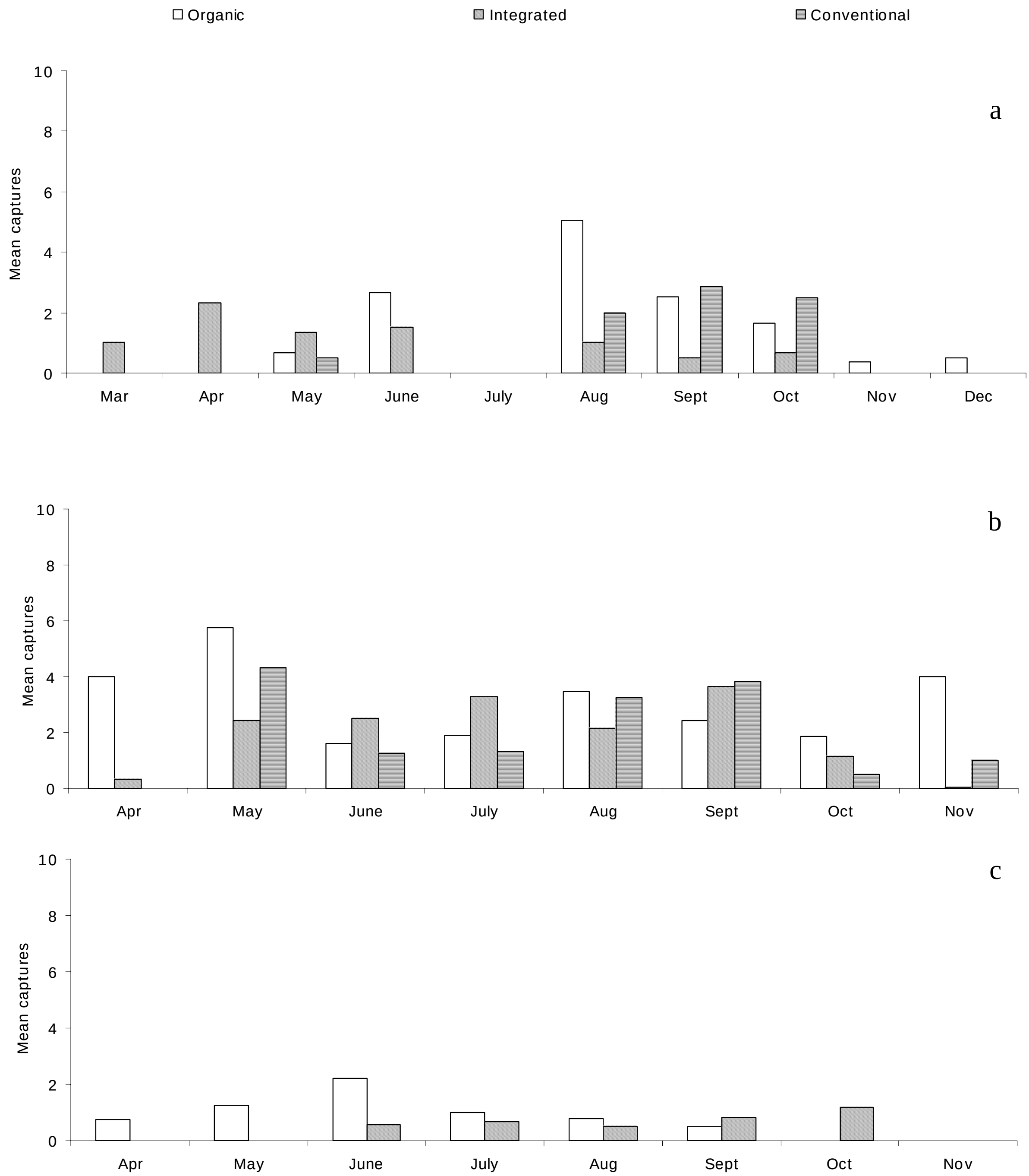

Figure 1. Mean *C. bifasciata* captures on California red scale pheromone traps in organic, integrated and conventional orchards in 2004 (a), 2005 (b) and 2006 (c).

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A new *Aphytis* species on *Aonidiella aurantii*?

T. Pina[1], M.J. Verdú[2], A. Urbaneja[1], B. Sabater-Muñoz[1]
[1]*Unidad Asociada de Entomología IVIA-UJI-CIB CSIC. Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Ctra. Moncada-Náquera Km. 4.5. 46113 Moncada, Valencia, Spain; tpina@ivia.es*
[2]*Centro Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias; Ctra. Moncada-Náquera km. 4.5. 46113 Moncada, Valencia, Spain*

In the last surveys of parasitoids conducted on Californian red scale, *Aonidiella aurantii* (Maskell), individuals of the genus *Aphytis* that present a black pigmentation of the pupa and exuvia have been detected. This description does not correspond to any of the parasitoids cited in Spain. *Aphytis chrysomphali* (Mercet), *Aphytis melinus* DeBach and *Aphytis lingnanensis* Compere, show a clearly differentiated pupa and exuviae pigmentation, without black pigmentation of pupa head in all cases. Therefore, these individuals have been assigned to a putative new species, *Aphytis* sp. *lingnanensis* group. Nevertheless, adult morphology corresponds to *A. melinus*, which pupa and exuviae are only black in the thorax. For this reason, the current taxonomic status of these individuals (black pupae) remains unclear, allowing us to suggest that it could be a cryptic species of *A. melinus*. Nowadays, cytochrome c oxidase I (COI) sequence is being considered as a taxonomic character (by Barcoding of Life initiative) and it is used for the description/unveiling of cryptic species. This work presents the use of the DNA barcodes (COI sequence) for the phylogenetic study of the black pupa individuals in the *Aphytis* group.

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Predation of *Aonidiella aurantii* (Maskell) crawlers by phytoseiids

A. Urbaneja[1], M. Juan-Blasco[2]; M.J. Verdú[2]
[1] *Unidad Asociada de Entomología IVIA-UJI-CIB CSIC. Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Ctra. Moncada-Náquera Km 4.5, 46113 Moncada, Valencia, Spain; aurbaneja@ivia.es*
[2] *Centro Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA); Ctra. Moncada-Náquera Km 4.5, 46113 Moncada, Valencia, Spain*

For our knowledge, there are few studies about the role of phytoseiids (Acari: Phytoseiidae) preying on *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) crawlers. The aims of the present work were to study in laboratory the development of three of the most abundant phytoseiids in Spanish citrus, *Euseius stipulatus* (Athias-Henriot), *Neoseiulus californicus* (McGregor) and *Typhlodromus phialatus* Athias-Henriot, and *Amblyseius swirskii* Athias-Henriot, recently introduced and released in Spain, feeding exclusively on *A. aurantii* crawlers. Furthermore, the ability of *A. swirskii* to reduce *A. aurantii* infestation on young citrus plants was studied under semi-field conditions by means of augmentative releases of this phytoseiid.

E. stipulatus was the only phytoseiid species tested which was not able to lay eggs on this prey. The progeny of *N. californicus* were not able to reach the protonymphal stage. On the other hand, *T. phialatus* and *A. swirskii* completed their development, from egg to adult preying exclusively on this prey. In the semi-field experiments, several doses of *A. swirskii* were tested. Once the phytoseiids were installed on the young trees, an artificial infestation of *A. aurantii* crawlers was promoted on each citrus plant. A significant reduction in *A. aurantii* infestation was observed in the trees where *A. swirskii* was previously released.

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A demonstrative program using augmentative releases of *Aphytis melinus* DeBach for the biological control of *Aonidiella aurantii* (Maskell) in Sicilian orchards

E. Raciti[1], A. Messana[2], G. Pasciuta[2], G. Perrotta[2], E. Sapienza[2], F. Saraceno[3], V. Sciacca[2], R. Finocchiaro[4], R. Maugeri[4], A. Strano[4]

[1] *Regione Siciliana, Assessorato Agricoltura e Foreste, Dipartimento Interventi Strutturali, Servizio Fitosanitario - U.O. 54 – Via Sclafani, 32, 95024 Acireale, Italy*

[2] *Regione Siciliana, Assessorato Agricoltura e Foreste, Dipartimento Interventi Infrastrutturali, XI Servizio – Servizi allo Sviluppo, V.le Regione Siciliana, 90100 Palermo, Italy*

[3] *Regione Siciliana, Assessorato Agricoltura e Foreste, Dipartimento Interventi Strutturali, Servizio Fitosanitario - U.O. 21 – Via Sclafani, 32, 95024 Acireale, Italy*

[4] *Soc. Coop. Microbios a.r.l., Via Lisi, 101, 95014 Giarre, Italy*

California red scale, *Aonidiella aurantii* (Maskell) is considered the key pest in Sicilian orchards. Experimental tests have been conducted in Sicily for several years, and are still being carried out, using augmentative releases of *Aphytis melinus* DeBach to control *A. aurantii*, with the aim to spread protection techniques that minimize chemical treatments. Such tests have not given sufficient and univocal indications on the effectiveness of augmentative releases. Local production of *A. melinus* and short storage times are crucial factors for a good parasitic activity. In 2005-06, a demonstrative program of *A. melinus* releases involved some Sicilian citrus farms located in eastern and western areas with good environmental conditions for citrus growing. The first area is important for the production of the pigmented orange “Tarocco” and the second one for the “Ribera” orange with the varieties of the “Navel” group. *A. melinus* has been produced in the laboratory of the Plant Disease Regional Service and the parasitoid has fulfilled the requirements of the quality control tests based on the standards of the I.O.B.C. The plots where the releases have been made were monitored monthly from July to September and they showed most limited presence of California red scale on the fruits in comparison with the control plots.

Further programs are in progress due to the start of an insectary realized by the Ente di Sviluppo Agricolo of the Sicilian Region which will allow to extend the areas submitted to releases of *A. melinus* At the same time the insectary also produces the beneficials *Leptomastix dactylopii* Howard and *Cryptolaemus montrouzieri* (Muls.) for the biological control of the citrus mealybug [*Planococcus citri* (Risso)]. The tests carried out on the biological control of *A. aurantii* have not given definitive results and they are still insufficient since they are limited by unsuitable plots extension, by availability and quality of the beneficial, by not fully controlled experimental conditions and by the insufficient availability of economical resources.

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Augmentative releases of *Aphytis melinus* (Hymenoptera: Aphelinidae) to control *Aonidiella aurantii* (Hemiptera: Diaspididae) in Sicilian citrus groves

Lucia Zappalà[1], Orlando Campolo[2], Francesco Saraceno[2], Saverio Bruno Grande[2], Ernesto Raciti[3], Gaetano Siscaro[1] & Vincenzo Palmeri[2]

[1] *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, via S. Sofia 100, 95123 Catania, Italy;*

[2] *Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università "Mediterranea" di Reggio Calabria, Località Feo di Vito, 89123 Reggio Calabria, Italy;*

[3] *Regione Siciliana, Assessorato Agricoltura e Foreste - Dipartimento Interventi Strutturali – OMP - U.O. 54, Via Sclafani, 32, 95024 Acireale, Italy.*

Abstract: Releases of *Aphytis melinus* DeBach were conducted to control the populations of the California red scale, *Aonidiella aurantii* (Maskell) in an orange orchard in Eastern Sicily. The trial was performed in 2004-2006 on 1-ha plots (3 replicates) releasing 120,000 adults/ha compared with untreated control. The releases started immediately after the first male captures on pheromone traps and were repeated on a biweekly basis releasing each time around 20% of the yearly per-hectare total, on ten release points per plot. To monitor the effect of wasp releases on scale densities, in coincidence with peak male flight activity, based on trap catches, and at fruit harvest, twigs (40cm 1 to 2 year-old from each cardinal direction, between 1.5 and 2m above the ground on 2 trees per plot) and fruits (1 fruit from each cardinal direction on 24 trees per plot) were sampled, observed under the binocular scope and all the California red scale stages recorded and identified as alive, dead and parasitized (by ecto- or endo-parasitoids).

The results showed that, at fruit harvest in 2006, the percentage of fruits having one or more second-instar or older California red scale in the released field was significantly lower than in the untreated control. Thus periodical augmentative releases of *A. melinus* appear to be a viable option for the California red scale control in an integrated pest management system.

Key words: California red scale, biocontrol, Aphelinid, ectoparasitoid

Introduction

The armored scale *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), commonly known as California red scale, can certainly be still considered as one of the key pests of citrus in arid and semiarid regions worldwide (Moreno & Luck, 1992; Franco et al., 2006; Grafton-Cardwell, 2006). This because of the direct damage to the trees, due to high infestations that may occur on trunk and branches, but also mainly because of the commercial damage linked to downgrading of fruit caused by the simple presence of instars on the peel (Walker et al., 1999).

The general difficulty in chemically controlling armored scales, the easy development of resistance by *A. aurantii* to chemical compounds (Forster et al., 1995; Grafton-Cardwell et al., 2004; Martínez Hervás et al., 2006) and the spread of integrated and organic citriculture, led to find biological methods to control this pest. The Aphelinid ectoparasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) is the most commonly used biocontrol agent of *A. aurantii* in Italy as well as worldwide through augmentative releases (Furness et al., 1983; Moreno & Luck, 1992; Forster et al., 1995; Luck et al., 1997; Rizqi et al., 2001; 2006).

In the last years some trials were conducted in Southern Italy (Tumminelli et al., 2000; 2006; Mazzeo et al., 2004) which gave inconsistent results and therefore the effectiveness of *A. melinus* releases has not been clearly demonstrated. The explanation of these results can be searched in the mutual relationship between the biology and behavior of the parasitoid and its host, in the methodology of release, in the difficulty to involve uniform areas and in the low quality of the parasitoids used. In this trial we tried to eliminate some of these elements of uncertainty therefore conducting the experiment in a uniform integrated citrus orchard, releasing *A. melinus* locally produced by the insectary of the Regional Phytosanitary Services regularly submitted to quality control tests (Zappalà et al., 2006) and using uniformely distributed release points according to a scheme supported by a parallel trial on the dispersal capacity of *A. melinus* (Palmeri et al., 2008).

Material and methods

The releases were conducted in 2005 and 2006 on a 1-ha plot of 20 year-old pigmented orange trees (cv. “Tarocco”, clone Scirè) planted at a distance of 6×4m. Ten release points were uniformely distributed in the plot, each one covering around 40 trees. The observations were conducted on a central area of 25 trees on which fruits (4 fruits per tree, one from each cardinal direction) and twigs (40cm, 1 to 2 year-old from each cardinal direction, on 2 trees per plot) were collected, between 1.5 and 2m above the ground, excluding the central plant (Moreno & Luck, 1992). The releases were repeated on 3 similar plots (replicates).

California red scale populations were monitored using pheromone yellow sticky traps placed on a 3-trees wide row, called “no release zone”, which separated the released plot from the untreated control, on which observations were carried out on a similar central area.

The releases were performed on a 2-weeks interval starting between mid-April and mid-May, after the first male captures, until mid-July before the temperature values peak. An average of 110,000 *A. melinus* adults per hectare were released in 2005 and in 2006. The samples in the observation area were collected at the main male flight peaks after the releases (in July, September and November) and at harvest (January 2006 and February 2007).

In the laboratory the number of alive, dead, ecto- and endo-parasitized instars were recorded; the percentage of infested fruits (with more than one second instar or older California red scale) was counted and at harvest the commercial damage (defined as percentage of fruits having more than 10 California red scale instars visible to the naked eye) was evaluated.

The percentage values were arcsin square root transformed before being submitted to statistical analysis. Repeated measures analysis of variance (ANOVA) was performed on all data, except those related to commercial damage at harvest which were submitted to one-way ANOVA. In both cases Least Significant Difference (LSD) test ($P= 0.05$) for separation of means was applied.

Results and discussion

In 2005 on fruits, the levels of ectoparasitization reached the highest values in September-November and after the month of July they were always higher in the released plots although the differences are not statistically significant ($F= 0.375$; d.f.= 1, 4; $P= 0.573$) (Figure 1). Similar results were obtained on twigs where the highest pecentage of ectoparasitized instars was recorded at harvest. The levels of endoparasitization were fairly low both on fruits (Figure 1) and twigs, however this parameter could have been underestimated due to the difficult assessment of endoparasitization at the initial stages. The mortality as well was higher in the released plot (Figure 1) and since we included also host-feeding among the causes of mortality together with predation and abiotic factors, the higher values could be related to this activity performed by *A. melinus*. The percentage of alive instars towards harvest and although it was lower in the released plot the differences were not significant (F= 0.618; d.f.= 1, 4; P= 0.476) (Figure 1).

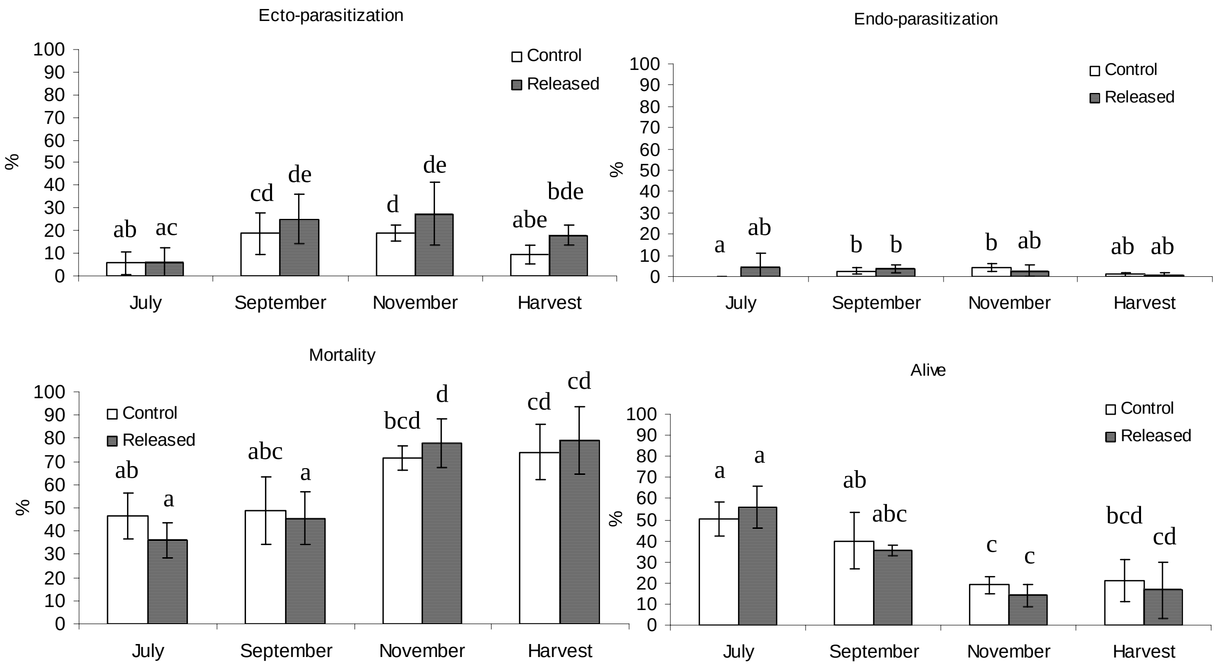

Figure 1. Percentage of ectoparasitized, endoparasitized, dead and alive California red scale instars on fruits in 2005 (mean ± SD). Columns bearing the same letter are not significantly different (P= 0.05).

Similar data were recorded in 2006 with regard to the four parameters observed (Figure 2). In particular, at harvest, in the released plot compared to the untreated control, the ectoparasitization was higher and the percentage of living instars was lower although not significantly (ectoparasitization: F= 1.584; d.f.= 1, 4; P= 0.276; alive: F= 0.258; d.f.= 1, 4; P= 0.638).

As regards the percentage of infested fruits (with more than one 2nd instar or older California red scale) no differences were highlighted in 2005 (F= 0.044; d.f.= 1, 4; P= 0.843) (Figure 3a) while at harvest in 2006 this value was significantly lower in the released plot (F= 4.362; d.f.= 1, 4; P= 0.105) (Figure 3b). Although this could be regarded as a difference in the level of infestation between the 2 treatments, the amount of fruits with more than 10 California red scale instars visible to the naked eye, what we considered commercial damage at harvest, was similar in the 2 treatments (2005: F= 1.584; d.f.= 1, 4; P= 0.276; 2006: F= 0.053; d.f.= 1, 4; P= 0.829) but it was anyway lower than 10% in the released plots in the 2 years of the trial (Figure 4).

The data obtained suggest that *A. melinus* gives a contribution to the control of California red scale infestations but cannot be considered as the key solution, at least in Sicilian conditions. In any case the results highlighted that the elimination of chemical treatments in the released plots as well as in the surroundings restored a biological equilibrium ensuring a consistent presence of fundamental natural enemies.

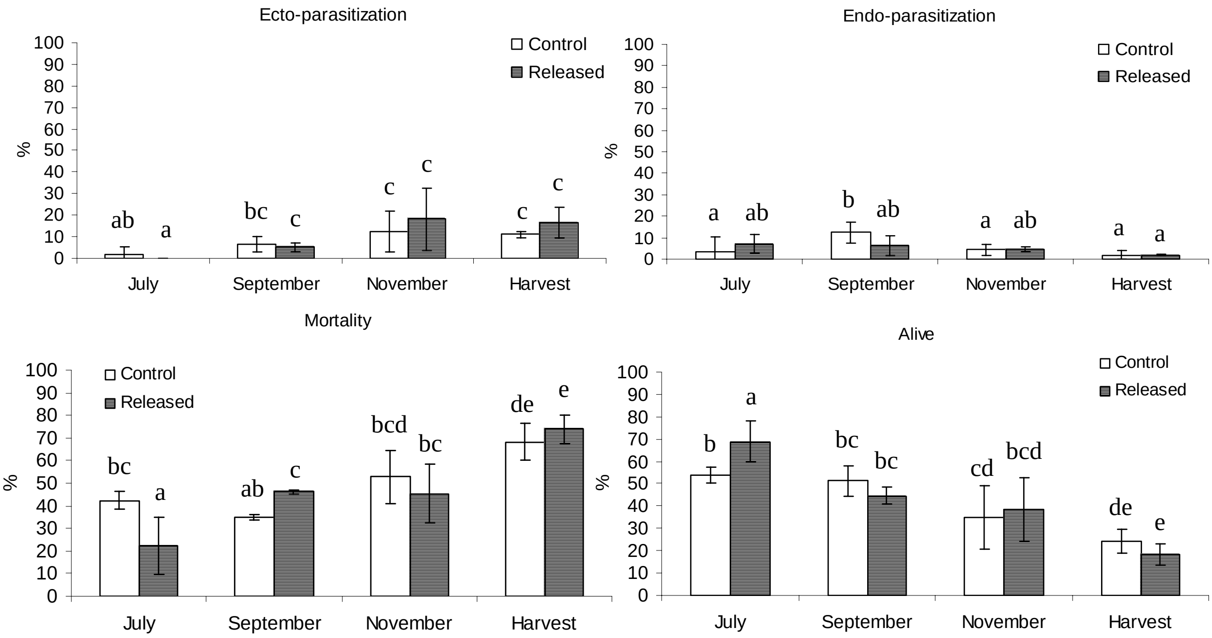

Figure 2. Percentage of ectoparasitized, endoparasitized, dead and alive California red scale instars on fruits in 2006 (mean ± SD). Columns bearing the same letter are not significantly different (P= 0.05).

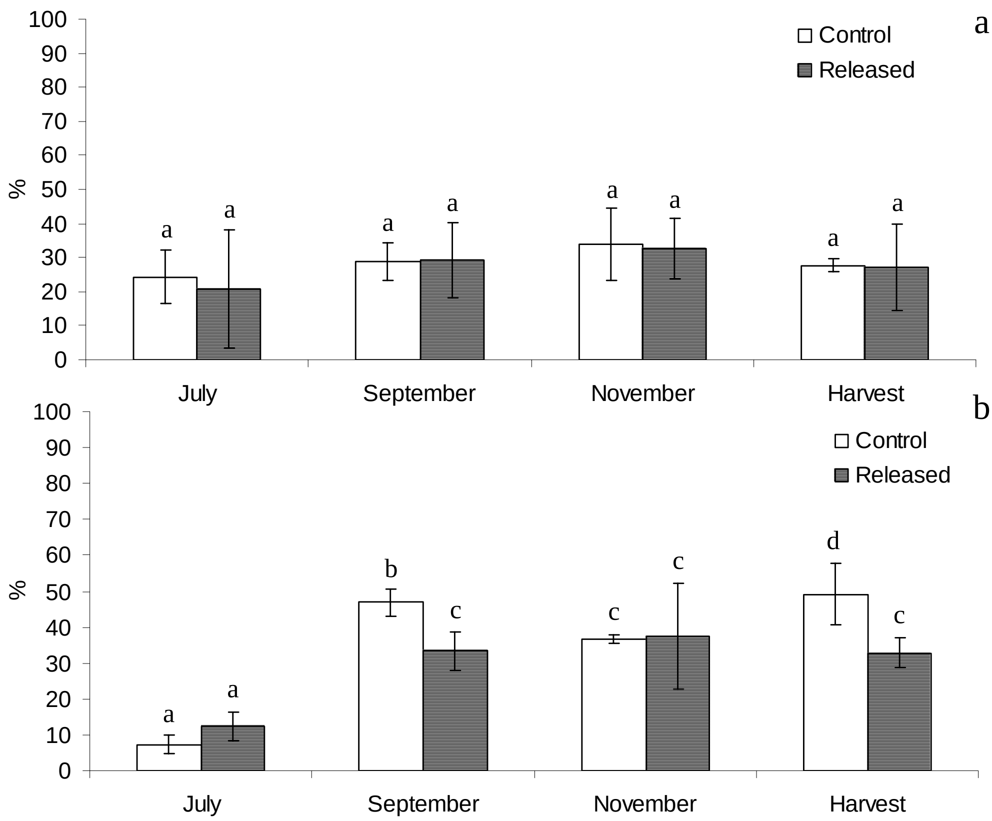

Figure 3. Percentage of fruits (mean ± SD) with more than one 2nd instar or older California red scale in 2005 (a) and 2006 (b). Columns bearing the same letter are not significantly different (P= 0.05).

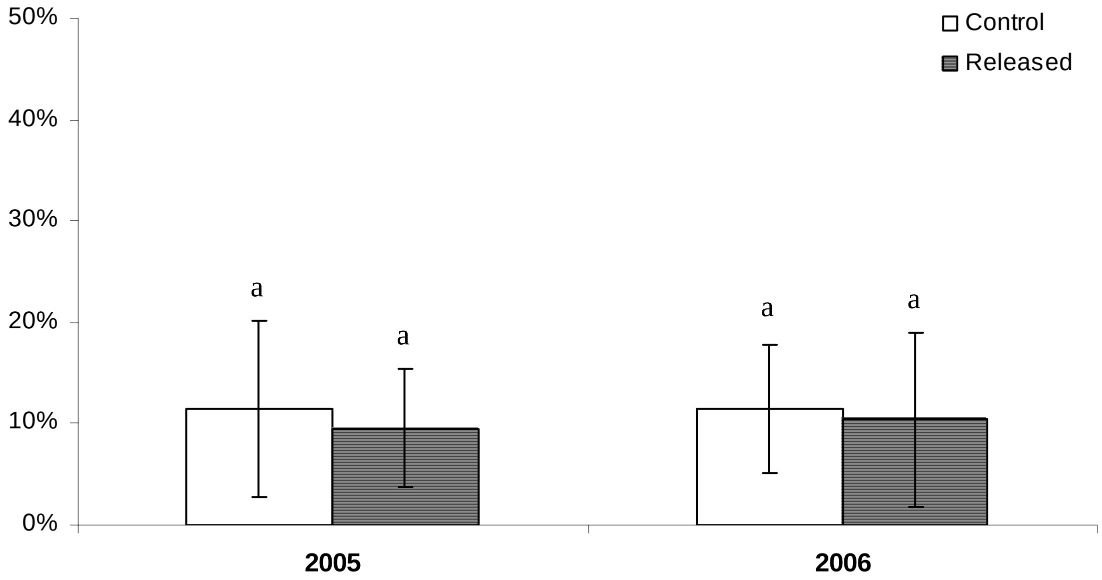

Figure 4. Commercial damage at harvest (mean ± SD) in 2005 (a) and 2006 (b). Columns bearing the same letter are not significantly different (P= 0.05).

Further investigations are presently being conducted on the evaluation of the actual role played by endoparasitoids, namely *Comperiella bifasciata* Howard (Hymenoptera: Encyrtidae), the interactions between *A. aurantii*, its natural enemies and the most common species of ants in Sicilian citrus orchards and interesting hints could come from the evaluation of the effect of joint releases of predators, such as for example *Chilocorus bipustulatus* (L.) (Coleoptera: Coccinellidae) which has an impressive "cleaning effect" on dense colonies, mostly on branches and trunk, and is less sensitive to high temperatures.

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Dispersal capacity of *Aphytis melinus* (Hymenoptera: Aphelinidae) after augmentative releases

Vincenzo Palmeri[1], Orlando Campolo[1], Saverio Bruno Grande[1], Francesco Saraceno[1], Gaetano Siscaro[2] & Lucia Zappalà[2]
[1] *Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università "Mediterranea" di Reggio Calabria, Località Feo di Vito, 89123 Reggio Calabria, Italy;*
[2] *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, via S. Sofia 100, 95123 Catania, Italy.*

Abstract: The authors report the results of a trial on the spatial dispersion of *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), ectoparasitoid of *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae), after augmentative releases. The experiment was conducted in May-June 2006 in a Sicilian integrated citrus orchard in a 1-ha plot divided in two halves: one where a single release of *A. melinus* (180,000 adults) was performed and the other left as untreated control. The flight range of the parasitoid was evaluated using yellow sticky traps activated with *A. aurantii* sexual pheromone. The total number of parasitoids trapped at the end of the trial was significantly different between the released plot and the control plot. The dispersal capacity of the parasitoid was assessed.

Key words: California red scale, biological control, ectoparasitoid, flight range

Introduction

Aonidiella aurantii (Maskell) (Homoptera: Diaspididae) is considered the most important pest of citrus in the Mediterranean basin as well as in other citrus growing areas worldwide (Franco et al., 2006). The species attacks all aerial parts of the tree including twigs, leaves, branches and fruits. Heavily infested fruit may be downgraded in the packinghouse and, if population levels are high, serious damage can occur to trees.

The parasitic wasp *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) plays an important role in controlling California red scale (DeBach, 1974; Lizzio et al., 1998) also by means of augmentative releases, but its effectiveness depends on scale careful monitoring and on the use of selective insecticides on other pests (Grafton-Cardwell et al., 2006). *A. melinus* could be monitored using yellow sticky traps activated with the sexual pheromone of *A. aurantii* (Sternlicht, 1973; Samways, 1988).

Aphytis spp. are known to have a limited capacity of dispersal; this aspect depends on behavioral, biotic and abiotic variables (Rosen & DeBach, 1979). The aim of this work was to evaluate the dispersal capacity of *A. melinus* after augmentative releases.

Material and methods

The trial was carried out in a Sicilian integrated citrus orchard (37°20’31’’ N; 14°49’42’’ E) located at Lentini (SR) at 80 meters above mean sea level where no chemical treatments have been performed for three years prior to the trial. The experiment was conducted in May-June 2006 in a 1-ha plot divided in two halves: the first one where a single release of *A. melinus* (180,000 adults) was performed and the second one used as untreated control. The wasps used in the experiment were reared on a uniparental strain of oleander scale, *Aspidiotus nerii*

Bouché (Homoptera: Diaspididae), fed on squash (*Cucurbita maxima* Duch. var. Butternut) (Raciti et al., 2003; Zappalà et al., 2006). The flight range of the parasitoid was evaluated using yellow sticky traps activated with *A. aurantii* sexual pheromone (AgriSense Ltd.) constituted by minute quantities of the components (3S,6R)-3-methyl-6-(1-methylenenyl)-9-decenyl acetate and (3S,6R)-3-methyl-6-(1-methylenenyl)-3,9-decadienyl acetate (Roelofs et al., 1977) impregnated into a pharmaceutical grade natural rubber controlled release medium. The traps were placed on the south-east outer part of the canopy, about 180 cm above the ground on trees forming circles around the central release point at regular distances (20 and 40 m). Seven weekly trapping periods were conducted the first two before the release and the others during the following five weeks. Both *A. aurantii* adult males and *A. melinus* adults were scored. In the released plot as well as in the control plot 17 traps were used including one on the central tree of both plots.

Spatial analysis was carried out using Surfer Version 8 (Golden software, Golden, CO, USA) with x, y representing the local coordinates and z the weekly data, expressed as number of individuals of *A. melinus* and *A. aurantii* trapped.

The data concerning *A. melinus* captures were small numbers, in some case equal to zero, therefore, they were square-root transformed ($\sqrt{x+1/2}$) before being analysed (Landi, 1987). Subsequently, they were subjected to a one-way analysis of variance (ANOVA) and means were separated by applying the Least Significant Difference (LSD) test.

Results and discussion

The total number of parasitoids trapped at the end of the trial was significantly different (Figure 1) between the released plot and the control plot ($F= 13.796$, d.f.= 32, $P= 0.00077$). In the released plot the average number of *A. melinus* captures was 6.70 (min 0; max 38), while in the control plot it averaged 0.529 (min 0; max 5).

In the released plot the number of parasitoids trapped in the circle of trees located at 20 m (mean 11.75; min 1; max 38) was significantly higher than those captured at 40 m (mean 2.12; min 0; max 5) ($F= 6.195$, d.f.= 14, $P= 0.0260$). Significant differences in the weekly captures of *A. melinus* between the two plots, were highlighted in the first, third and fifth week after the parasitoid release (Figure 2); in the second and fourth week after the release only very few specimens were trapped. The specimens captured during the first week are most likely those released. In the third and fifth week the adults of *A. melinus* captured can be ascribed to the progeny produced by the released specimens. Infact, the mean temperatures and relative humidity registered during the post-release period (27.90°C; 37.60%RH) are compatible with the development time of *A. melinus* and the captures intervals (Rosen & DeBach, 1979). The LSD test showed a significant difference ($P\leq 0.05$) in the total number of *A. melinus* trapped at the end of the trial between the trees located at 20 and 40 m in the released plot (Figure 2).

The spatial analysis showed that *A. melinus* is able to progressively disperse from the release point, essentially following the distribution pattern of California red scale adult males. However, the data obtained during this trial confirm the reduced dispersal capacity by *A. melinus* also after augmentative releases. This aspect should be taken into account when defining the release methodology and, in this perspective, the hypothesis of mechanical distribution of the parasitoid might be evaluated.

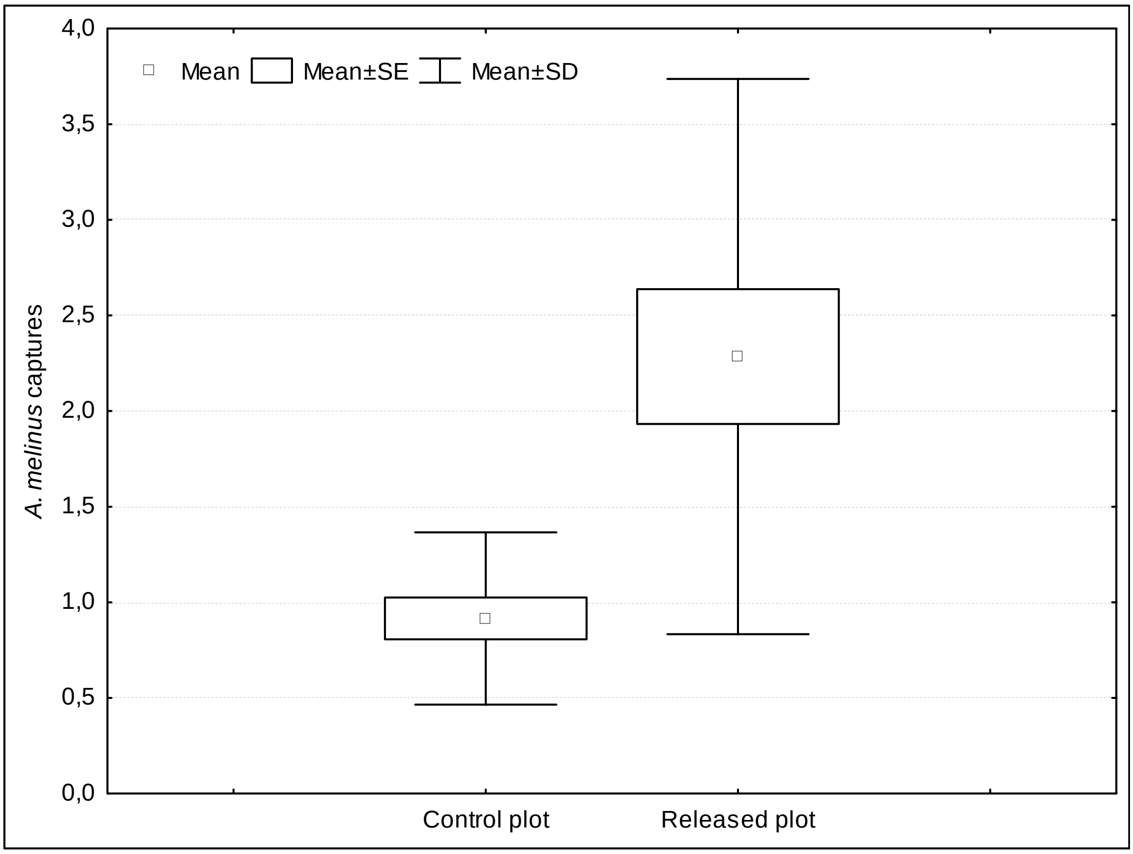

Figure 1. Total captures of *A. melinus* in the control and released plot (y=$\sqrt{x+1/2}$).

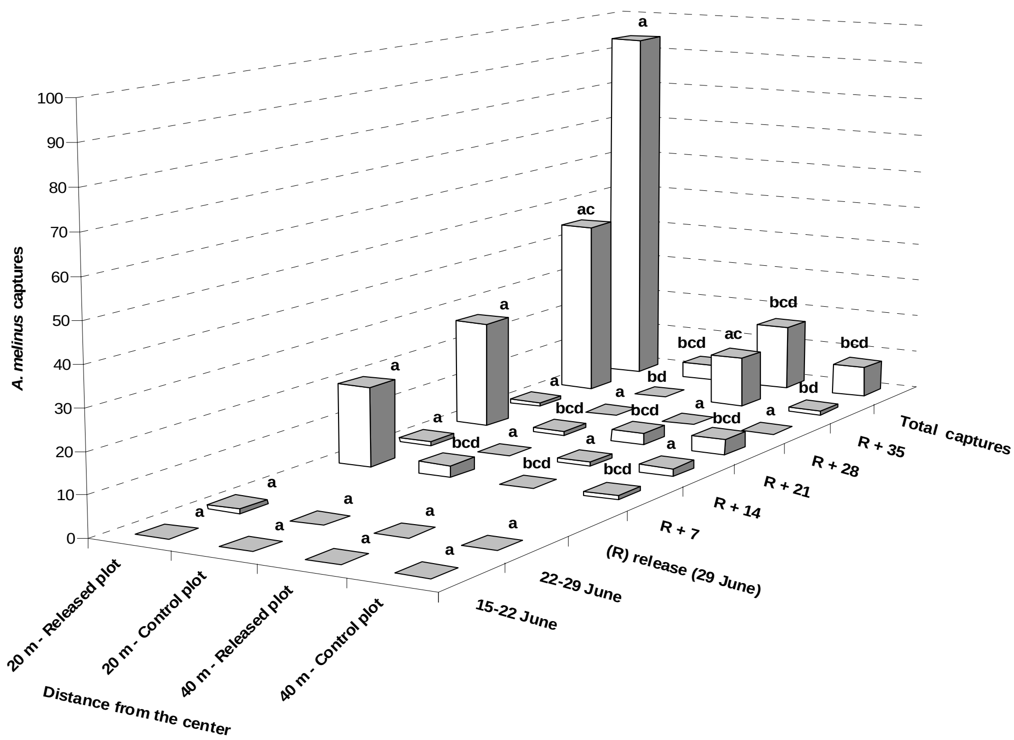

Figure 2. Weekly and total captures of *A. melinus* in the control plot and in the released plot at 20 and 40 meters from the center. Columns bearing the same letter in the same time interval were not significantly different (one-way ANOVA; LSD test) (P≤0.05).

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Petroleum spray oils and releases of *Aphytis melinus* to control *Aonidiella aurantii* in Spain

A. Urbaneja[1], P. Vanaclocha[1], A. García[2], M. Laurín[2], J.L. Porcuna[2], A. Marco[3], M.J. Verdú[4]
[1] *Unidad Asociada de Entomología IVIA-UJI-CIB CSIC. Centro de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA). Ctra. Moncada-Náquera Km. 4.5, 46113 Moncada, Valencia, Spain; aurbaneja@ivia.es*
[2] *Sanidad Vegetal. CAPA. Apdo. Correos 125, 46160 Silla, Valencia, Spain*
[3] *Grupo Sancho. C/Molins 6, 12590 Almenara, Castellón, Spain*
[4] *Centro Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA); Ctra. Moncada-Náquera km. 4.5, 46113 Moncada, Valencia, Spain*

California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) is one of the main pests of citrus in Spain. Until now the control of this pest has been conducted exclusively with the use of insecticides. The release of parasitoids of the genus *Aphytis* (Hymenoptera: Aphelinidae) in combination with petroleum spray oils is a biorational control strategy that is successfully applied in other parts of the world. However, this strategy has not been tested in Spain. In this work, we investigated, first the *Aphytis* species to be released under the Spanish citrus conditions, and second, we conducted preliminary assays to test the efficacy of both, releases of *Aphytis melinus* DeBach and petroleum spray oils.

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Control of California red scale in Citrus orchards, using mineral oil and biological control

S. Eltazi[1], A. Mazih[2], I. Srairi[1], Y. Bourachidi[2]
[1] *Domaines Abbes Kabbage-Souss, AGADIR, Morocco*
[2] *Institut Agronomique et Vétérinaire Hassan II, AGADIR, Morocco; mazih@iavcha.ac.ma*

Almost all citrus, in the Souss Valley, is grown for the fresh market, which requires pesticides in order to ensure yield and prevent cosmetic damage on fruits. *Aonidiella aurantii* (Maskell) is considered as one of the most concerning citrus pest in this area. Growers mostly use organophosphate pesticides or IGR. However, by the late 1990s, they began to realize the importance of pesticidal effects on secondary pests and their natural enemies.

Changes and improvements in pest management approaches have occurred in response to the spectacular invasion of Citrus leafminer and the pest outbreaks (i.e. *I. purshasi*, *P. citri*) as the consequence of secondary effects of some very disruptives pesticides used for the control. Therefore, several CLM parasitoids were introduced. Also biological control has been employed mainly against California red scale populations. Changes in the future availability of pesticides also have affected pest control strategies. In this context, and as a first step to implement an IPM system in our orchards (several varieties), we tried to set up a control based mainly on the use of mineral oils (i.e. Sunspray) 1 to 2%v/v alone, or combined with organophosphate insecticides (e.g. Chlorpyrifos, Methidathion).In order to enhance the biological control in our orchards, we built a rearing facility to rear *Aphytis melinus*. In this paper, preliminary results are presented and discussed.

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Preliminary data on mating disruption of red scale in Portugal

Helena Sousa[1], Celestino Soares[2], Nídia Ramos[2], Hugo Laranjo[3], Isabel Gonçalves[4], Márcia Rosendo[4], Marta Neves[5], José Carlos Franco[1]

[1] *Dep. Protecção de Plantas e de Fitoecologia, Instituto Superior de Agronomia, Universidade Técnica de Lisboa, 1349-017 Lisboa, Portugal;* [2] *Direcção Regional de Agricultura e Pescas do Algarve, Braciais, Patacão, 8001-904 Faro, Portugal;* [3] *APICITRO - Associação para a Produção e Protecção Integrada de Citrinos, Rua Dr. Manuel de Arriaga, 12, 8300-169 Silves, Portugal;* [4] *CACIAL – Cooperativa Agrícola de Citricultura do Algarve, CRL, Apartado 128 – Vale da Venda, 8001-902 Faro, Portugal;* [5] *Cooperativa Agrícola "A Esperança" de Moncarapacho, Av. D. Maria Lizarda Palermo, 8700-081 Moncarapacho, Portugal*

Abstract: The use of Red Scale Down™ for mating disruption (MD) and pest management of the red scale (CRS), *Aonidiella aurantii* (Maskell), was evaluated in a field experiment carried out in 2007, in the Southern region of Portugal (Algarve). A total of 250 dispensers of Red Scale Down™ per hectare were installed in four 1-2 ha sweet orange orchards, in two applications (March and ca. 3 months later). Each dispenser contains 0.4 mg of the active ingredients, i.e., (3S, 6R)-3-methyl-6-isopropenyl-9-decen-1-yl acetate (0.041%) and (3S, 6S)-3-methyl-6-isopropenyl-9-decen-1-yl acetate (0.025%). Three modalities of RS management were compared in each orchard: 1) MD; 2) MD + 1 insecticide application (chlorpyriphos) RS; 3) MD + 2 or 3 insecticide applications (chlorpyriphos, mineral oil). Male captures in pheromone traps were monitored every two weeks. The level of fruit infestation by CRS was estimated before the experiment and in the end of season in order to evaluate the effectiveness of each management modality. The results suggest that mating disruption of CRS may be an effective tactic for pest management of CRS populations when infestation levels are low.

Key words: *Aonidiella aurantii*, mating disruption, pheromones, citrus

Introduction

The citrus red scale (CRS), *Aonidiella aurantii* (Maskell) (Hemiptera, Diaspididae), is a major pest in most of the citrus regions in the world. It was considered a key-pest in 73% of the Mediterranean countries surveyed by Franco et al. (2006). In Portugal, CRS was detected for the first time in Moncarapacho in 1998 (DRAALG, 1998) and it has been dispersing all over the region of Algarve.

Actually, CRS management is mostly dependent on chemical control, using organo-phosphate insecticides (OP). However, OP's as broad-spectrum insecticides are common causes of natural enemies disruption (Smith et al., 1997). Ineffectiveness due to resistance and/or poor application techniques has also been reported in different regions (e.g., Grafton-Cardwell, 2006; Martínez et al., 2006). Therefore, alternative tactics are needed as a basis to develop sustainable IPM strategies.

Red Scale Down™ is a commercial formulation for mating disruption of CRS in citrus orchards. It was specifically designed for low CRS populations in order to keep them in low densities for long periods of time without the use of insecticides (RSD, 2005). In 2007, we carried out a field experiment in Algarve to evaluate the performance of Red Scale Down™ in comparison with the combined application of mating disruption and insecticides.

Material and methods

Citrus orchards
The experiment was carried out in four 1-2 ha sweet orange orchards in Algarve: Tavira (var. Valencia late), Olhão (var. Newhall), Moncarapacho (var. Valencia late) and Algoz (var. Rohde).

Modalities
Three modalities of CRS management were compared in each orchard:

A) Mating disruption - A total of 250 dispensers of Red Scale Down™ per hectare were installed in two applications (March and ca. 3 months later). Each dispenser contains 0.4 mg of the active ingredients, (3S, 6R)-3-methyl-6-isopropenyl-9-decen1yl acetate (0.041%), (3S, 6S)-3-methyl-6-isopropenyl-9-decen1yl acetate (0.025%); other ingredients (99.934%);

B) Mating disruption and one insecticide application (chlorpyriphos) - The insecticide was sprayed in May-June (1st generation of CRS);

C) Mating disruption and two or three insecticide applications - The first insecticide treatment was the same of modality B; depending on the orchards, one or two sprays of mineral oil were applied in August-September.

In the case of Moncarapacho orchard only modalities B and C were installed.

Monitoring
Male captures in pheromone traps were monitored every two weeks. The level of fruit infestation by CRS was estimated before the experiment (except in Olhão orchard) and in the end of season (October 2007), by sampling 100 fruits per plot. The following indices were used to classify fruit infestation level:

Index	Nº scale insects/fruit
0	0
1	1 a 3
2	4 a 10
3	11 a 30
4	31 a 100
5	> 100

Results and discussion

No shutdown effect was observed in male captures on pheromone traps installed in the four orchards.

Before the experiment, the percentage of fruit infested with CRS ranged between 14 and 75, with a percentage of CRS fruit waste (fruits with more than 10 scale insects per fruit) varying between 1 and 44 (Table 1). The evaluation carried out in the end of season, after the experiment, showed that none of the tested modalities of CRS management was able to significantly reduce the infestation level (Table 2; Fig 1-4). Only in two (Moncarapacho and Tavira) out of four orchards the combination of matting disruption and insecticides originated a reduction on the percentage of fruit infestation compared to the initial estimate (Table 1-2; Fig. 4). However, in the case of Tavira orchard, where the initial CRS infestation level was relatively low, matting disruption was able to prevent a significantly increase of CRS population (Table 1-2; Fig. 2).

Despite the fact that the effectiveness of the tested management tactics was conditioned by the relatively high CRS infestation levels of the selected orchards, the results suggest that mating disruption of CRS using 250 dispensers of Red Scale Down™ per hectare may be an effective tactic for IPM of CRS populations when infestation levels are low (fruit infestation index < 1). As a selective tactic, it is expected that the continuous application of mating disruption will contribute to the conservation of natural enemies and consequently will improve natural control of CRS.

Table 1 – Percentage of citrus red scale infested fruits and fruit waste estimated per orchard, before the experiment, in the plots used for each modality: A – mating disruption (MD); B- MD + chlorpyriphos (MD+C); C – MD+C+ mineral oil.

Orchard	% Infested fruits			% Fruit waste		
	A	**B**	**C**	**A**	**B**	**C**
Algoz	75	69	65	26	35	32
Moncarapacho	-	89	88	-	44	30
Tavira	27	57	42	1	13	4

Table 2 – Percentage of infested fruits and fruit waste estimated per modality and orchard, after the experiment (October 2007): A – mating disruption (MD); B- MD + chlorpyriphos (MD+C); C – MD+C+ mineral oil.

Orchard	% Infested fruits			% Fruit waste		
	A	**B**	**C**	**A**	**B**	**C**
Algoz	100	97	93	97	85	75
Moncarapacho	-	74	76	-	28	33
Tavira	33	46	52	4	15	17
Olhão	48	35	31	23	10	11

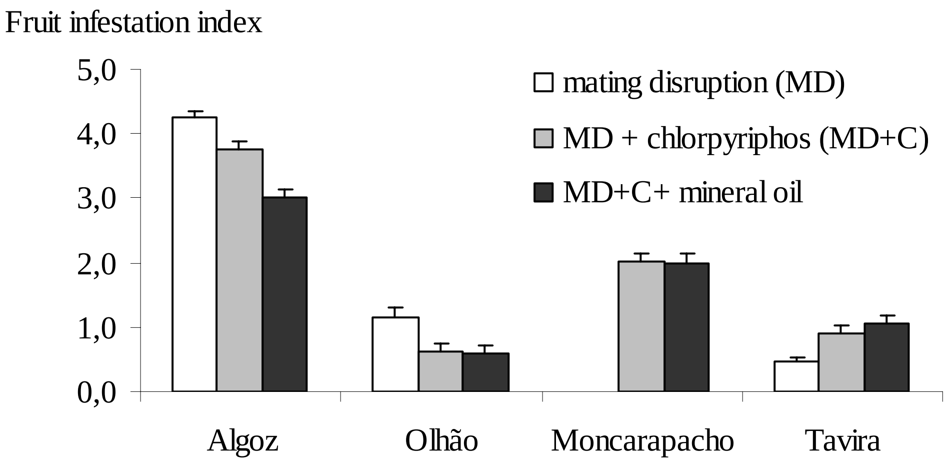

Figure 1 – Fruit infestation level (mean + SE) per modality and citrus orchard in the end of season (October 2007)

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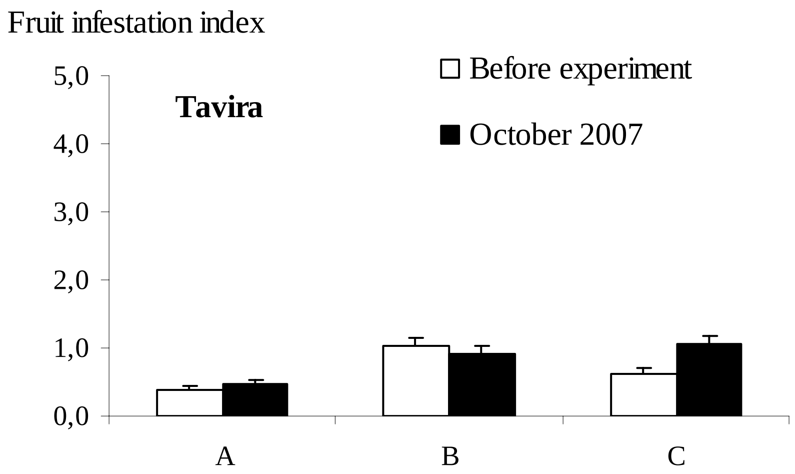

Figure 2 – Fruit infestation level (mean + SE) in Tavira orchard before and after the experiment: A – mating disruption (MD); B – MD + chlorpyriphos (MD+C); C – MD+C+mineral oil

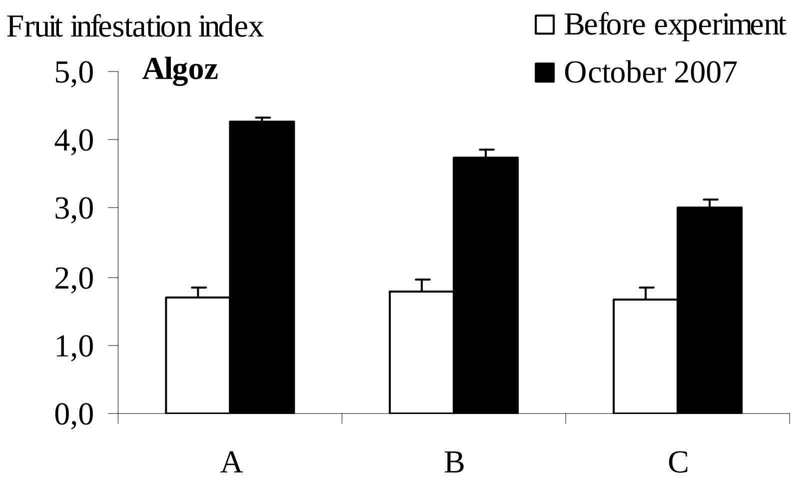

Figure 3 – Fruit infestation level (mean + SE) in Algoz orchard before and after the experiment: A – mating disruption (MD); B – MD + chlorpyriphos (MD+C); C – MD+C+mineral oil.

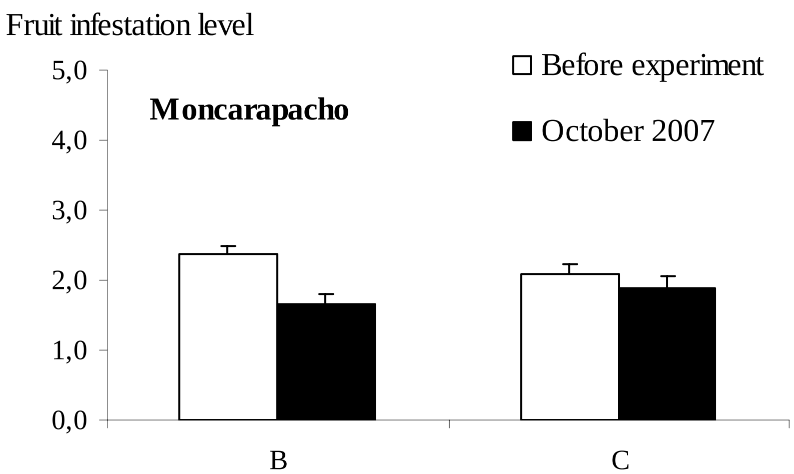

Figure 4 – Fruit infestation level (mean + SE) in Moncarapacho orchard before and after the experiment: A – mating disruption (MD); B – MD + chlorpyriphos (MD+C); C – MD+C+mineral oil.

Acknowledgements

Thanks are due to the growers and the Direcção Regional de Agricultura e Pescas do Algarve for allowing us to use their farms in field experiments. The study was carried out with collaboration of Biosani Ltd. (http://www.biosani.com).

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Mating disruption to control California Red Scale, *Aonidiella aurantii* Maskell (Homoptera: Diaspididae)

S.Vacas González, C. Alfaro Cañamás, V. Navarro Llopis, J. Primo Millo
Centro de Ecología Química Agrícola. Universidad Politécnica de Valencia, Camino de Vera s/n. Edificio 6C, 5ª planta. 46022 Valencia, Spain

A field trial was carried out in Valencia, Spain, in order to test the efficacy of mating disruption treatment against *Aonidiella aurantii* Maskell (CRS). Two doses of CRS pheromone were formulated in biodegradable dispensers and tested at 500 dispensers per ha before second generation of CRS occurs. Trials were conducted in a 3 ha citrus orchard, *Citrus sinensis* Osbeck, cultivar “Lane-late”. Results showed that to higher pheromone dose, less catches of CRS males in monitoring traps were observed. However, no differences in fruit damage were obtained when comparing check field without treatment and the two tested doses. Due to these results, new field trials, hanging dispensers before the first generation occurs, with higher pheromone doses will be conducted next year.

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Biological efficacy of two organophosphate insecticides against California red scale (*Aonidiella aurantii* Maskell) related to deposition parameters under laboratory conditions

Garcerá C.[1], Chueca P., Santiago S., Moltó E.
Centro de Agroingeniería, Instituto Valenciano de Investigaciones Agrarias. Ctra. Moncada-Náquera, km. 4,5 46113Moncada (Valencia, Spain) [1]e-mail: cgarcera@ivia.es

Abstract: California red scale (*Aonidiella aurantii* Maskell) is a major economic pest of citrus in many countries. It is mostly controlled by organophosphate insecticides. The present work is aimed at adjusting an optimal dosage of two insecticides, in order to reduce the presence of residues on the fruit while assuring their efficacy. The paper establishes, under laboratory conditions, the relationship between the deposition characteristics of these insecticides and their efficacy. It takes into account the influence of the development stage of the scale to build curves of expected mortality against the amount of active ingredient deposition. The results demonstrate the importance of applying the insecticides in the early stages of the pest and shows that the amount of active ingredient has to be doubled or even quadruplicated when treating the adult phases (pre-pupae, young female, adult female) with respect to the amounts required for the young ones (L1 and L2).

Key words: *Aonidiella aurantii*, organophosphate, coverage, efficacy

Introduction

California red scale (*Aonidiella aurantii* Maskell) is the most harmful pest in Spanish citrus growing regions, as well as in many other countries, being especially well established in almost all citrus areas of its Mediterranean coast (Durbá Cabrelles et al., 2006). Its management includes insecticide treatments, being chlorpyrifos, methidathion, pyriproxyfen, buprofezin and mineral oils the most used in Spain (M.A. Martínez Hervás et al., 2005). The use of pesticides represents a serious problem for the environment and their application is also dangerous for the field workers, who need special protections for the application. Inadequate dosage also induces resistance on the pest. However, very little research has been done to study the optimal dosage in order to decrease the quantity of applied products.

The aim of the present work is to relate the deposition of two organophosphate insecticides with the expected mortality of the California red scale in its different development stages. This is the first step to establish the dose and time of application for an optimal control of the pest, with the intention of reducing the presence of residues of insecticides while assuring their efficacy.

Therefore, in this work it has been sought to determine, on the one hand, the characteristics of the depositions of the products when applying different volumes and, on the other hand, the efficacy obtained on the mortality of California red scale in different development phases. Finally, the characteristics of the deposits have been related to their efficacy.

Material and methods

Product application

The pesticides were applied by means of a Potter precision laboratory spray tower (C. Potter, 1952) (Figure 1). It consists of a metallic central tube, with has a small deposit on the top, connected to a pneumatic nozzle. At the bottom of the tube there is a platform where the specimen that receives the spray is situated. It performs a pneumatic spray and in all the trials 1 bar of air pressure was used.

In previous experiences using this device, it was realized that an important part of the sprayed volume did not reach the target, because it evaporated or adhered to the tube walls. In order to know the actual volume that reaches the target a preliminary trial was performed to calibrate the procedure. In this experiment 5 plastic Petri dishes were dried and weighed (Precision scales, XR 205 SM-DR) for each tested volume (500 µl, 1000 µl, 2000 µl, 3000 µl and 4000 µl). Then, they were sprayed with the corresponding volumes of water and weighed again, thus obtaining the weight of the deposited spray by calculating the differences. Finally, this data was converted to volume and expressed in percentage of the total volume that had been added to the tower deposit. This is what was called the recovery percentage.

Figure 1. Potter tower Figure 2. Lighting system

Deposition studies

Square, white PVC collectors (4x4 cm), which have drop retention behaviour similar to that of the citrus leaves (G. Mercader et al., 1995), were used as artificial targets. They were sprayed with water, adding 1% p/p of an iron quelate (Sequestrene 138 Fe G-100, Syngenta) as a red colouring agent, to generate a high contrast with the background, necessary for the subsequent image analysis. An experimental design of one factor, the volume (µl) with 5 levels (500, 1000, 2000, 3000 and 4000) and 5 replicates, was performed.

These collectors were then photographed with a digital camera (Canon PowerShot A70) under a lighting system (Figure 2) composed by an aluminium hood, which was the support, and two circular fluorescent lamps (Philips, TLE 22W/54 and TLE 32W/54). The photographs were taken with a resolution of 2 pixels per mm, and compressed to JPG format. The camera was set in a Polaroid MP-4 Land Camera support at a vertical distance of 27.5 cm over the samples, to make all the photographs in similar conditions.

In order to calibrate the images spatially, a ruler was photographed in the same enlargement conditions of the collectors and the number of pixels contained between two marks of the ruler in this image was counted. This gave the scaling factor to convert pixels in µm.

After the calibration, the images were analysed with commercial software (Matrox Inspector v. 2.2, Matrox Electronic Systems Ltd.). The analysis consisted of 4 phases:

- A representative region of the observed deposition on the image was manually selected.
- This region was converted to a 256 grey level image.
- On the latter image, a grey level threshold to separate droplets from background was set by an operator. This person compared visually the segmentation result with the original image until obtaining a satisfactory accuracy in the representation of the spray
- Once the impact of the droplets was isolated, three features were calculated from the image: coverage (percentage of total surface covered by the spray), Feret mean diameter (FMD) of each of the impacts (µm) and number of impacts per square centimetre.

Efficacy trials

Biological efficacy of treatments was estimated based on insect absolute mortality, this defined as the percentage of individuals that did not evolve from the total, since mortality of the control was almost zero.

California red scale infested lemons, reared under a protocol developed by the Entomology Unit of the IVIA, were employed along the experiments (Figure 3).

Figure 3. California red scale infested lemon

The life cycle of the California red scale was divided in four phases, based on the cycle stages described by L.D. Foster et col. (1995), which were supposed to have different sensitivity to the treatments:

- L1, which includes Instar 1 and Molt 1 stages
- L2, which includes Instar 2 and Molt 2 stages
- H, which includes young female, adult female and gravid female stages (H1, H2 and H3)
- PP, which includes prepupae male and pupae male stages

To obtain individuals in a specific phase for the trial the larvae were let evolve during a prefixed number of days since they fixed in the lemon surface. This number was 5 days for L1, 9 days for L2 and 15 days for H and PP.

At least 50 live individuals in each lemon were identified by marking them with a permanent pen (Figure 4) before performing the treatments. Ten days after treatments, the marked individuals that had not evolved were counted to estimate the percentage of mortality, because they were supposed to have died because of the treatment.

Figure 4. Surface of an infested lemon, prepared for its spraying

An experimental design of two factors with 5 replicates was performed: the first factor was the water volume rate, with 5 levels, 4 volumes (µl) (1000, 2000, 3000 and 4000) plus a control; and the second factor was the life phase with the four levels the life cycle of the California red scale had been divided in (L1, L2, H, PP). This experimental design was carried out with two organophosphate insecticides (Product A and Product B), both of them applied at the label maximum concentration, prescribed for the treatment of this pest in citrus.

It is important to remark that the dose of treatment 1 is half of that of the treatment 2, third part of the treatment 3 and fourth part of the treatment 4, which means that different treatments implies a dose increase by means of increasing the volume while the product concentration is always the same.

Statistical analysis

The Analysis of Variance (ANOVA) was employed in its factorial version to study the results of the efficacy trials (two factors: volume and development phase) and in its simple version to evaluate the deposition parameters (one factor: volume). The Shapiro-Wilks test was used over the model residues to test the normality of the data and the Levene test to evaluate their homocedasticity (homogeneity of variances). All these tests were performed with 95% confidence interval. The non-parametric Tukey test was used for the comparison of means, which lowers Type I error.

When some of the assumptions of the ANOVA were violated, the Kruskal-Wallis non-parametric variance analysis was performed. In this case, the Box and Whisker plot with the median confidence intervals was used to compare the means. However, in this work only ANOVA results are presented, because results were identical and interpretation is straightforward.

Results

Deposition studies: Recovery percentage

It is important to remark that the obtained recovery percentages were very low, going from 4.8% to 7.1%. The volume was not statistically significant for this factor (Figure 5), that is to say, the percentage of recovered volume was almost the same whichever the applied volume was, so the volume reaching the target was proportional to the initial volume that is located on the tower deposit. Therefore, the active ingredient dose that later on would be effectively put on the lemons would be proportional to the water volume.

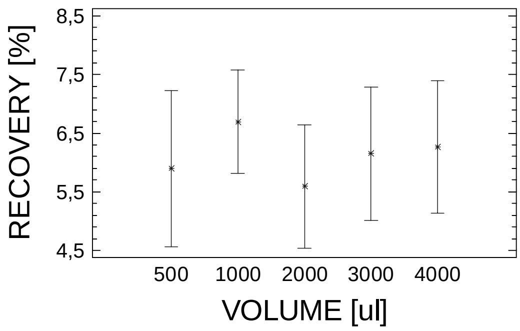


Figure 5. Tukey HSD-Intervals for the recovery percentage

Deposition studies: Deposit characterization

Regarding the coverage percentage, although there was no significant difference between 1000 µl and 2000 µl, the volume was statistically significant, as it can be observed on the Figure 6. The coverage percentage increased proportionally with the volume.

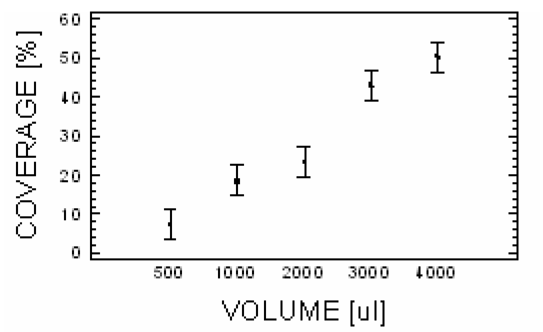
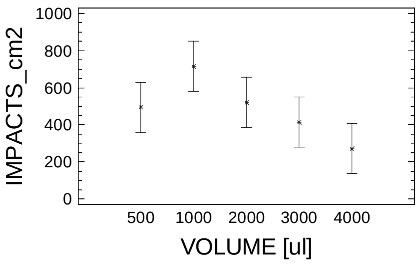


Figure 6. Tukey-HSD Intervals for coverage percentage

It was also noticed that when the volume was higher than 1000 µl, the impact size increased too, and the number of impacts decreased (Figure 7), which seemed to happen because as the volume increased, the impacts aggregate and give rise to larger impacts.



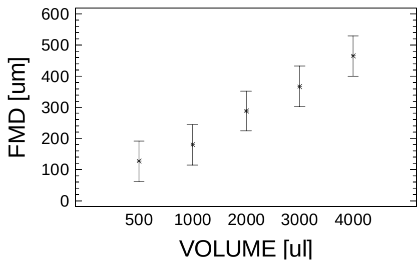


Figure 7. Tukey-HSD Intervals for the number of impacts per square centimetre and FMD (µm)

Efficacy trials

First of all, the quantity of sprayed active ingredient with each volume (µg a.i./cm^2) was estimated on the basis of the recovery percentage, the employed concentration and the content of active ingredient in each product (Table 1).

Table 1. Estimation of active ingredient sprayed (µg a.i./cm^2) (Average ± Standard Error)

TREATMENTS	**µg a.i. A/cm^2**	**µg a.i. B/cm^2**
1000 µl	0.88 ± 0.10	0.99 ± 0.10
2000 µl	1.64 ± 0.18	2.17 ± 0.21
3000 µl	2.82 ± 0.25	3.47 ± 0.11
4000 µl	3.82 ± 0.28	4.74 ± 0.23
Control	Control	Control

Regarding the mortality, significant differences between the control and the other treatments and among the different phases of the scale were observed (Figure 8).

L1 and L2 phases were the most sensitive since they presented the highest mortality (96%) and because high mortality levels were achieved even with the minimal volume, which implied both the minimal coverage and the minimal quantity of active ingredient (0.88 µg a.i. A/cm^2 and 0.99 µg a.i. B/cm^2).

PP phase was less sensitive. Its mortality increased between treatments of 1000 and 2000 µl (from 0.88 to 1.64 µg a.i./cm^2) with Product A, reaching 30% of coverage, and between 1000 and 3000 µl (from 0.99 to 3.47 µg a.i./cm^2) with Product B, reaching almost 40% of coverage, with 83% of mortality in the first case and 98% in the second one. For higher volumes, its mortality did not increase significantly.

The H phase was the least sensitive, showing the lowest mortality levels. The increase of volume up to 2000 µl (1.64 µg a.m. A/cm^2 and 2.17 µg a.m. B/cm^2), which implied an increase of coverage up to 30%, did not result in a significant increase of mortality. It only increased when the volume was raised up to 3000 µl (2.82 µg a.i. A/cm^2 and 3.47 µg a.i. B/cm^2), with almost 40% of coverage, going the mortality up to 65% with Product A and 60% with Product B. From there on its mortality did not change even if the volume increased up to 4000 µl (3.82 µg a.i. A/cm^2 and 4.74 µg a.i. B/cm^2) and therefore the coverage up to 43%.

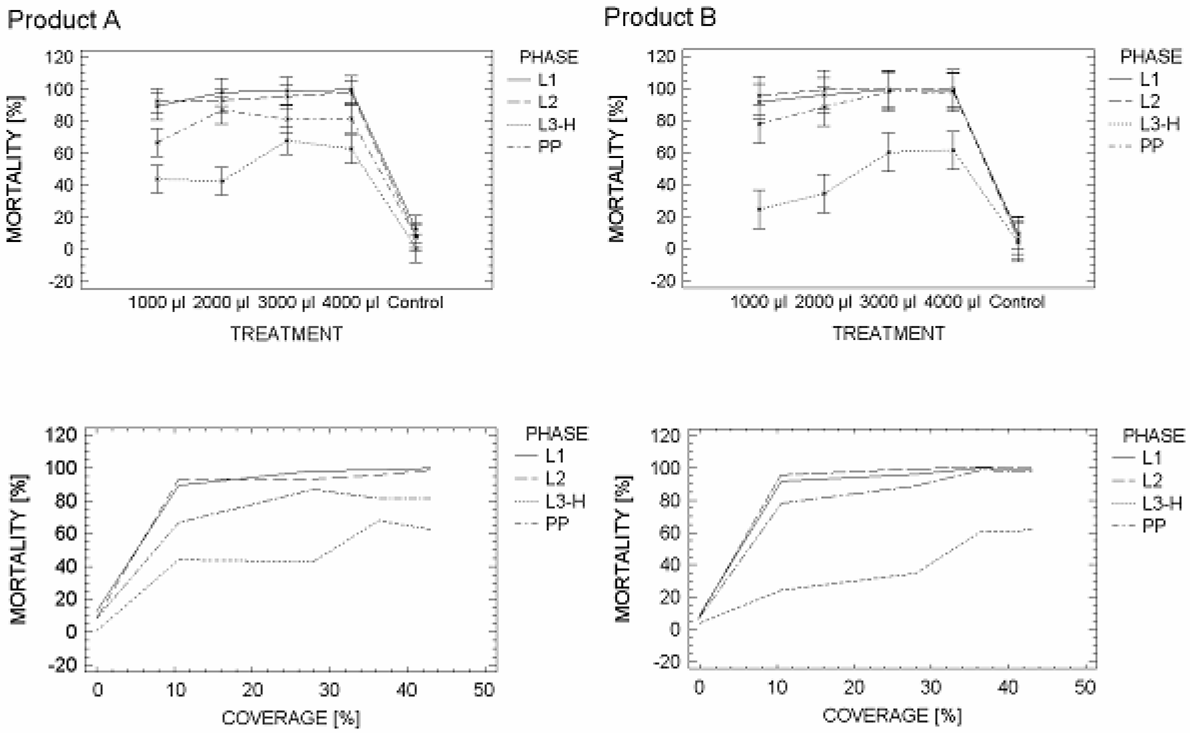


Figure 8. Mortality of California red scale based on the treatment and coverage percentage in each development phase

Conclusions

Regarding the obtained depositions, first of all it was determined that the applied doses (µg a.i./cm^2) were proportional to the volume. On the other hand, it was observed that, when applying 1000 µl or more, the more volume is applied, the more coverage is obtained, by means of increasing the impact size and decreasing the number of impacts, which could be because the adjacent impacts aggregate.

Regarding the biological efficacy, the highest mortality was obtained for L1 and L2 when applying 0.88 µg a.i. A/cm^2 and 0.99 µg a.i. B/cm^2. Their mortality did not increase although the dose was increased by means of increasing the volume, that is to say, the increase of active ingredient, coverage and impact size did not produce significant changes.

PP phase presented lower mortality and a slight increase was observed when raising the quantity of active ingredient up to 1.64 µg a.i. A/ cm^2 and 3.47 µg a.i. B/cm^2. The mortality remained around 85-90% for higher doses, but it is not known if the maximum was reached.

The H phase was the least sensitive, showing the lowest mortality levels. The highest level reached by this stage was 60-65%.

To obtain levels of efficacy in the PP phase similar to those of the young phases, depositions of the active ingredient should be doubled, whereas depositions with the quadruple of the active ingredient only would raise the mortality of the H phase up to 65%.

Hence, this work demonstrated the importance of performing field treatments when the pest is in its first phases of development, since later on treatment is not going to be as effective as it could be.

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A binomial sampling method for the California Red Scale (*Aonidiella aurantii*) in Citrus groves

J.R. Boyero[1], N. Rodríguez[1], J.M. Vela[1], R. Moreno[1], F. Pascual[2]
[1] *IFAPA. Centro Churriana (Málaga). Cortijo de la Cruz s/n. 29140 Churriana (Málaga), Spain; juanr.boyero@juntadeandalucia.es*
[2] *Dpto. de Biología Animal. Facultad de Ciencias, Universidad de Granada. 18071 Granada, Spain*

The California red scale (CRS), *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) is one of the main pests of *Citrus* all around the World. Making available methods for measure its occupancy in the groves is very important for control in IPM programs. Bietapic sampling is specially indicated for estimates of insect populations when a tendency to aggregation and high population means exist. Besides, this method is easy and quick to do in the field, and can be applied so in ecological studies as in pest management.

Our work describes a bietapic sampling method that employs a presence-absence (incidence) variable, in order to estimate the occupancy by CRS in Citrus groves. We select the secondary and primary units having in mind two criteria: i) they must be representatives of the level of occupancy in the tree and in the grove, and ii) they must be time and energy savers. The efficacy of this methodology could be tested. The concrete sampling structure (n. of secondary per primary unit and n. of primary units) was stated as a function of the values of inter and intravariance of the incidence in the trees obtained, in the former sampling date. The results during the sampling period are showed. The applied methodology in two Citrus groves in fortnight samplings along two years also yields good results, with a precision level for the estimation of the incidence of 15.5%.

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Host preference of *Aonidiella orientalis* on citrus in South Baghdad (Homoptera: Coccidae)

M.Z. Khalaf, A.K. Abed, H.M. Alrubaie, R.A. Okaily, A.K. Minshed
Integrated Pest Control Research Center, Ministry of Science & Technology, P.O. Box: 765 Baghdad, IRAQ; mzkhalaf2007@yahoo.com

Field and laboratory studies were conducted in South Baghdad aiming at determining population dynamics and the host preference of *Aonidiella orientalis* on lemon (*Citrus limon*), bergamot (*C. aurantium*), mandarin (*C. reticulata*) and orange (*C. sinensis*) throughout the season 2002.Results showed that the numerical density in higher on fruits than leaves (20.4, 6.0 insects/cm^2 respectively), As regards fruits, lemon was the most susceptible to the infestation by *A. orientalis*, while for the leaves, mandarin leaves was the most susceptible. The results indicated that lower parts of citrus tree were best preferred than mid and upper part by *A. orientalis*: 10.0, 7.6 and 0.51 insect per cm^2 of leaves surface respectively and 31.8, 28.3 and 0.96 insect per cm^2 of fruit surface respectively. In general, the inner part of citrus canopy was found to be nearly similarly susceptible to *A. orientalis*. We recorded the presence of *Chilocorus bipustalatus* L. as a predator to this pest. The results of this study can be used in the practical application of biological and chemical control of this pest.

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Chrysomphalus aonidum (Hemiptera: Diaspididae) in Spain. Studies on its biology and population dynamics

Antonia Soto, María Borrás, Rosa Vercher and Ferran Garcia-Marí
Instituto Agroforestal Mediterráneo, E.T.S.I.Agrónomos, Universidad Politécnica de Valencia. Camino de Vera s/n 46022 Valencia. asoto@eaf.upv.es

Abstract: The diaspidid scale *Chrysomphalus aonidum* was first detected in Valencia in 1999. In 2004, studies on its biology were initiated due to the importance of the damages caused by this insect in most citrus regions of the world. The objectives of this work were, first, to investigate the distribution and behaviour of *C. aonidum* in the area of appearance in order to control the pest and prevent its dispersion towards commercial citrus fields. Second, to detect its natural enemies and to determine their relative abundance. *C. aonidum* monitoring was carried out in 2004 and 2005, locating all the infested citrus trees, starting from the first point of infestation. In two selected groves, periodic samplings were made, consisting of fifteen leaves and some fruits. In the laboratory, individuals of different development stages in the population were counted. In addition, adult males were captured with sexual pheromone traps. Very high levels of this scale were detected. *C. aonidum* completes from three to four annual generations, with higher populations in summer. Sex related differences were observed in the distribution of *C. aonidum*, males showing preference for the upper side of the leaves whereas females were located preferently on the lower side. Several natural enemies were identified along the period of the study. *Aphytis chrysomphali* was the most frequent, but only parasitized *C. aonidum* males.

Key words: *Crysomphalus aonidum*, citrus, distribution, population, *Aphytis chrysomphali*.

Introduction

Chrysomphalus aonidum (L.) is a species native of tropical regions. Nowadays it is present in all five continents, being a common and frequent citrus pest in some countries (CAB International, 2005). In the Iberian Peninsula it was first recorded on leaves of *Mirtus communis* L (Gómez-Menor, 1937) and was not found again until 1999, when it was identified in an abandoned orange orchard in the vicinity of the urban area of the town of Valencia (García Marí *et al.,* 2000). *C. aonidum* was very abundant on leaves and fruits in that orchard, and thus probably the population was present several years before it was detected.

C. aonidum is a diaspidid scale with sexual reproduction, ovoviviparous, with an average of 150 eggs laid per female. Females develop a slightly convex, circular scale while male shield becomes more oval. The population trend of this species on citrus is different depending on climatic conditions. Shows preference for citrus, but it is very polyphagous and can feed and develop on many plant hosts (Quayle, 1941).

C. aonidum management is carried out in many countries through biological control. It has a wide complex of natural enemies, being especially remarkable *Aphytis holoxanthus* DeBach (Hymenoptera: Aphelinidae), with levels of parasitism that can reach up to 90-100% (DeBach, 1975; Bedford & Cilliers, 1994).

The objective of this work was the study of the seasonal trend along the year in *C. aonidum* populations in Valencia (Spain). In addition, the natural enemies which attack or feed on this species in the area of study were collected and identified.

Material and methods

***Prospection of* C. aonidum**

After the detection of *C. aonidum* in Valencia in 2000, we decided to study its presence in all citrus trees within a distance of 2 to 3 km around the first focus of infestation. In total, approximately 15km^2 were surveyed, with 8,052 citrus trees observed (mostly sweet and sour orange, and lemon trees) Trees were carefully inspected visually for the presence of *C. aonidum.* The prospected area included urban areas with citrus trees in gardens and streets in the city of Valencia and others areas in the periphery of Valencia with commercial plantations and gardens.

***Distribution and seasonal evolution of* C. aonidum**

Weekly samplings were conducted during one year, except in winter when sampling was done every two weeks. Citrus leaves were collected and 150 randomly selected live scales were observed at the laboratory with the aid of a binocular. The distribution of *C. aonidum* on the upper and lower sides of the leaf was evaluated. Immature stages considered were first instar fixed and second instar. Four types of adult females were differentiated, H1 corresponding to adult females not yet fully developed, H2 fully developed females, H3 gravid females, and H4 gravid females with external eggs. Among males, three levels of development were differentiated, prenymph, nymph and adult.

The male flight was monitored in three plots using sticky traps with the sexual pheromone of the diaspidid *Aonidiella aurantii.* This synthetic pheromone is recorded in the bibliography as attractive for *C. aonidum* (Su, 1983). The pheromone was changed monthly. Traps were collected weekly and the males counted in the laboratory.

Natural enemies

On each sample, parasitoids and predators observed were identified. Organisms in an immature stage were allowed to develop under controlled conditions to the adult stage to be identified.

Results and discussion

***Prospection of* C. aonidum**

Prospection started from the original focus where in 1999 *C. aonidum* was detected for the first time. In this orchard, all the citrus trees showed the presence of this species with high levels of infestation. In an area of 3 km around this first focus, nine additional foci were detected, four in rural areas and the other five in urban areas. Only 3% of the 8,052 observed citrus trees showed presence of *C. aonidum*. Citrus trees attacked showed usually very high population levels of the pest, especially lemon trees. In addition, the presence of this diaspidid was detected in 33 non citrus plants, including *Hedera helix* L., *Laurus nobilis* L., *Prunus laurocerasus* L., *Ligustrum ovalifolium* Hassk, *Ligustrum lucidum* Ait., *Nerium oleander* L., *Hibiscus rosa-sinensis* L. and *Howea forsteriana* (C. Moore &F.L. Muell) Becc.

Two different kinds of damage caused by *C. aonidum* to the plant were observed. First, the effect of toxic substances injected on the plant tissues by the insects while feeding produced yellowish areas on leaves, shoots and fruits. Second, fruits and leaves become covered with scales in high numbers and this caused early fall of the leaves and depreciation of the fruits.

Distribution and seasonal evolution
Monitoring conducted during one year allowed us to study the distribution of the individuals of both sexes, observing a clear preference of the males for the upper side of the leaves, while females preferred the lower side (fig.1).

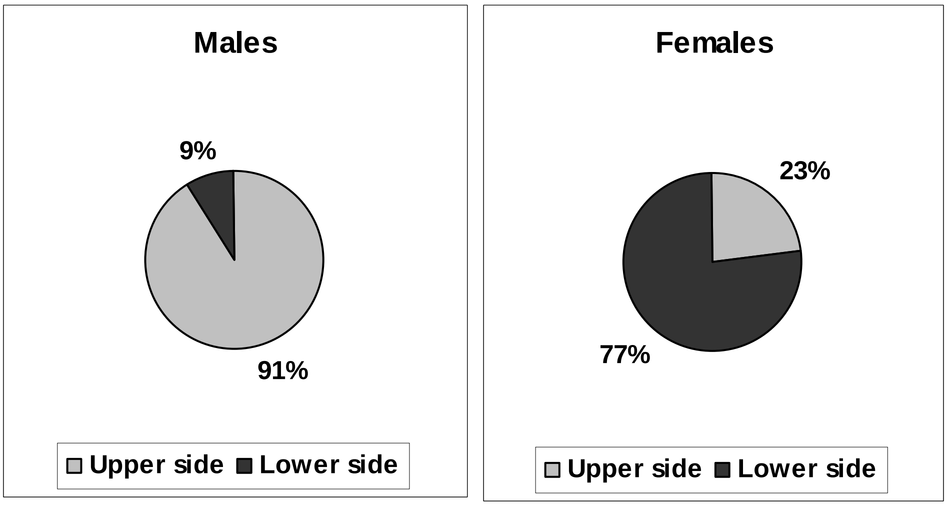

Figure 1. Relative abundance of males and females of *C. aonidum* on citrus leaves.

Determining the distribution of the different development stages along the time (fig.2), adult females in all its forms were the most abundant. From May onwards there was a slight increase in the percentage of immature. During the summer there were two main peaks, one in July another in September. A small increase in the percent of immatures was seen during November.

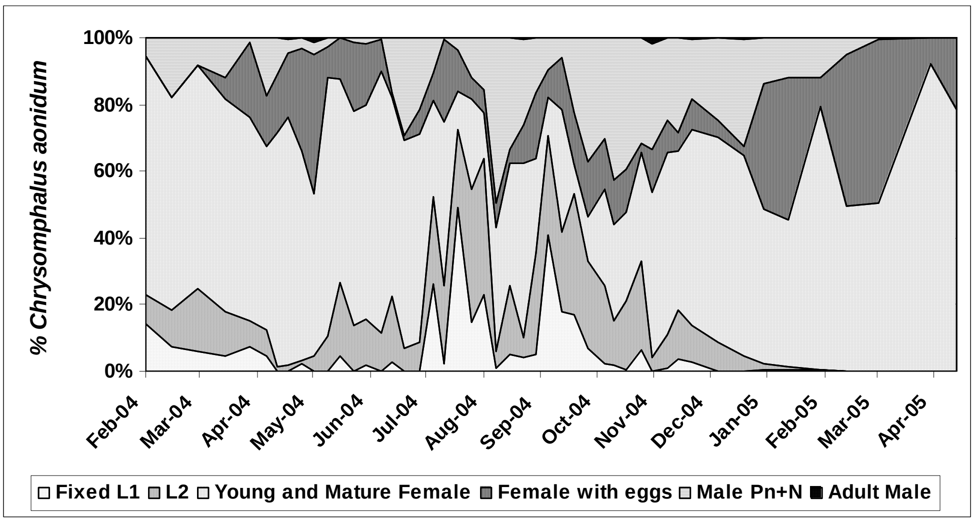

Figure 2. Evolution along the year in the proportion of development stages, in a population of *Chrysomphalus aonidum* in Valencia (Spain).

Trapping for adult males started in June. A small flying period was observed in July, followed by two periods of high captures, the first in August-September, and the second in October (fig.3).

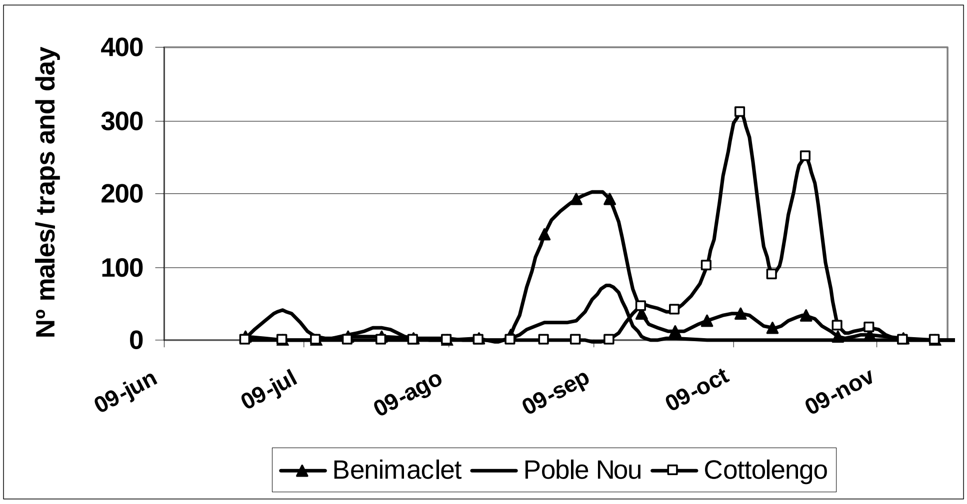

Figure 3. Trend of *Chrysomphalus aonidum* males captured on yellow sticky traps with pheromone.

***Native natural enemies of* C. aonidum**

Parasitoids identified were the ectoparasitoid *Aphytis chrysomphali* (Mercet) and the endoparasitoid *Encarsia perniciosi* (Tower). *A. chrysomphali* was the more common and widespread, but it was found parasitizing only males. Weekly captures of *A. chrysomphali* in yellow traps detected a higher abundance of this species during June and July, and from September onwards (fig. 4).

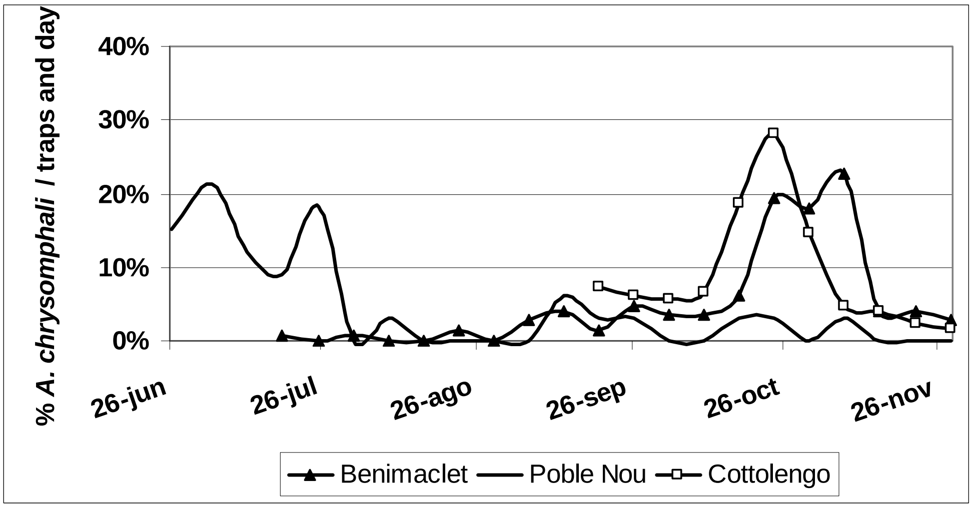

Figure 4. Evolution of the proportion of *Aphytis chrysomphali* captured on yellow sticky traps in three citrus orchards from Valencia (Spain).

Several species of predators were observed preying on *C. aonidum*, though their abundance was usually low. *Rhyzobius lophanthae* (Blaisdell) (Coleoptera: Coccinellidae) and *Semidalis aleyrodiformis* (Stephens) (Neuroptera: Coniopterygidae) were the species more frequently found.

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Parasitoid complex of black scale *Saissetia oleae* on Citrus: species composition and seasonal trend

Alejandro Tena, Antonia Soto, Ferran Garcia-Marí
Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camí de Vera 14, 46022 Valencia, Spain

Abstract: The parasitoid complex of black scale *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) was studied on citrus to determine their relative abundance, seasonal trend, geographical distribution, and their incidence on black scale populations. Branches and leaves of ten citrus groves infested with black scale were periodically collected over the period March 2003-December 2005 in eastern Spain, covering an area of 10,000 km^2. Adult parasitoids were also sampled with a portable engine-powered suction device. Black scale females were often attacked by *Scutellista caerulea* (Fonscolombe) (Hymenoptera: Pteromalidae), which was found beneath 35.4 ± 7.5% female scale's body. However, it attacked the scales when most of their eggs had already hatched. The parasitic mite *Pyemotes herfsi* (Oudemans) (Prostigmata: Pyemotidae) fed on all development stages of *S. caerulea*. The gregarious female's endoparasitoid *Metaphycus lounsburyi* (Howard) (Hymenoptera: Encyrtidae) was commonly found, but the parasitism rates it reached were low. Second and third instars of black scale were parasitized by the solitary endoparasitoid *Metaphycus flavus* (Howard), and secondarily by *Metaphycus helvolus* (Compere) which was much less abundant and limited in distribution. Thus, *M. helvolus*, introduced 30 years ago, has not displaced *M. flavus* as in other Mediterranean areas. According to their abundance, distribution and incidence, *M. flavus* and *S. caerulea* appeared as the main parasitoids of black scale in eastern Spain, whereas *M. helvolus* and *M. lounsburyi*, considered the main parasitoids in other citrus areas of the world, had a limited incidence.

Key words: *Saissetia oleae*, parasitoids, parasitism rates, seasonal trend

Introduction

Black scale *Saissetia oleae* (Olivier) (Hemiptera: Coccidae) is an important citrus pest in the Mediterranean basin (Franco et al. 2006). Satisfactory biological control of black scale has been achieved through the releases of parasitoids or through the introduction of a complex of parasitoids. In Valencia, different parasitoids were introduced but their establishment and incidence on black scale remain unclear (Carrero, 1981). Thus, we initiated a study of the parasitoid complex of black scale to determine the main species present in citrus.

Material and methods

Ten citrus groves were sampled twice a month between March 2003 and December 2005. In each grove: sixteen, 15-cm long twigs with green-wood and leaves were collected and they were processed to determine the phenology of black scale population and the identity and incidence of its parasitoids. Adult parasitoids were also collected from the tree canopy with a portable, engine-powered, suction device.

Results and discussion

The most abundant and widely distributed parasitoids in both olive and citrus groves were *Metaphycus flavus* (Howard), *Scutellista caerulea* and *M. lounsburyi* (Table 1).

Among the parasitoids of the immature scales, *M. flavus* was the most abundant and widely distributed. It was present in all the groves sampled, whereas *M. helvolus* was much less abundant and was present only in five of the 10 groves sampled. Both parasitoids were recovered in the emergence cages mainly when black scale occurred as 2nd and 3rd instars (between September and May each year), similarly to other parasitoids of immature scales as *Coccophagus lycimnia* and *C. semicircularis* (Fig 1). In the suction samples *M. flavus* was present throughout the year, even in summer when suitable black scale stages for oviposition by *M. flavus* were absent (Fig 2). During that time, *M. flavus* might have emerged from alternative hosts present in spanish citrus groves, as brown soft scale *Coccus hesperidum* L. (Hemiptera: Coccidae) (Llorens, 1984). The availability of using alternative host species could explain, at least in part, the superiority of *M. flavus* over *M. helvolus* observed in our study, because *M. helvolus* suffers high encapsulation rates when developing in brown soft scale (Blumberg, 1977). The encapsulation rates of *M. helvolus* when developing in *C. hesperidum* decrease at low temperatures (Blumberg and DeBach, 1981), and in our observations this parasitoid was found at high levels just in the most interior and, consequently, the most continental citrus grove sampled, being scarce or absent in the other nine groves.

Table 1. Relative abundance of *Saissetia oleae* parasitoids, observed using two sampling methods from March 2003 to December 2005) in Valencia.

Species	% Abundance[a]		Grove presence
	Emerging parasitoids	Suction-sampled	
Aphelinidae			
Coccophagus lycimnia	4,0	6,2	7/10
C. semicircularis	0,8	0,7	3/10
Encyrtidae			
Metaphycus flavus	22,3	69,7	10/10
M. helvolus	6,1	3,2	5/10
M. lounsburyi	19,1	7,1	9/10
Pteromalidae			
Scutellista caerulea	47,7	13,2	10/10
Total Number	2174	3460	10

[a] Parasitoid species percentage in each sampling method.

The parasitoids of adult females *S. caerulea* and *M. lounsburyi* were widely distributed, because they were collected in almost all the groves sampled (Table 1). Their abundance peaked at the beginning of summer (June-July), just at the end of the female black scale's development in both sampling methods (Fig 1 and 2). The parasitism rates of *S. caerulea* reached high values (>80%) at the end of the development of the black scale females (end of

June-beginning of July) (Fig 3), being lower during the maximum of black scale females (May-beginning of June). Thus, an important part of the eggs laid by black scale females usually escaped predation by *S. caerulea* larvae. The mite *Pyemotes herfsi* (Oudemans) (Prostigmata: Pyemotidae) was observed feeding on the larvae, pupae and adults of *S. caerulea*. Although *P. herfsi* had not been previously cited in Spain, we have found it as a common natural enemy regulating *S. caerulea* populations and, consequently, decreasing its efficacy as a biological control agent.

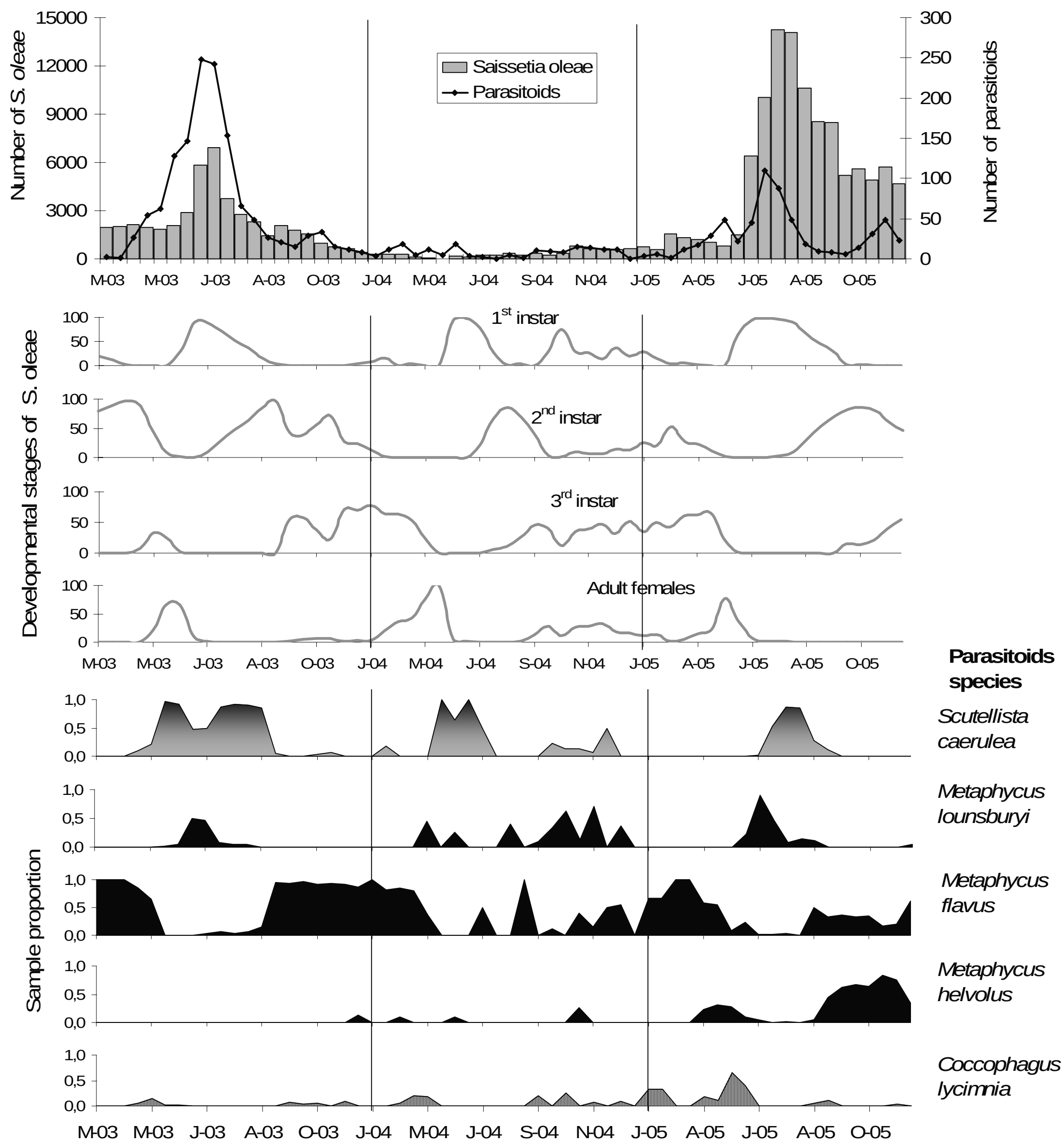

Fig 1. *Saissetia oleae* phenology and the relative abundance of its main parasitoids collected in the emerging cages in Valencia from March 2003 to December 2005.

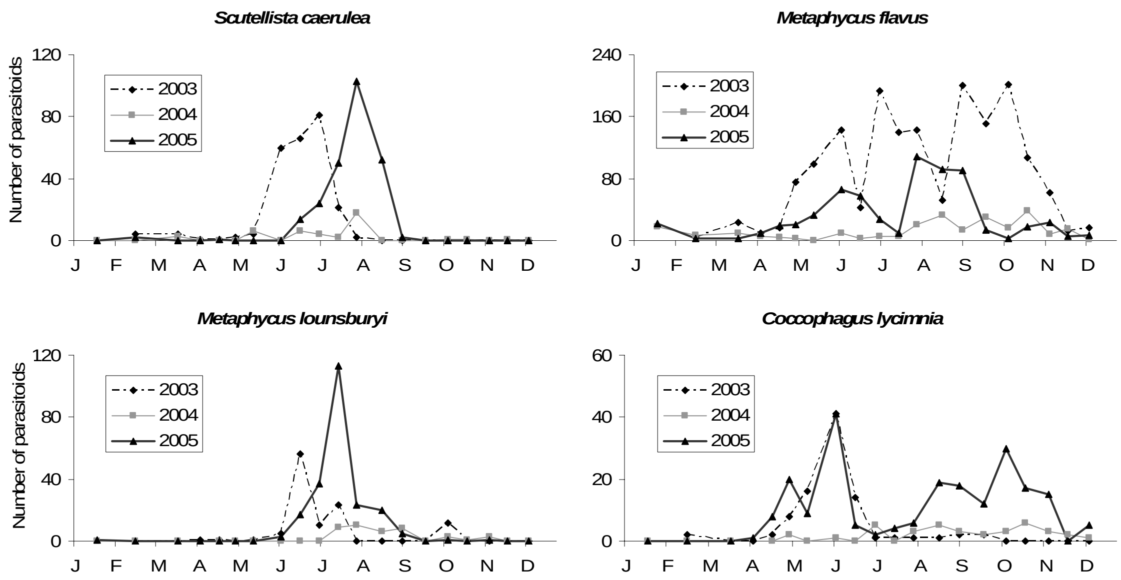

Fig 2. Seasonal abundance of *Saissetia oleae* parasitoids collected with a suction engine-powered device in Valencia from February 2003 to December 2005.

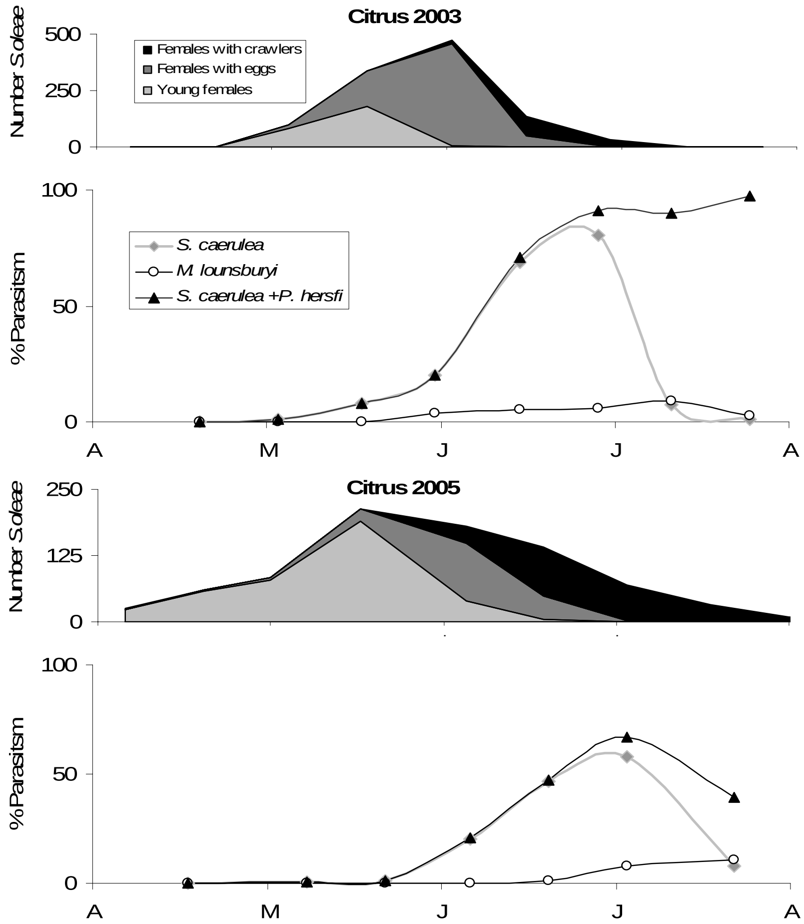

Fig 3. Changes in parasitism rates by *Metaphycus lounsburyi* and *Scutellista caerulea* (either alone or with its parasite *Pyemotes herfsi*) related with the phenology of *Saissetia oleae.*

Overall, our results show that the most abundant and widely distributed parasitoids of black scale in citrus in eastern Spain are *S. caerulea, M. flavus* and *M. lounsburyi*. These parasitoids should be considered when determining the side effects of pesticides on beneficials, as an important component of Integrated Pest Management strategies. We also recommend the rearing and augmentative release of *M. flavus* instead of *M. helvolus* for black scale outbreaks in citrus, because the native parasitoid appears to be better adapted and, moreover, mass-production of *M. flavus* is less costly than that of M. helvolus (Schweizer et al. 2003).

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IOBC/wprs Bulletin Vol. 38, 2008
pp. 87-93

Scale insect fauna (Hemiptera, Coccoidea) of citrus in Cap Bon region (Tunisia)

Hanen Jendoubi[1], Kaouthar Lebdi Grissa[1], Pompeo Suma[2], Agatino Russo[2]
[1] *Département de Protection des Plantes et Maladies Post-récolte, INAT, Tunis, Tunisie;*
[2] *Dipartimento di Scienze e tecnologie fitosanitarie, Università degli Studi di Catania, Italy*

Abstract: The authors report faunistic observations carried out on most infested citrus groves of Cap Bon region (Tunisia) during the year 2007. Eleven species of scale insects have been detected: *Icerya purchasi* Maskell (Margarodidae); *Planococcus citri* (Risso) (Pseudococcidae); *Ceroplastes rusci* (Linnaeus), *Coccus hesperidum* Linnaeus, *C. pseudomagnoliarum* (Kuwana), *Saissetia oleae* (Olivier) (Coccidae); *Aonidiella aurantii* (Maskell), *Chrysomphalus dictyospermi* (Morgan), *Lepidosaphes beckii* (Newman), *Parlatoria pergandei* Comstock and *P. ziziphi* (Lucas) (Diaspididae). *C. pseudomagnoliarum* is a new record for Tunisian fauna. For each species, brief data on distribution and density are given.

Key words: Tunisia, citrus, Coccoid fauna, field surveys

Introduction

Among fruit species grown in Tunisia, citrus is one of the most important fruit commodities with a potential for local consumption and export. Cap Bon is considered the most important citrus region, located in the North- East of Tunisia and containing 75% of the citrus growing area. Maltaise orange is the major commercialized variety, presenting 47% of the citrus cultivated area (GIF, 2006). However, researches regarding the scale insect fauna associated to the citrus trees are up to now limited. There is in fact a lack of literature including regional monographs and review articles, treating the population dynamic, incidence of the scale species and the efficient control methods. According history records, 17 scale species are reported attacking citrus in Tunisia: *Icerya purchasi, Planococcus citri, Pseudococcus longispinus, Coccus hesperidum, Ceroplastes rusci, C. sinensis, Saissetia Oleae, Parthenolecanium persicae, Eucalymnatus tessellatus, Pulvinaria psidii, Aonidiella aurantii, Aspidiotus hederae, Chrysomphalus dictyospermi, Fiorinia theae, Parlatoria pergandei, P. zizyphi, Lepidosaphes beckii* (Pagliano, 1938, 1951; CAB International, 1964, 1968; Jerraya, 2003; Ben-Dov & Miller, 2007). Between them, only three diaspidid species *P. zizyphi, C. dictyospermi* and *L. beckii* were subjected to expand studies on their population biology (Benassy & Soria, 1964).

In this study, a contribution to the knowledge of the scale insect fauna and their distribution in the citrus area with the assessment of their incidence were given. The associated auxiliaries were explored too. The ultimate aim of the work was to give an useful tool to improve the IPM strategies in Tunisian citriculture, considering the scale insects between the key pests of citrus.

Material and methods

Surveys were carried out from January to March 2007, in the citrus growing area of Cap Bon, in 6 localities Menzel Bouzelfa, Beni Khaled, Soliman, Takelsa, Grombalia and Bou Argoub (Figure 1). 37 samples were randomly collected from the most infested citrus groves in Cap Bon region (25 sites). Hence, ten trees were chosen randomly in each citrus grove. From each tree 5 fruits, 5 twigs of 20 cm of length were picked from the four quadrants and the center. A part of collected material was used to identify scale species and record the specimen state (living, dead and parasitized). The remaining part of material was maintained in plastic boxes until the emergence of natural enemies occur.

Figure 1. Surveyed areas in Cap Bon region

A hierarchic ascendant sort was applied in order to define the different density classes. The density of each species, expressed as a number of living individuals per organ examined in each site, is indicated in table 2. Four density classes were considered: the first includes the species present in the samples at density more than 0 and equal to or less than 1 specimen per organ (symbol ○), the second class includes the species present at density more than 1 and equal or less than 2 specimens per organ (symbol●), the third class includes the species density more than 2 and equal or less than 5 (symbol●●), the fourth class includes the species density higher than 5 (symbol●●●). We report here the density value registered on the most invaded organ (fruit, leaf, twig) depending of the species (table 1).

The data were treated by statistical program SPSS; statistical comparisons were performed by the Duncan t-test (p=0.05). An analysis of variance was used for the study of scale insects fauna at locality and site.

Results and discussion

Composition of the scale insect fauna

During the survey, 65% of the 17 species associated with citrus trees in Tunisia have occurred in the citrus growing areas of Cap Bon: *I. purchasi*, *Pl. citri*, *C. rusci*, *C. hesperidum*, *C. pseudomagnoliarum*, *S. oleae, A. aurantii*, *C. dictyospermi*, *L. beckii*, *P. pergandei* and *P. ziziphi*. The most numerous families were the Diaspididae (5 species) and Coccidae (4 species). The Margarodidae and Pseudococcidae families each include only one species. Between the 11 scales species detected, *C. pseudomagnoliarum* is a new record for Tunisian fauna. Many of them as the citrus mealybug, the chaff scale, the california red scale, the red scale and the fig wax scale are polyphagous and worldwide, considered as notorious pests of citrus in the Mediterranean area (Raymond, 1997; Rose, 1997; Franco *et al*., 2004). Compared with the other citrus regions, the scale insect fauna recorded in Tunisia is less rich than those of Southern Italy where more than 20 species were detected (Longo *et al*., 1994).

The citrus area needs therefore more collecting and studies on scale insect fauna through a more intensive sampling of size more important, in order to confirm the presence of the other recorded species in the past.

Relative importance of scale insect species and their distribution

According to the table 1, major of scale species recorded are eurymureous, feeding on more than two parts of the citrus trees. Globally, *P. pergandei, P. ziziphi* and *Pl. citri* constituted the key scale insect species of citrus, reaching the highest densities 2.08 individuals/fruit, 1.05 individuals/leaf and 0.27 individuals/fruit, respectively. The other scale species belong to the coccoid fauna of citrus in the area causing relatively somehow damages; the density varied in fact from 0.01 to 0.15 individuals per organ.

Table 1. Relative importance of species densities by organ.

Families	**Scale species**	**Fruit**	**Leaf**	**Twig**	**Densities individual per organ**
Margarodidae	*I. purchasi*	0.00	0.30	0.14	0.15
Pseudococcidae	*Pl. citri*	0.45	0.24	0.13	0.27
Coccidae	*C. pseudomagnoliarum*	0.00	0.17	0.11	0.13
	C. hesperidum	0.00	0.17	0.22	0.09
	S. oleae	0.00	0.01	0.07	0.05
	C. rusci	0.00	0.08	0.08	0.03
Diaspididae	*P. pergandei*	2.76	1.89	1.58	2.08
	P. ziziphi	0.58	2.32	0.26	1.05
	C. dictyospermi	0.25	0.10	0.05	0.13
	L. beckii	0.04	0.00	0.00	0.12
	A. aurantii	0.18	0.18	0.00	0.01

As showing in table 2, the scale insect species incidence was apparently considerable; 24% of species densities registered belong to the classes 4 and 3 in which the densities of species can reach more than 5 individuals by organ.

P. pergandei, P. ziziphi and *Pl. citri* showed a wide spread at high densities in all the localities, followed by *C. dictyospermi* and *I. purchasi.* Even though the population densities of the other species were inferior, their presence in the region was frequent, showing an important dispersal particularly in the regions of Menzel Bouzelfa and Beni Khaled. We note in particular *S. oleae* and *C. rusci*. However, some of them showed infestation foci localized mainly in Beni Khaled as *C. pseudomagnoliarum, A. aurantii,* and *L. beckii*. While Grombalia and Bou Argoub localities were the least rich regions on scale species in which 7 species, present at low density, were detected.

This large dispersal of scale species detected could be attributed both to the mobility of pseudococcid species and to the capacity of armored scale to colonize areas with success as reported by many authors (Kosztarab, 1987, 1996). Moreover, some cultivation practices as the mixture of several citrus varieties in the same citrus orchard (43% of farmers), inappropriate chemical control measures and mainly citrus commercial exchanges of infected plant material between localities, contributed to increase the infestation and create new foci. The differences on the vigour, age of trees and the climatic conditions offer a different microclimates and vegetation canopies affecting considerably the development of insects and thus the heterogeneity species distribution observed among the different surveyed citrus groves.

The margarodid scale species *I. purchasi* was spread enough widely in the different citrus groves, attaining the highest density in Takelsa locality. This should be related to a restricted presence of *Rodolia cardinalis* Muls, the Vedalia ladybird that is well acclimatized allover the Mediterranean Basin.

As Pseudococcidae, the citrus mealybug *Pl. citri* has been harmful in many sites, 23, at moderate densities. As auxiliaries, the endogenous parasitoid, *Leptomastidea abnormis* (Gyrault), and the introduced predator *Cryptolaemus mountrouzieri* Mulsant seemed not to be able to maintain *Pl. citri* population below the economic threshold; in despite that these two biocontrol agents and *Leptomastix dactylopii* (Howard) are among the most used auxiliaries, knowing to control the citrus mealybug successfully (Cadee and Van Alphen, 1997). Hence, *Pl. citri* is a damaging and difficult to control pest.

Among coccid species, the citricola scale *C. pseudomagnoliarum* and the brown scale, *C. hesperidum* were reported. The latter was spread in the region at low level, without causing great damages. The brown scale populations were generally controlled by indigenous natural enemies especially, *Metaphycus* parasites (Hart, 1972; Copland and Ibrahim, 1985). It was the same case in the different prospected areas in Cap Bon; it seemed to be associated to a well parasitized complex (*Microterys nietneri* (Motschulsky), *Metaphycus flavus* (Howard) and other *Metaphycus* spp.) controlling successfully its population.

C. pseudomagnoliarum has been surveyed sporadically in quantities which were harmless for citrus. It developed already an infestation focus in Beni Khaled reaching 4 specimens by leaf. Considering, that an average of more than 0.5 scale insects per leaf requires treatment if sampling was done at January-March (Phillips, 2005), a further spread of the species may cause its inclusion among the species which has already shown to be dangerous in the area. Additionally, only one parasitoid, *Coccophagus* spp, was identified.

As far as other coccids are concerned, we found the black scale, *S. oleae* and the fig wax scale *C. rusci*. The populations of these species were usually at low level. The second that is very polyphagous species was harmful only in one sample in the Soliman area.

Among Diaspididae, the chaff scale *P. pergandei* was present in almost all surveyed citrus areas, and considerably damaged all the vegetative organs of the citrus tree especially the fruits which remain with a greenish blotch at the feeding point. It is diffused at high population level in the different localities in spite of the parasitoids presence (*Aphytis* spp. and *Encarsia* spp.).

The black parlatoria, *P. ziziphi*, make a generalized phytosanitary problem, usually, reaching moderate to high population densities particularly on leaves. The parasitism action was still insufficient to control the species and no parasitoids have been determined. In fact, as it was reported by Fasulo and Brooks (2004), *P. ziziphi* is inefficiently controlled by the different biological control agents. The intervention is hence necessary for the two diaspids.

The california red scale *A. aurantii* has a limited diffusion in the surveyed areas. The diffusion of this scale, considered the most injurious species to citrus in the Mediterranean area (Raymond, 1997; Rose, 1997), could represent a serious menace for citrus groves and fruit cultivations.

Table 2. Distribution and density of the scale species collected in 37 samples in Cap Bon region.

Localities	Species sites	*I. purchasi*	*Pl. citri*	*C. pseudomagnoliarum*	*C. hesperidum*	*C. rusci*	*S. oleae*	*P. pergandei*	*P. ziziphi*	*C. dictyospermi*	*A. aurantii*	*L. beckii*
Beni Khaled	1		○	○	○			●	○	○		
	2		○	●●		○	○	●●●	●●		●	
	3	○	○					●●●	●●●			
	4	○	○	○	○			●●●	○	○		
	5	○	○		○			●●	○	○		
	6	○	●		●	○		●●	●●●	○		
Menzel Bouzelfa	7	●●	○		●	○	○	●●	●	○		
	8		○		○	○		●●	○			
	9		○					○	●	○		
	10	○	○		○			○	○	●		
	11	●	○		●			○	●●			
	12							●●	●●●	○		●
Soliman	13	○				●		○				
	14					○		○				
	15		○					●●	●	●		
	16							●●	●●			
	17			○				○	●●			
	18						○	●●●	●●			
	19		○	○		○	○	●	●●●			
	20		○					●●	○			
	21						○	○				
Takelsa	22											
	23		○			○		●●				
	24	○	○		○		○	●●	●	○		
	25		○					●				
	26		●●					○	○	○		
	27	●●●	○						●●	●		●●
	28											
Bou Argoub	29	○						○	○			
	30			○				○	●			
	31							●●	●●			
	32		○				○	●	●●	○		
Grombalia	33	○	○					○	○			
	34							○	●●			
	35			○				○	●●			
	36							●	●●			
	37						○		●	○		

The purple scale *L. beckii* has been sporadically surveyed only in two citrus groves at low densities. The red scale *C. dictyospermi* was more common species, presenting a density and distribution relatively more significant. These two species had been considered between the most injurious scale insect species in Tunisian citrus groves until sixties years according to Benassy (1961). Today, they are only met sporadically and in not seriously infesting state, especially the purple scale.

Conclusion

To conclude, we should continue surveys on the scale insect species associated with citrus trees in the entire governorate, especially in the richest regions on scales species, and the new planted areas. Moreover, we should also deepen studies on the population dynamics and specific control interventions for *P. pergandei, P. ziziphi* and *Pl. citri* species. Monitoring the other scale species having a limited diffusion in the area should be carried out, in order to acquire the useful knowledge to prevent insect outbreaks. The natural enemies fauna should be investigated more and their identification should be completed too.

Acknowledgements

Thanks are due to all of the staff at the regional services in Cap Bon for their devotion and generous assistance in the field surveys.

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IOBC/wprs Bulletin Vol. 38, 2008
pp. 94-98

May vine mealybug sex pheromone improve the biological control of the citrus mealybug?

José Carlos Franco[1], Taiadjana Fortuna[1], Elsa Borges da Silva[1], Pompeo Suma[3], Agatino Russo[3], Leonor Campos[1], Manuela Branco[2], Anat Zada[4], Zvi Mendel[4]

[1] *Dep. Protecção de Plantas e de Fitoecologia, Instituto Superior de Agronomia, Universidade Técnica de Lisboa, 1349-017 Lisboa, Portugal;*

[2] *Dep. Engenharia Florestal, Instituto Superior de Agronomia, Universidade Técnica de Lisboa, 1349-017 Lisboa, Portugal;*

[3] *Dip. Scienze e Tecnologie Fitosanitarie, via S. Sofia, 100 – 95123 Catania, Italia*;

[4] *Dep. Entomology, Volcani Center, ARO, Bet Dagan 50250, Israel*

Abstract: It was recently showed that *(S)*-lavandulyl senecioate, the sex pheromone of the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), attracts the females of the parasitoid *Anagyrus* spec. nov. near *pseudococci* (Hymenoptera: Encyrtidae). In a further study we examined whether this behaviour increases parasitization of this wasp in citrus mealybug *Planococcus citri* (Risso), in citrus orchards. As an experimental tool we exposed, in field trials, sentinel mealybugs (3^{rd} instar nymphs and adult females) on sprouted potatoes (= potato traps), to allow the access of the parasitoids to the mealybug colony. Three modalities were compared: 1) potato traps baited with dispensers loaded with the sex pheromone of *P. ficus*, 2) potato traps baited with dispensers loaded with the sex pheromone of *P. citri*, and 3) same trap design without pheromone (control). A similar set-up was conducted in citrus orchards in Portugal, Italy and Israel. Based on the number of trapped and emerged parasitoids and the minimal number of days of first parasitoid emergence we concluded that the presence of *(S)*-lavandulyl senecioate significantly increases the parasitization rate of *P. citri* colonies by *Anagyrus* spec. nov. near *pseudococci*. The results and their possible applications in future management of *P. citri* are discussed.

Key words: *Planococcus citri*, *Anagyrus pseudococci*, parasitoid, citrus, biological control

Introduction

Anagyrus pseudococci (Girault) (Hymenoptera, Encyrtidae) has been used in classical biological control programmes and augmentative releases to control the citrus mealybug, *Planococcus citri* (Risso) (Noyes & Hayat, 1994). Triapitsyn et al. (2007) showed that the taxon formerly known as *A. pseudococci* in fact comprises two sibling species, i.e. *Anagyrus pseudococci* (Girault) and *Anagyrus* spec. nov. near *pseudococci*. Except for the coloration of F1 of the female antenna, these two species are morphologically indistinguishable.

Recently, Franco et al. (2008) concluded that *A.* spec. nov. near *pseudococci* is apparently the most common species in citrus orchards and vineyards in Portugal and demonstrated that this wasp is attracted to the sex pheromone of the vine mealybug, *Planococcus ficus* (Signoret), (S)-(+)-lavandulyl senecioate (LS), but not to the sex pheromone of the citrus mealybug, (+)-(1*R*,3*R*)-*cis*-2,2-dimethyl-3-isopropenyl-cyclobutane-methanol acetate (PcA, namely Planococcyl acetate).

In this study, we present experimental evidence showing that the application of LS can enhance parasitism performance of *A.* spec. nov. near *pseudococci* in relation to the citrus mealybug in citrus orchards.

Material and methods

Chemicals

LS and PcA were synthesized in the chemical unit of the Department of Entomology, at the Volcani Center (Agricultural Research Organization, Bet Dagan, Israel), according to Zada et al. (2003) and Zada et al. (2004), respectively. All dispensers were loaded with the pheromones in hexane solution.

Trials

Sentinel mealybugs (3^{rd} instar nymphs and adult females of *P. citri*) were exposed in the field on sprouted potatoes, within plastic cylindrical containers (11 cm diameter, 13 cm height) with circular openings (3 cm diameter) (= potato traps), to allow the access of the parasitoids to the mealybug colony. All traps were suspended inside the tree canopy, at 1.0-1.5 m height, in the southeast quadrant, distributed about 20 m apart in a complete randomised design according to position, with 10 replicates. After about one week of exposure, the potato traps were transported to the laboratory and the number of *A.* spec. nov. near *pseudococci* females present within the traps was counted. Then, after removing ants and predators, the mealybug colonies were kept in the laboratory to allow the emergence of the wasps from the parasitized mealybugs. The number of days needed for the first parasitoid emergence was also determined per replicate, aiming at estimation of the efficiency of *A.* spec. nov. near *pseudococci* females to locate the trap.

Three modalities were compared: 1) potato traps baited with dispensers loaded with 200 µg of LS, 2) potato traps baited with dispensers loaded with 200 µg of PcA, and 3) same trap design without pheromone (control).

The trials were conducted in 2006-2007, in citrus orchards, in Portugal (Trial 1 and 2), Italy (Trial 3) and Israel (Trial 4).

Results and discussion

No *A.* spec. nov. near *pseudococci* females were present within the traps when these were collected from the field in Trial 1 (Table 1). Only the mealybugs from the traps baited with LS were parasitized.

The number of emerged wasps in LS baited traps was significantly higher than in control traps, in both Trial 2 (Table 2) and Trial 3 (Table 3). No significant differences were observed between PcA and control traps in Trial 2. In the case of Trial 3, the number of emerged wasps in PcA baited traps significantly differed from both LS and control traps, showing intermediate values (Table 3).

The number of captured *A.* spec. nov. near *pseudococci* females in LS baited traps was significantly higher than in control traps, in both Trial 2 (Table 2) and Trial 3 (Table 3). No significant differences were observed between PcA and control traps in Trial 2 (Table 2) and between PcA baited traps and the other modalities in Trial 3 (Table 3).

The number of days for the first wasp emergence in LS baited traps was significantly lower than in both PcA and control traps, in both Trial 2 (Table 2) and Trial 3 (Table 3). No significant differences were observed between PcA and control traps in Trial 2 (Table 2). The number of days for the first wasp emergence in PcA baited traps was significantly lower than in control traps in the case of Trial 3 (Table 3).

In Trial 4, the number of emerged *A.* spec. nov. near *pseudococci* females was very low and no significant differences were observed between modalities (Table 4).

Table 1. Number (mean±SE, n=10) of captured *Anagyrus* spec. nov. near *pseudococci* females, and number of emerged wasps from potato traps exposed in a citrus orchard, in Tavira (Portugal), from 19 to 27 June 2006, in function of the modality: traps baited with (S)-(+)-lavandulyl senecioate (LS) and control traps.

Modality	Nº wasp females collected per trap	Nº emerged wasps per trap
LS	0	34.20±10.0
Control	0	-

Table 2. Number (mean±SE, n=10) of captured *Anagyrus* spec. nov. near *pseudococci* females, number of days for the first wasp emergence and number of emerged wasps from potato traps exposed in a citrus orchard, in Tavira (Portugal), from 4 to 12 September 2006, in function of the modality: traps baited with (S)-(+)-lavandulyl senecioate (LS) or Planococcyl acetate (PcA), and control traps.

Modality	Nº wasp females* collected per trap	Nº days for the first wasp emergence	Nº emerged wasps per trap
PcA	1.80±0.47a	20.75±0.33b	8.90±2.23a
LS	15.70±2.56b	18.90±0.74a	44.70±9.99b
Control	2.10±0.53a	22.44±0.59b	19.90±5.88a

* Numbers followed by the same letter within each column do not differ significantly (SNK, P=0.05)

Table 3. Number (mean±SE, n=10) of captured *Anagyrus* spec. nov. near *pseudococci* females, number of days for the first wasp emergence and number of emerged wasps from potato traps exposed in a citrus orchard, in Sicily (Italy), from 20 to 27 October 2006, in function of the modality: traps baited with (S)-(+)-lavandulyl senecioate (LS) or Planococcyl acetate (PcA), and control traps .

Modality	Nº wasp females* collected per trap	Nº days for the first wasp emergence	Nº emerged wasps per trap
PcA	0.70±0.30ab	16.60±0.16c	23.20±3.76b
LS	1.44±0.34b	15.78±0.22a	36.33±4.60c
Control	0.40±0.16a	17.44±0.29b	11.00±3.73a

* Numbers followed by the same letter within each column do not differ significantly (SNK, P=0.05)

The number of emerged wasps from potato traps, obtained in Trials 1-3, reveals that LS, the pheromone of *P. ficus*, may enhance the parasitism of citrus mealybug in citrus orchards by *A.* spec. nov. near *pseudococci* . This is the first experimental evidence that this parasitic wasp uses LS as a kairomone in host location. The data collected on the number of captured wasp females and on the number of days for the first wasp emergence suggest that the enhancement of the parasitoid performance is the result of both a higher number of wasp females that were attracted to the vicinity of the pheromone source and a faster host detection as compared with the mealybug colonies without the pheromone.

Table 4. Number (mean±SE, n=10) of emerged *Anagyrus* spec. nov. near *pseudococci* from potato traps exposed in a citrus orchard, in Coastal plain (Israel), from 17 to 28 May 2007, in function of the modality: traps baited with (S)-(+)-lavandulyl senecioate (LS) or Planococcyl acetate (PcA), and control traps.

Modality	Nº emerged wasps per trap*
PcA	2.54±0.80a
LS	7.04±2.23a
Control	4.75±1.50a

* Numbers followed by the same letter do not differ significantly (SNK, P=0.05)

The lack of kairomonal response observed in Trial 2 in relation to PcA, the sex pheromone of *P. citri,* is consistent with previous field studies (Suma et al., 2001; Franco et al. 2008), electro-antennography (Suma et al., 2004) and olfactometer tests (Franco et al., 2008). However, a lower (in relation to LS) but significant response to PcA was registered in Trial 3, in Sicily. Considering the period this trial was carried out (end of October) and that previous field studies conducted in the same region registered no response to PcA (Suma et al., 2001), this result might be related to parasitoid learning/experience (e.g., Vinson, 1998). We might hypothesize that an expected longer life span of wasp females (the average temperature is lower, as compared with summer) and relatively high abundance of the citrus mealybug, in begin of autumn, in Sicily, could have resulted in learning response of *Anagyrus* females to the *P. citri* sex pheromone. A longer life span and high host abundance are expected to favour higher frequency of successful parasitism events and eventually learning/experience of wasp females. Further studies are needed to elucidate this issue.

The results obtained in Trial 4 are possibly originated by very low population density of the parasitoid, reason why the response to LS was higher but not significantly different from the other modalities. In addition, possible differences in the kairomonal response to LS may exist between populations of *Anagyrus* (or species?) of different geographical origins in the Mediterranean basin. Further studies are needed to elucidate this issue.

Possible applications of the results in biological control tactics for the citrus mealybug, in citrus orchards, include the use of LS-baited potato traps: i) as a tool to evaluate the population level of *Anagyrus* in citrus orchards, in spring or early summer; ii) to anticipate the natural build up of *Anagyrus* population by creating artificial hotspots of mealybugs within the orchard; and iii) to enhance the early and effective colonization of *Anagyrus* after inoculative releases in the spring.

Acknowledgements

Thanks are due to Manuel Cariano for field and laboratory assistance, Celestino Soares and Hugo Laranjo for their help in the selection of experimental plots in Algarve, and to the growers and the Direcção Regional de Agricultura do Algarve for allowing us to use their farms in field experiments. This research was funded by Fundação para a Ciência e Tecnologia (FCT) and co-funded by FEDER, as part of the Project n. POCI/AGR/57580/2004. JCF and EBS received grants from FCT (SFRH/BSAB/645/2006 and SFRH/BPD/22145/2005).

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IOBC/wprs Bulletin Vol. 38, 2008
pp. 99-103

Laboratory evaluation of pesticide secondary effects on *Anagyrus* sp. nov. near *pseudococci,* parasitoid of the citrus mealybug *Planococcus citri*

Pompeo Suma, Gaetana Mazzeo
Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, via S. Sofia 100, 95123 Catania, Italy

Abstract: The encyrtid *Anagyrus pseudococci* (Girault) *s.l.* is an important parasitoid widely used for biological control of pseudococcids and in Sicilian citrus groves it is the main indigenous natural enemy of the citrus mealybug. Laboratory-reared females were exposed to insecticides both sprayed on the internal surfaces of a glass box and supplied as food in a mixture with honey (1:1). The toxicity by tarsal contact, by ingestion and the effects on fecundity under laboratory conditions were evaluated. The tested insecticides were mineral oil, spinosad, chlorpyrifos-methyl, pyriproxyfen and buprofezin applied at the highest recommended field rate marked on the label. Mineral oil, spinosad and chlorpyrifos-methyl were highly harmful in the contact toxicity tests (100% mortality), therefore they have been excluded for the ingestion toxicity evaluation. No significant differences in mortality and longevity were observed between buprofezin and the untreated control; pyriproxyfen caused more than 50% of adult's mortality and significantly reduced the longevity. The ingestion experiments showed that the two IGRs significantly affected the longevity of the parasitoid females. The tested insecticides showed a variable level of toxicity in relation to the way they came in contact with parasitoids, and due to this fact the use of different testing methods in evaluating the effects of pesticides on natural enemies is suggested.

Key words: insecticides, toxicity, side effects, natural enemies, IPM

Introduction

The citrus mealybug (CM), *Planococcus citri* Risso (Hemiptera: Coccoidea) is an important worldwide distributed pest on agricultural and horticultural plants, field crops, ornamentals and in protected crops (Williams, 1962; Ben-Dov, 1994) that in several citrus growing areas is considered a key-pest. Its control commonly requires the use of insecticides, but the high levels of resistance, due to the wax-like coating on its body may favour pest resurgence (Hardin *et al*., 1995). In last decades several mealybug outbreaks were recorded, i.e. in Israel and Southern Africa; the phenomenon was correlated with the use of the juvenoid pyri-proxifen for the control of California red scale *Aonidiella aurantii* (Maskell) (Mendel *et al*., 1994; Hatting & Tate, 1997). Several attempts were therefore made to control the insects with other tactics, such as use of beneficials (Smith et al., 1988; Mendel et al., 1999). Effective integration of biocontrol agents and compatible insecticides could play a crucial role in citrus IPM. Biological control of CM by using parasitoids and/or predators was effective in interior plantscapes (Kole & Hennekam, 1990) and, although some Authors report that the complex of indigenous natural enemies is unable to prevent outbreaks of *P. citri* under particular environmental conditions (Longo & Russo, 1986; Barbagallo et al. 1992), their protection is an aspect of primary importance for IPM in citrus groves.

In this study, we analyzed the compatibility of five insecticides, some of them frequently employed in Italian citriculture, with *Anagyrus pseudococci* that, in Sicilian citrus groves, represent the main indigenous natural enemy of the CM. Based on the coloration of the first funicular segment (F1) of the female antenna we can assume that the parasitoid populations used in this work could be ascribed to *A.* spec. nov. near *pseudococci* (Triapitsyn et al., 2007).

Material and methods

Insect rearing

The study was conducted at the Laboratory of Applied Entomology, Faculty of Agriculture, Catania University, Italy. Rearings of both *P. citri* and *A.* spec. nov. near *pseudococci* were carried out under laboratory conditions from specimens collected in the field and maintained on potato sprout and pumpkin squash in plastic boxes (37 W×50 L×25 H cm) with openings covered with net to ensure proper ventilation. Parasitization of *P. citri* nimphs by *A. pseudococci* was conducted in plastic cages kept in insect-rearing room at temperature of 26±1°C, 65-80% RH and 14L:10D. For the experiments, mummified mealybugs were collected from the mass cultures and put into glass vials (length 5.0 cm, diameter 2.5 cm), which were then kept in an incubator at 26±1°C under continuous light. They were daily checked for parasitoid emergence. Newly emerged parasitoid females were individually isolated with a male until mating. The wasps used in the experiments were 1-2-day-old adult males and females never tested more than once.

Pesticides

Five insecticides were bioassayed. They were selected according to the following criteria: one insecticide frequently used in Citrus pest protection in organic and IPM (narrow range mineral oil); two insecticides generally used in case of high infestation (chlorpyrifos-methyl in conventional management and buprofezin in IPM); one IGR recently registered in Italy (pyriproxyfen) and the last one registered on Citrus in other countries (spinosad).

Each commercial product was tested at the highest dose reported on the label. For each assay, the insecticides were diluted with distilled water and distilled water was used as controls. According to Hassan et al. (1994), pesticides were classified into four categories in function of the reduction in parasitization: 1, harmless (< 30%); 2, slightly harmful (30-79%); 3, moderately harmful (80-99%); 4, harmful (>99%).

Toxicity assays

The effects of the insecticides were evaluated through the estimation of the toxicity by ingestion and tarsal contact. In this last case, the exposure cage consisted of six glass plates (9.5×9.5 cm) assembled forming a cube. Each glass plate was sprayed with the insecticides using a Potter spray tower (Burkard Manufacturing Co. Ltd.) and the cages were built after the treated surfaces had dryed. Five newly emerged females and males were introduced in the cages for 24 hours. Ten replicates were performed. After this period the survived females for each treatment were placed individually in clean plastic boxes containing an excess of suitable CM hosts. The parasitoids were allowed to oviposit for 24 h. The parasitized mealybugs were then removed and placed in new boxes until the emergence of offspring recording its number and the sex ratio. Further hosts were then offered to the survived parasitoid females to maintain a continuous presence of victims suitable for parasitism, in order to define the total progeny production. Mortality of the parasitoid females was recorded 72 hours after exposition to the treated glass surface.

A no-choice test was used to evaluate the ingestion toxicity of the selected pesticides on parasitoid survivorship rate. On emergence, a single wasp female was isolated in a Petri dish (∅ 5 cm) and provided with a droplet of a 50% aqueous honey solution diet incorporating either the insecticide at the highest dosage reported on the label. After 24 h in each Petri dish a continuous presence of victims suitable for parasitism was maintained. Mortality, longevity and progeny production of the treated females were determined.

Results and discussion

In relation to tarsal contact test, both buprofezin and pyriproxyfen seem to be compatible with *A.* spec. nov. near *pseudococci*. However, pyriproxyfen was slightly detrimental with regard to mortality (table 1) and longevity (figure 1). Mineral oil, chlorpyrifos-methyl and spinosad were extremely toxic to adult parasitoids, causing 100% mortality at the rates tested.

Table 1. Mortality (%±SD), total number of progeny produced by each single *A.* sp. nov. near *pseudococci*,female, reduction in the parasitism rate, progeny sex ratio (M:F) and toxicity categories of the tested compounds according to Hassan et al., (1994).

Insecticides	Mortality	progeny/female*	PR%**	Progeny sex ratio	Toxicity categories
control	—	28.64±5.98	—	1.2:1	—
buprofezin	6±0.89	23.02±8.61***a***	19.62	1.2:1	1
pyriproxyfen	56±3.77	31.36±13.04***a***	0	1.1:1	1
n.r. mineral oil	100	—	100	—	4
chlorpyrifos-methyl	100	—	100	—	4
spinosad	100	—	100	—	4

*Numbers followed by the same letter within each column do not differ significantly (LSD test, p=0.05). **PR is the reduction in the parasitism rate compared with the control.

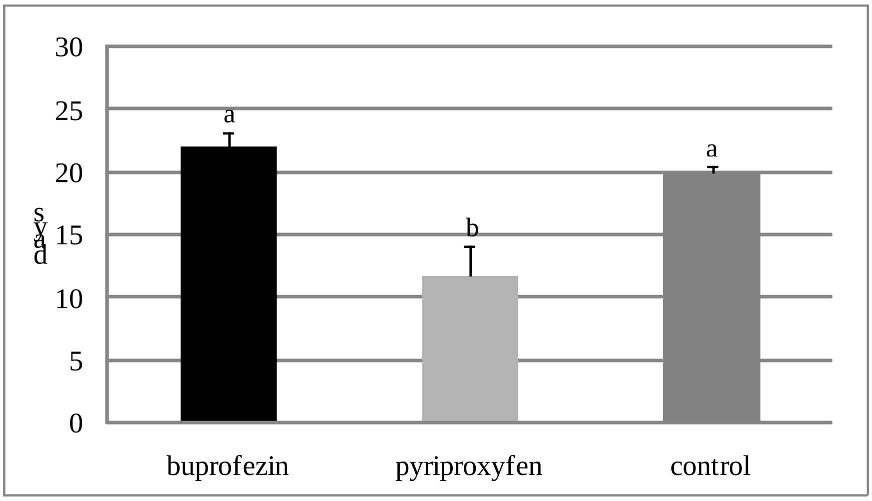

Figure 1. Mean longevity of *A.* sp. nov. near *pseudococci* adult females (n=25) exposed to the surface sprayed with different insecticides, for a period of 24 hours. Columns bearing the same letter were not significantly different (LSD test, p=0.05).

Based on the results of the previous experiments in the ingestion toxicity assay, the solely activity of the two IGRs was evaluated. No mortality was recorded 72 h after food supplying. In contrast, comparing their activity with the untreated control, parasitoid longevity (Fig.2) and progeny production were significantly affected (Fig. 3).

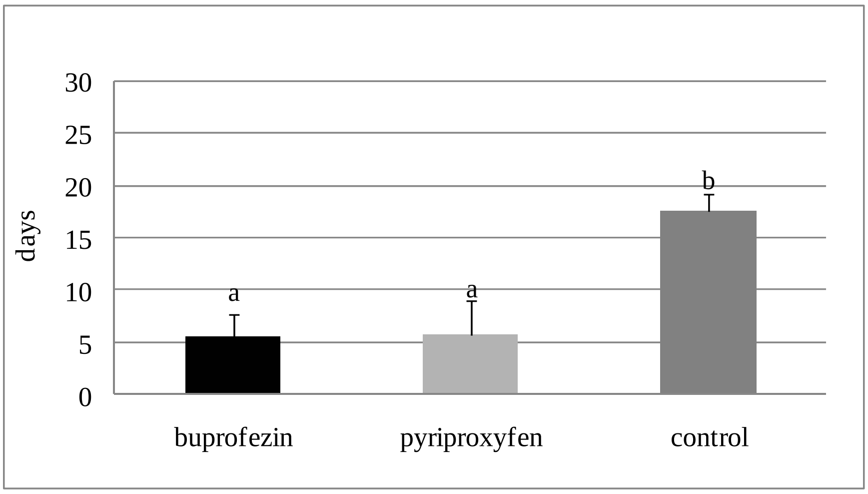

Figure 2. Mean longevity of *A.* sp. nov. near *pseudococci* adult females (n=20) fed with an aqueous honey solution diet incorporating the two IGRs tested. Columns bearing the same letter were not significantly different (LSD test, p=0.05).

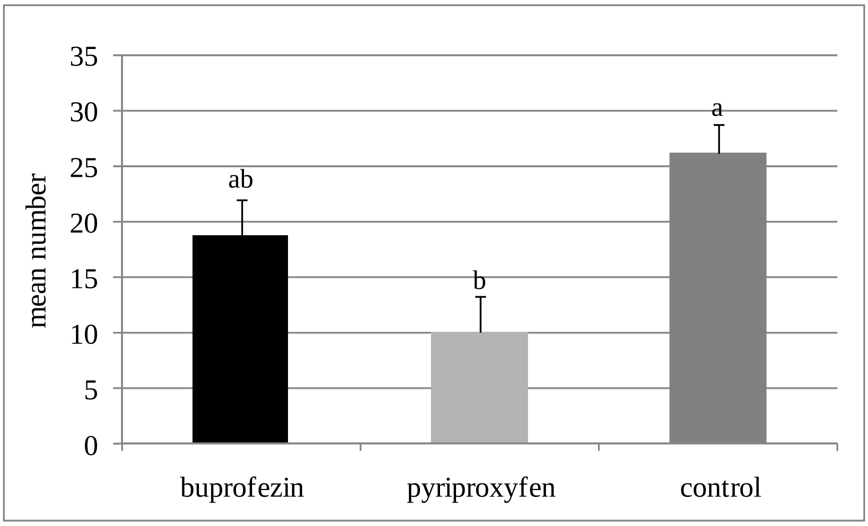

Figure 3. Progeny production of *A.* sp. nov. near *pseudococci* adult females (n=20) fed with an aqueous honey solution diet incorporating the two IGRs tested. Columns bearing the same letter were not significantly different (LSD test, p=0.05).

Mortality induced by buprofezin and pyriproxyfen was not significantly different from that of the untreated control in the two bioassays. Different indications were obtained when longevity and progeny production were measured with the two experimental methods adopted. These results suggest that, in evaluating the effects of pesticides on insects, the method used may really have a decisive effect on the results; therefore, further studies on the secondary effects under semi-field and field conditions should be performed to have a more complete evaluation, especially in relation to mineral oil, chlorpyrifos-methyl and spinosad.

Acknowledgements

Thanks are due to Dr. Lucia Zappalà for comments and critical revision of the manuscript.

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Control in Citrus Fruit Crops
IOBC/wprs Bulletin Vol. 38, 2008
pp. 104-110

Influence of ant-exclusion on *Planococcus citri* density in a citrus orchard

Piera Maria Marras[1], Francesco Sanna[2], Roberto A. Pantaleoni[3,4]
[1] *Dipartimento per la Ricerca nella Arboricoltura, AGRIS Sardegna, 07100 Sassari, Italy*
[2] *Dipartimento per la Ricerca nelle Produzioni Vegetali, AGRIS Sardegna, 09123 Cagliari, Italy*
[3] *Dipartimento di Protezione delle Piante, Università di Sassari, Italy*
[4] *Istituto per lo Studio degli Ecosistemi, CNR, 07100 Sassari, Italy*

Abstract: Formicids have been observed to protect mealybugs by aggressive behaviour towards their natural enemies. In field studies carried out over five growing seasons (1996-2000) in an organic citrus orchard in Sardinia, Italy, the influence of ant-exclusion on the population density of *Planococcus citri* Risso was examined. In the last three years sticky trunk barriers were used to exclude ants from orange tree canopies for at least 7 months every year. The presence of isolated *P. citri* specimens (1-10) or mealybug colonies (>10) on orange fruits was recorded. Randomized intervention analysis (RIA) was used to detect a change in the ant-excluded plot relative to the undisturbed one. It was applied to paired time series of data from both plots before and after manipulation over five years. Four species of ants were found on fruits and *Lasius niger niger* (L.) was the most common species (> 80%). The percentage of *P. citri* infested fruits in ant-free trees was significantly lower than that in ant-present ones, besides less fruit damage, due to black sooty mould, has been observed in the ant-excluded plot.

Key words: Formicid, *Lasius niger niger*, mealybugs, orange, sticky barriers

Introduction

Many honeydew-producing Homoptera, such as aphids, mealybugs and soft scales, have mutualistic relationships with ant species which feed on their honeydew. Homoptera attended by ants appear to change their life cycle as well as some behavioural and structural chacteristics as adaptations to live with ants (Way 1963).

Attendant ants benefit Homoptera by transporting them to feeding sites, removing honeydew and protecting them from unfavourable weather. Moreover they protect or partly protect Homoptera from natural enemies by disturbing or killing parasitoids and predators, thereby reducing natural enemy effectiveness (Bartlett 1961, Way 1963, DeBach 1965, Cudjoe *et al*. 1993, Fontanari *et al*. 1993, Itioka & Inoue 1996, 1999, Kaneko 2003).

Ants have also shown to disrupt parasitism and predation of phytophagous insects and mites that do not produce honeydew (Haney *et al*. 1987, Murdoch *et al*. 1995, Martinez-Ferrer *et al*. 2002). This is generally considered an indirect relationship, resulting from an interest in associated honeydew-producing insects (Bartlett 1961, James *et al*. 1999). Ants may play an important role in hindering key pest biological control by reared parasitoids and predators which are released in orchards to augment the population of natural enemies (Martinez-Ferrer *et al*. 2002).

The citrus mealybug *Planococcus citri* (Risso) is one of the six mealybug species reported as citrus pests in the Mediterranean basin (Franco *et al*. 2003). In numerous citrus orchards, *P. citri* population management has been based on biological control, with inoculative release of the parasitoid *Leptomastix dactylopii* Howard and the predator

Cryptolaemus montrouzieri Mulsant. However these natural enemies could not effectively reduce the citrus mealybug population. In a trial conducted in Spain by Villalba *et al.* (2006) no significative decrese in *P. citri* population has been observed in release orchards compared with non-release ones. Moreover ant-free orchards showed a significantly lower mealybug population level. The authors consider the presence of ants an important factor which hinders biological control of *P. citri* by *L. dactylopii* and *C. montrouzieri.*

Lasius niger (Laitrelle), the most common ant species in Spanish citrus orchards, appeared to significantly reduce *L. dactylopii* and *Anagyrus pseudococci* (Girault) parasitism on *P. citri* by about 50% (Campos *et al.* 2006). The management of ant populations is one of the different strategies in enhancing biological control of mealybugs and honeydew-producing insects (Moreno *et al.* 1987, Fontanari *et al*. 1993, James *et al.* 1997, Tumminelli *et al*. 1997, Benfatto 1999). In this work the influence of ant-exclusion on the population density of *P. citri*, in an organic citrus orchard in Sardinia, Italy, was examined.

Material and methods

The study was carried out over five growing seasons (1996-2000) in an organic citrus grove [*Citrus sinensis* (L.) cv. Washington navel] in southern Sardinia, Italy. During our study *L. dactylopii* was released to control the *P. citri* population. The grove (0,6 ha), containing 126 trees more than 20 years old, was divided in two equal plots including seven rows of nine trees each.

In the first two years two trees in each plot were chosen to record the presence of isolated *P. citri* specimens (1-10) or mealybug colonies (>10) on orange fruits. Ten fruits were observed randomly round each tree every two weeks from late June through September.

During the last three years in one of the two plots, ants were prevented from moving onto trees by applying 20-cm sticky barriers directly onto the bottom of the trunks. All trees within the other plot were not subject to treatment (control). In both plots the ground vegetation was mowed and the trees' lower branches were trimmed to stop them touching the ground. Sticky trunk barriers were applied in June and repeated monthly through November; mowing of ground vegetation and trimming of trees branches was repeated routinely during the study. Fifteen trees per plot were chosen to record the presence of *P. citri* on ten fruits/tree. Isolated *P. citri* specimens or mealybug colonies were surveyed every ten days from late June through early December (November in 1998). Ant presence on fruits was detected on the same days by sampling, at randomly chosen compass directions, one fruit per tree from thirty trees in each plot. Ants were surveyed only in 1998-99. At harvest-time the presence of black sooty mould was observed on one thousand fruits per plot.

Randomized intervention analysis (RIA) was used to detect a change in the percentage of *P. citri* infested fruits in the ant-excluded plot relative to the undisturbed one (Carpenter *et al.* 1989). RIA was applied to a paired time series of *P. citri* (isolated specimens + colonies) density data from both plots before (1996-1997) and after manipulation (1998-2000). A time series of inter-plot differences was calculated and from these, mean values relative to the PRE ant-exclusion and POST ant-exclusion differences were calculated. The absolut value of their difference is the test statistic. So the $|\overline{D}\,(\text{PRE}) - \overline{D}\,(\text{POST})|$ distribution was estimated by one-million random permutations of the sequence of inter-plot differences in a Monte Carlo simulation. RIA was also applied to a paired time series of percentages of *P. citri* infested fruits which includes mealybug colonies only.

Results

In two years (1998-99) 87 specimens of ants were sampled and four species were identified: *Lasius niger niger* (L.), the most common species (> 80%), *Formica rufibarbis rufibarbis* Fabricius (~14%), *Tetramorium brevicorne* Bondroit and *Camponotus aethiops aethiops* (Latreille). All ants were collected in the non-manipulated plot except two specimens collected in the manipulated plot on 1 July 1998 and 2 July 1999.

The percentage of *P. citri* infested fruits was significantly lower in ant-free trees than that in ant-present ones. P value from RIA applied to total *P. citri* (isolated specimens + colonies) infested fruits was 0,000012, its very low value indicates that a non random change in the interplot difference occurred (Fig. 1). RIA computed for fruits infested by *P. citri* colonies indicated the same result (P = 0,001893) (Fig. 2). At harvest-time less fruit damage, due to black sooty mould, was observed in the ant-free plot compared to the ant-present one (Tab. 1).

Discussion

Several authors reported that ant exclusion affects various honeydew and non-honeydew producing citrus pests (Haney *et al*. 1987, Moreno *et al.* 1987, Murdoch *et al*. 1995, Itioka & Inoue 1996, James *et al.* 1997, Tumminelli *et al*. 1997, Benfatto 1999, Martinez-Ferrer *et al*. 2002, Villalba *et al.* 2006). The results of our study supported these findings. Among citrus pests *P. citri* is considered one of the main honeydew sources on which ants feed. The more numerous ants are the more natural enemies could be disturbed or attacked (DeBach *et al.* 1951). *L. niger,* the most common species in our orchard, was observed to protect mealybugs by an aggressive behaviour towards its natural enemies and to be a very effective mutual partner (Way 1963, Itioka and Inoue 1996, 1999). By preventing ants, particularly *L. niger*, from moving onto trees we permitted a more effective *P. citri* biological control and a significant decrease of its population density as the RIA response indicated.

Sticky trunk barriers are an effective means of ant-exclusion utilizable in organic citrus orchards. Nevertheless together with ants they may capture several other insects which include predators such as Coleoptera Coccinellidae, Neuroptera, Araneida and Heteroptera. A constant use of this kind of barrier may result in a decreased predator population on orange trees. Further long term experiences and an economic analysis are necessary in order to state the real applicability of this practice in commercial citrus orchards.

Table 1. Sooty mould presence on orange fruits at harvest-time (1998-2000).

	sooty mould presence (%) on one thousand fruits					
	ant-excluded trees			control trees		
year	slight*	severe**	total	slight*	severe**	total
1998	18,4	0,4	18,8	32,8	0,0	32,8
1999	23,9	1,8	25,7	20,7	4,1	24,8
2000	40,2	14,6	54,8	59,4	21,6	81,0

*presence of light sooty mould on less than 1/8 of fruit surface
**presence of thick sooty mould on more than 1/8 of fruit surface

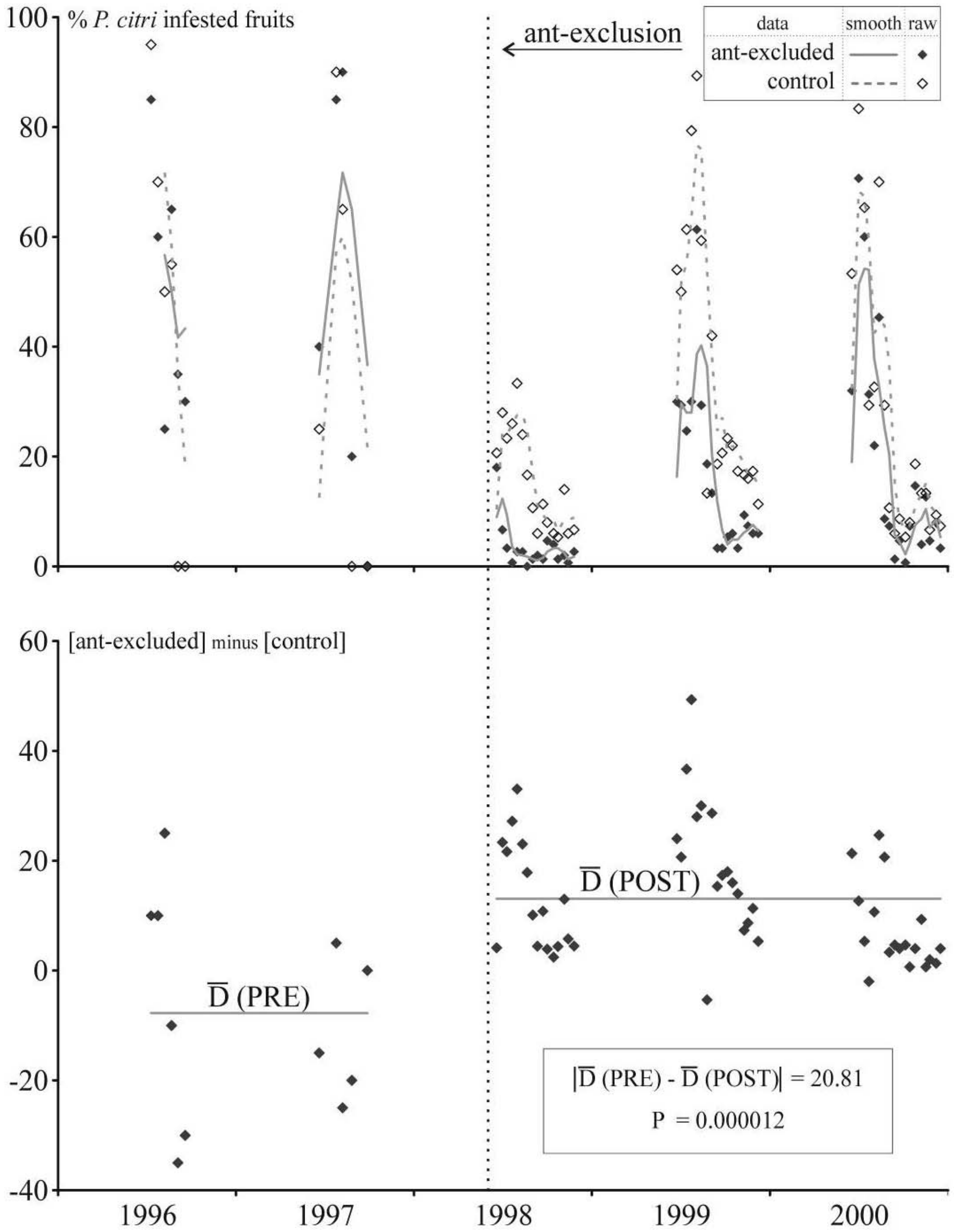

Figure 1. RIA applied to *P. citri* infested fruits (isolated specimens + colonies) from ant-excluded and control plots. Inter-plot differences (ant-excluded − control) are calculated from the time paired data from both plots before and after ant-exclusion. Mean inter-plot differences before [$\overline{D}$ (PRE)] and after [$\overline{D}$ (POST)] ant-exclusion are then calculated.

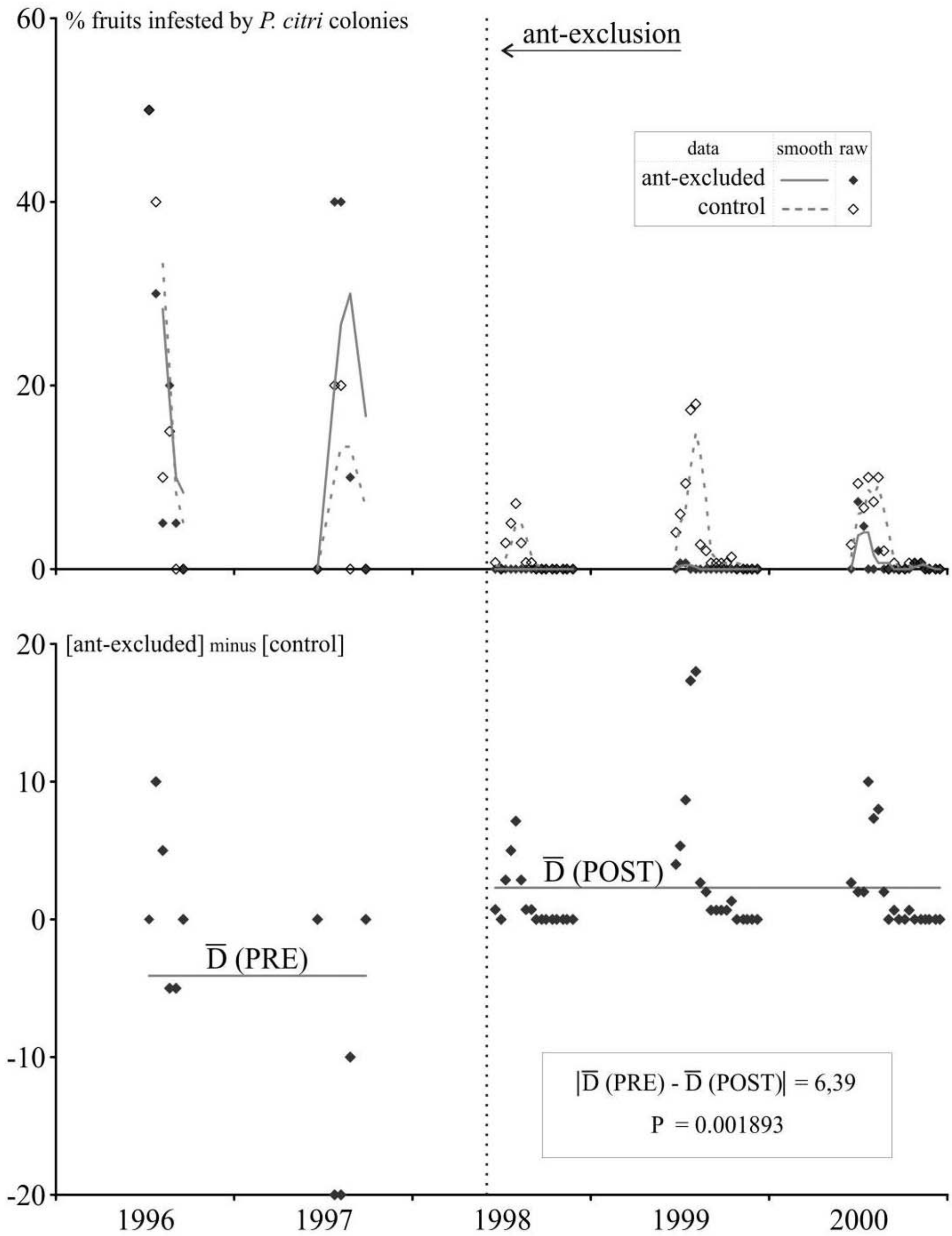

Figure 2. RIA applied to *P. citri* infested fruits (only colonies) from ant-excluded and control plots. Inter-plot differences (ant-excluded − control) are calculated from the time paired data from both plots before and after ant-exclusion. Mean inter-plot differences before [$\overline{D}$ (PRE)] and after [$\overline{D}$ (POST)] ant-exclusion are then calculated.

Acknowledgements

We thank Dr. Pier Carlo Cresto for statistical calculations. We are also grateful to Dr. Marcello Verdinelli for the identification of ants and Loreta Porcu for technical assistance.

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Secondary effects of seven pesticides on *Anagyrus pseudococci* (Girault) and *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae), parasitoids of *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae)

Campos, J.M.; Martínez-Ferrer, M.T; Forés, V.
IRTA Amposta, Ctra. de Balada, Km 1, 43870 Amposta (Tarragona), Spain

Abstract: The toxic residual activity of 7 pesticides, abamectin, petroleum spray oil, buprofezin, carbosulfan, chlorpyrifos, hexitiazox and pyriproxifen, was tested against adults of *Leptomastix dactylopii* and *Anagyrus pseudococci*, natural enemies of the citrus mealybug *Planococcus citri*. Insects were exposed for 7 days in small containers to pesticide residues on leaves of sprayed citrus at intervals of 1, 3, 8, 21 and 30 days post-treatment to determine their susceptibility to the residues and the persistence of the residues. The effect on the longevity and progeny production of parasitoids was studied. Carbosulfan residues showed high toxicity to both beneficial insects, due to both initial toxicity and persistence. Chlorpyrifos caused a slight toxicity in the beginning, but its persistence was low. Abamectin caused a high initial toxicity in both parasitoids but also with a low persistence. Insect growth regulators buprofezin and pyriproxifen residues, along with petroleum oil, appeared to be the less toxic for the parasitoids. *A. pseudococci* was more sensitive than *L. dactylopii* to the more toxic pesticides, carbosulfan and abamectin. None of the pesticides altered the progeny production of *L. dactylopii*; however the *A. pseudococci* progeny was adversely affected by both buprofezin and abamectin.

Key words: Secondary effects, *Leptomastix dactylopii, Anagyrus pseudococci*, extended-laboratory, *Planococcus citri.*

Introduction

Planococcus citri is a pest with a rich beneficial complex, which include *A. pseudococci* and *L. dactylopii.* From late spring to early summer, activity of both species against *P. citri* is important to control the pest under the calyxes (Martínez-Ferrer *et al*., 2003). At this time treatments against aphids, mites, armored scales, leafminers and mealybugs tend to be applied in Spanish citrus orchards. Populations of these parasitoids are thereby exposed to residues from several commercially applied pesticide combinations present on citrus leaves. Accordingly, an extended laboratory trial was performed to simulate field conditions, with leaves containing pesticide residues. The main objectives of this study were: to determine the residual toxicity of 6 pesticides that are commonly used in the spring-summer period and assess their influence on the survival and reproduction of the two parasitoids, and to study the persistence of the harmful effects of pesticide residues on leaves in the field after application.

Material and methods

Two-year-old mandarin potted plants (*Citrus reticulata* Blanco.) of the Clemenules variety were used for the test. Sixteen plants, two for each of the pesticides assayed, were chosen. They each had a similar number of leaves, and there were enough of them to conduct the whole experiment. Treatments were performed at the maximum recommended field dose (Table 1) with a conventional 1 l. hand sprayer. A separate hand sprayer was used for each pesticide. The products were applied from a distance of approximately 20 cm. After spraying, plants were taken to different locations and left to dry in well-ventilated areas partially protected from sunlight and they were irrigated twice a week.

Table 1. Pesticides: Active ingredient, trade name, dose and volume of chemical sprayed over 2-year-old mandarin plants (Clemenules variety).

Active ingredient	Trade name	Dose (%)
Abamectin 1,8% p/v EC	BERMECTINE® Probelte Jardin	0,04
Carbosulfan 25% p/v CS	MARSHAL® 25 CS FMC Foret	0,15
Buprofezin 25% p/p WP	APPLAUD® Syngenta	0,1
Chlorpyrifos 44,6% p/v	DURSBAN® Syngenta	0,2
Pyriproxifen 10% p/v	ATOMINAL® 10 EC. C.Q. Massó	0,075
Hexythiazox 10% WP	ZELDOX® Syngenta	0,015
Petroleum Spray Oil 83% p/v	VOLK® Miscible Agrodán	2,5

In each experiment, 2 leaves from each of two plants exposed to the same treatment were randomly removed and introduced into small containers (140 x 60 x 80 mm) with a 110 x 40 mm cloth mesh window in its lid to permit ventilation. Two holes were also made in the lateral surfaces in order to enhance air circulation within each container. Three replicates were performed for each treatment. The petiole of the leaf was placed inside a small vial (1.5 ml) containing water in order to keep the leaf fresh throughout the exposure period. Once the leaves were inside the small containers, 5 male and 5 female parasitoids of each species were released inside them. Small droplets of honey were left inside the containers to provide a source of carbohydrates for the parasitoids.

Five different experiments were conducted on the sprayed plants: 1, 3, 8, 21 and 30 days after treatment. Mortality was evaluated (ABBOTT mortality %) by counting the number of dead insects at 24, 48 hours and 7 days for parasitoids exposed to 2 sprayed leaves. Fertility assays were conducted with surviving females from the 1 and 3 days experiment (Fig 1). Female parasitoids were mated and then kept with approximately 30 *P. citri* individuals for 24 hours. After the offspring emerged, the number of parasitoids was recorded. When appropriate, data were transformed $\sqrt{x+0.05}$ before means separation. Analysis of variance (ANOVA) was conducted in order to analyse the results (PROC GLM, SAS institute 1998). Significant differences between means were determined by Duncan's Multiple Range Test, with a 95% level of significance.

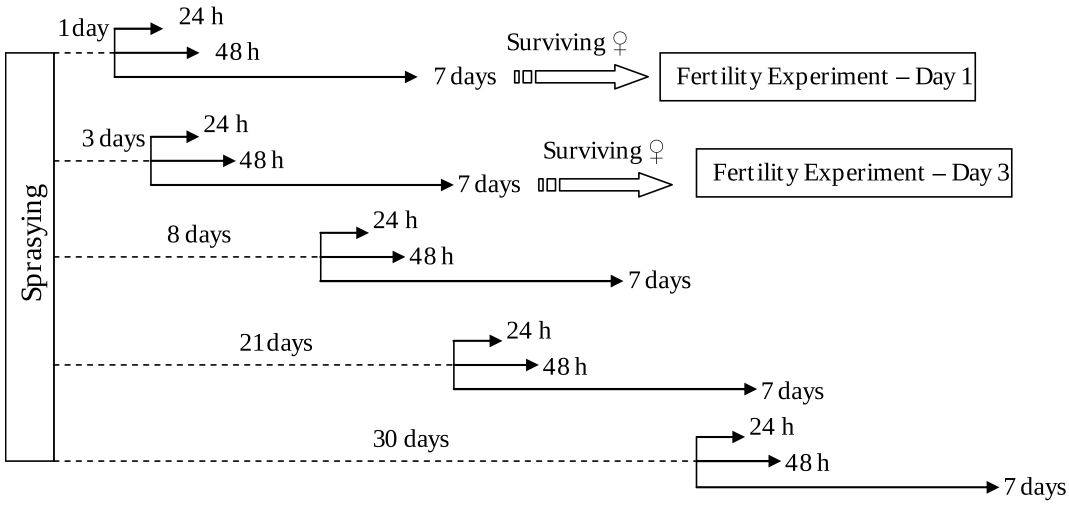

Figure 1. Mortality and fecundity trial scheme. Mortality evaluation at 24, 48 hours and 7 days for parasitoids exposed to sprayed leaves. Persistence evaluations conducted 1, 3, 8, 14 and 31 days after treatment.

Results

Except for carbosulfan, the pesticides tested showed low toxicity and persistence. The high toxicity of carbosulfan residues along with its persistence, with effects lasting for up to 3 weeks, was similar for both parasitoid species. Carbosulfan caused 100% mortality amongst encyrtids within 3 days of treatment.

Residues of the insect growth regulators buprofezin and pyriproxifen were, together with mineral oil, the least toxic substances for the parasitoids that we tested. IRG's have been considered toxic for coccinellid predators (Garrido, 1995; Ripollés, 1997; Grafton-Cardwell ang Gu, 2003). Buprofezin and pyriproxifen have been respectively considered toxic and highly toxic for *Cryptolaemus montrouzieri*, a predator of *P. citri* (Ripollés, 1992; Garrido, 1999), however they showed low toxicity for the two parasitoid species tested. Mendel *et al.* (1994) affirmed that insect growth regulators are non-toxic for these hymenopteran parasitoids. Low toxicity was shown by mineral oil on both parasitoids, since pests must be covered with a film of oil to be killed (Davidson *et al.*, 1991). Buprofezin and pyriproxifen are used in citrus crops to control armoured scale insects, *Parlatoria pergandei*, *Aonidiella aurantii* and *Cornuaspis beckii*, and buprofezin is also used against whiteflies. As armoured scales are key pests in IPM programs in Spain, the impact of these pesticides on both of the parasitoids tested is crucial.

Abamectin is a pesticide that is used to control leafminers and mites in late spring and summer. In our studies, the high toxicity but low persistence observed during the first evaluation, mainly on *A. pseudococci* should be kept in mind when releasing parasitoids in the field.

Chlorpyrifos is the most commonly used pesticide for controlling mealybugs and armoured scales in citrus orchards. In our experiment, *L. dactylopii* mortality due to chlorpyrifos was higher than *A. pseudococci*, but both with a low persistence. However, *L. dactylopii* has sometimes been cited as a hymenopteran that is extraordinarily tolerant to organic phosphate pesticide (Bartlett, 1963; Meyerdick *et al.*, 1979). Chlorpyrifos low persistence, constitutes a great advantage for its application in the field.

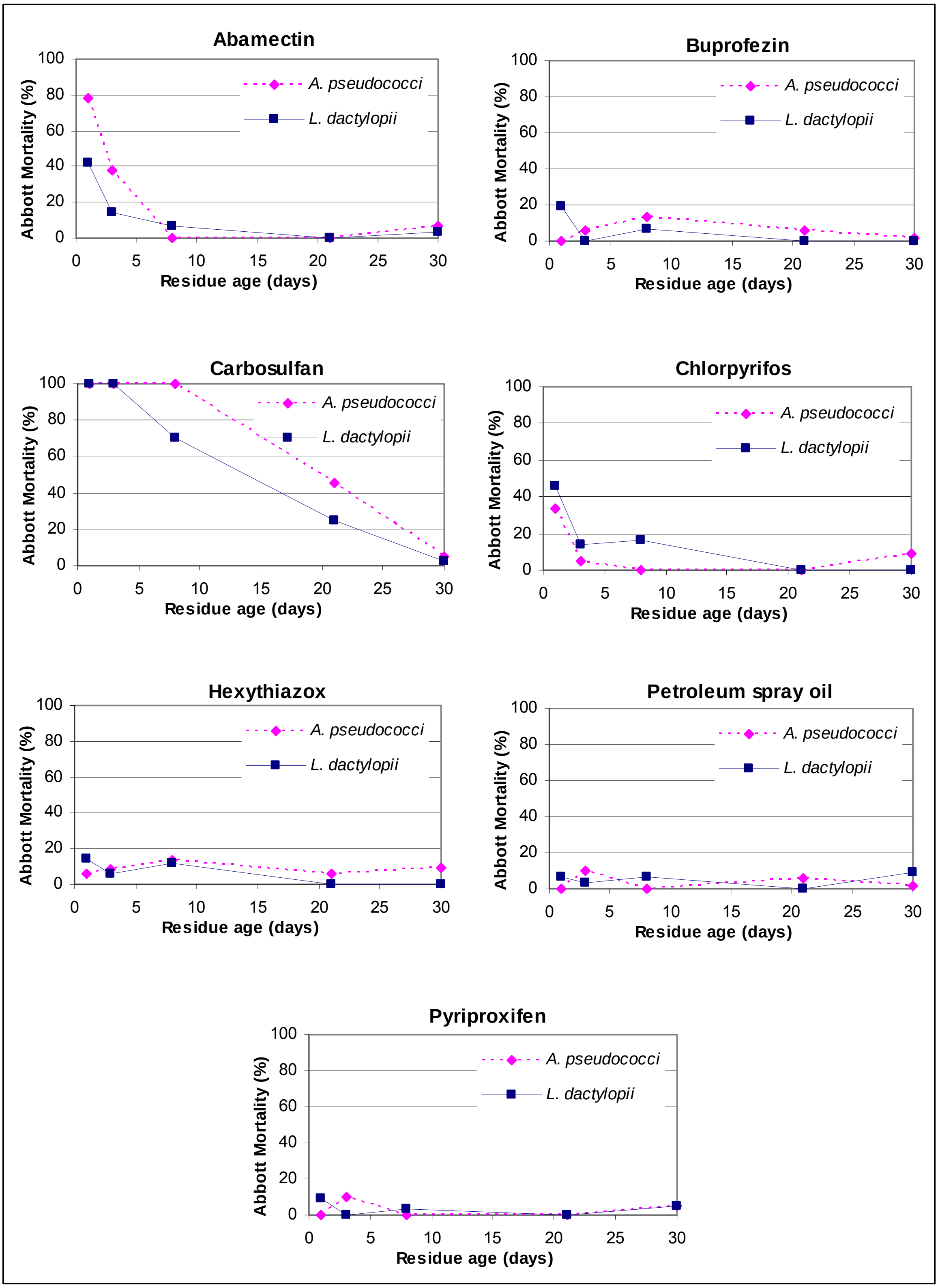

Figure 2. Persistence of pesticide residues on *A. pseudococci* and *L. dactylopii* after 7 days on sprayed Clemenules mandarin leaves.

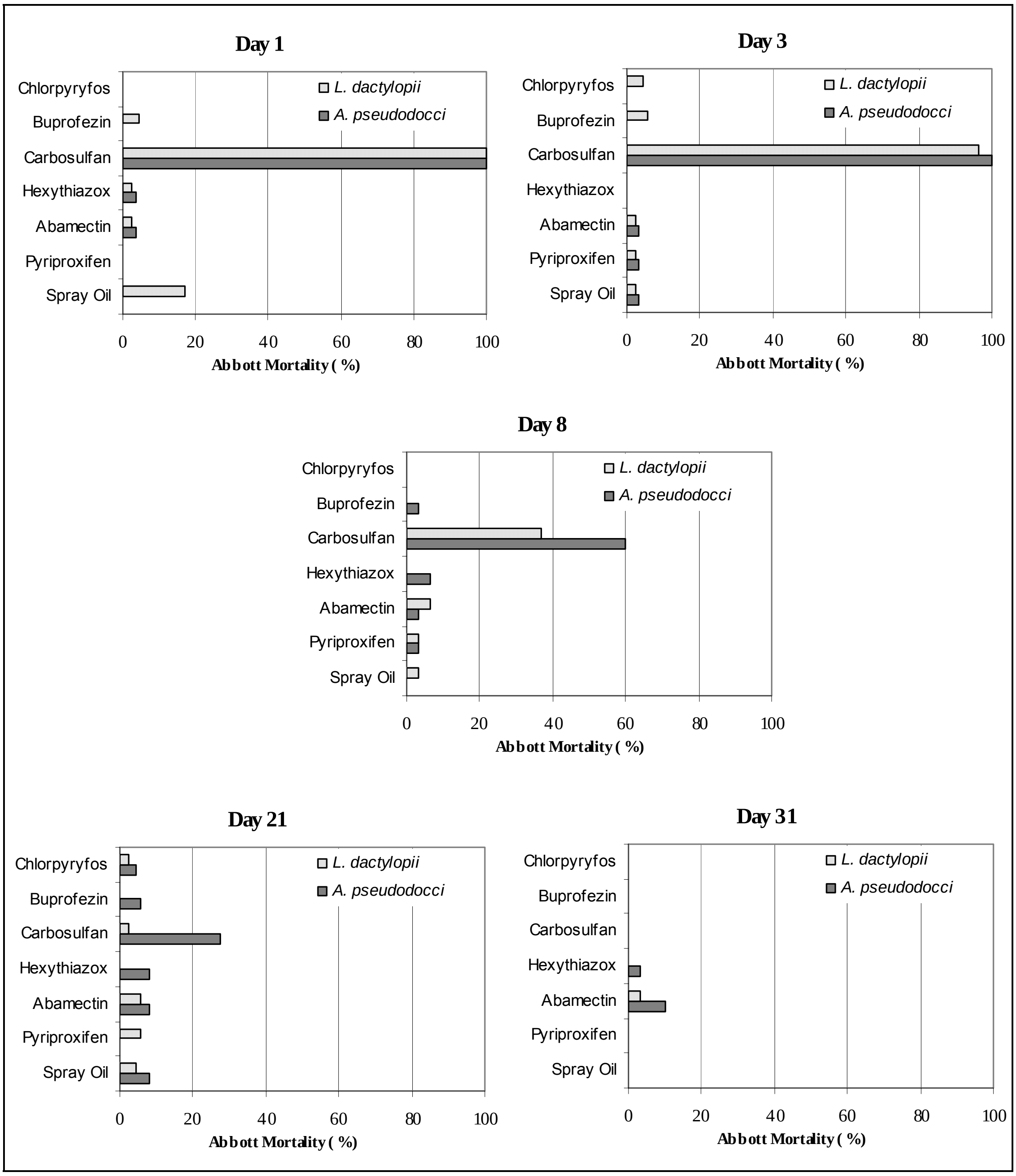

Figure 3. Abbott Mortality (%) of the two parasitoids after 24-hour exposure to sprayed Clemenules mandarin leaves.

The number of *L. dactylopii* progeny was not adversely affected by any of the pesticides tested in the extended-laboratory experiment confirming findings from similar experiments (Rothwangl *et al.*, 2004). However, buprofezin and abamectin residues reduced the progeny production by *A. pseudococci* (Table 2). It was not possible to evaluate the effect of carbosulfan on progeny production because no female parasitoids survived the toxicity experiments with 1 and 3-day-old residues. The same happened with 1-day-old abamectin residues.

Table 2. Progeny per female and day of female parasitoids after 7-day exposure to sprayed Clemenules mandarin leaves.

	Age residue - 1 day		**Age residue - 3 days**	
Treatment	*A. pseudococci*	*L. dactylopii*	*A. pseudococci*	*L. dactylopii*
Abamectin	–	6.43 ± 1.23 a	1.17 ± 0.75 b	8.67 ± 3.18 a
Petroleum Spray Oil	5.22 ± 0.71 ab	10.50 a	7.00 ± 1.46 a	6.00 ± 1.58 a
Buprofezin	3.68 ± 0.2 b	5.55 ± 1.01 a	6.00 ± 1.26 a	10.50 ± 2.49 a
Carbosulfan	–	–	–	–
Chlorpyrifos	5.14 ± 0.98 ab	9.17 ± 2.33 a	4.50 ± 1.82 ab	9.00 ± 2.54 a
Hexythiazox	5.00 ± 0.65 ab	7.17 ± 1.20 a	6.50 ± 1.91 a	5.33 ± 1.63 a
Pyriproxifen	7.25 ± 0.91 a	4.94 ± 1.35 a	7.40 ± 1.60 a	9.83 ± 2.04 a
Control	7.20 ± 1.41 a	6.62 ± 0.96 a	7.67 ± 1.52 a	6.40 ± 1.69 a

Means within a given column followed by the same letters did not differ significantly. Duncan's Multiple Range Test $P<0.05$

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Treatment thresholds for the Citrus Mealybug *Planococcus citri* (Hemiptera: Pseudococcidae) based on the relationship between male's abundance and fruit infestation

María Teresa Martínez-Ferrer[1], José Luis Ripollés Moles[1] and Ferran Garcia-Marí[2]
[1] *IRTA Amposta. Carretera de Balada, Km 1. E-43870 Amposta (Tarragona-Spain)*
[2] *Institut Agroforestal Mediterrani - Universitat Politècnica de València (Spain)*

Abstract: Citrus Mealybug is a polyphagous pest that on citrus attacks mainly navel varieties. Cosmetic damage on fruit due to large citrus mealybug colonies and honeydew and sooty mold *Capnodium* spp., chlorotic spots, and rind hypertrophy, are commonly observed on fruits at harvest. This causes economic loss for the citriculture export industry in Spain. Treatment thresholds have been determined based on these injuries observed at harvest, and depending on looses the grower is ready to take up office. For instance, if 8 to 12% of fruits with symptoms were accepted, then treatment threshold would be 15 to 20% of attacked fruits. No injuries were observed when population on fruit during the season was under 5% of attacked fruits, so this would be the lower treatment threshold. A positive relationship was found between all the male flights and the population of *P. citri* on the fruits, so traps with pheromone detected the abundance of population, both for number of insects per fruit and percentage of attacked fruits. This relationship was delayed in time, and there were from one to two months of difference between the male flight considered and the population on fruit, depending on the season. First male flight (May) correlated with population under the calyxes of the fruits in July; second (June-July) and third (August) male flights were related to population on fruit in August, and third and forth (September) male flights were correlated with fruit population in September.

Key words: citrus mealybug, treatment thresholds, male's abundance, integrated control

Introduction

The Citrus Mealybug, *Planococcus citri* (Risso) is a poliphagous and cosmopolitan insect pest. It damages many outdoor crops in the tropics and subtropics and also greenhouse crops in temperate regions. In citrus, it is an occasional but serious pest, which mainly attacks Navel oranges and lemons. In cases of major infestation, fruit is rendered non commercial as a result of both the sooty mould growing on the honeydew excreted by *P. citri* and the loss of colour and hypertrophy that result from feeding (Franco et al., 2000, Martínez-Ferrer et al., 2003).

In Spain, five flights are detected in the months of May, June-July, August, September and November. Each flight lasts between one and two months. The different flights identified therefore imply five generations of *P. citri* per year in the citrus groves in the area studied. (Martínez-Ferrer et al., 2003).

The sex pheromone of *P. citri* has been used for monitoring citrus Mealybug, but there are no estimations of the relationship between number of males captured per trap and percent of fruit infestation in a particular citrus grove (Ortu and Delrio, 1982, Rotundo et al., 1979, Moreno et al. 1984).

Material and methods

The study was carried out over a 5-year period (1992-1995 and 1998) in nine unsprayed 0.1-0.5 ha orange groves (*Citrus sinensis* (L) Osbeck, var Navelina and Washington Navel) located in Tarragona and Castellón (Spain).

Population under the calyx was estimated by sixty-six samplings made during May, June and July. Each sampling consisted of randomly selecting 8 fruits per tree from 25 trees per grove. These fruits were then taken to the laboratory and examined using a stereoscope microscope. All *P. citri* on the fruit, mainly located under the calyx, were counted. Citrus mealybug on the fruit was studied from July, when *P. citri* become visible on fruit, until December. A total of one hundred and thirty two samplings were made, consisted of 8 randomly chosen fruits per tree picked from 25 trees per grove. In the field, the number of individuals, belonging to third instar, young female and female with eggs stages of development was counted on these fruits.

Male flights were monitored with traps 3 pheromone traps per grove, settled out in the groves from 1st January. Each trap consisted of a yellow wooden frame containing a piece of glass coated with a sticky spray (Souverode) and baited with a pheromone capsule (Inagra). Pheromone capsules were changed every six weeks and the pieces of glass were checked on a weekly or fortnightly basis, according to the season. *P. citri* males captured on 6 x 10-cm glass surfaces were then counted under a microscope. 22 one-year periods were sampled.

Results and discussion

We have tried to correlate *P. citri* population along the year on the fruit with the observed injuries at harvest. The best correlation was found to the maximum attack in the year, normally occurring on August and September. The relationship between percentage of maximum attacked fruits with the percentage of injuries at harvest was defined by the equation: $y=0.7911\ x - 3.5608$ ($n=14$; $R=0.53$; $P=0.05$). For instance, if 8 to 12% of fruits with symptoms were accepted, then treatment threshold would be 15 to 20% of attacked fruits. No injuries were observed when population on fruit during the season was under 5% of attacked fruits, so this would be the lower treatment threshold. The treatment threshold was related to the population under the calyx on July, being 70 % of invaded calyxes corresponded to 20% of attacked fruits on August or September (Martinez-Ferrer, 2003). So, these were the thresholds we used for male’s relationships.

For all the males' flights we have stated the existence of some correlation with the population of *P. citri* on the fruits, which indicates that the males' traps detect the abundance of population, in number of insects per fruit and in the percentage of infested. Nevertheless, this relation expresses in a relatively brief period of time, just between one and two months, from the captures in traps up to the assessment of fruit population.

The males' first flight takes place during May. This flight was closely correlated with *P. citri* population observed under the calyx of the fruit on July, not only for the number of insects for fruit under the calyx but also with the percentage of calyxes occupied by *P. citri*. This flight is usually slightly abundant; nevertheless, captures of few males in this flight involve already the existence of important population of *P. citri* under the calyx on July (Fig. 1 and 2).

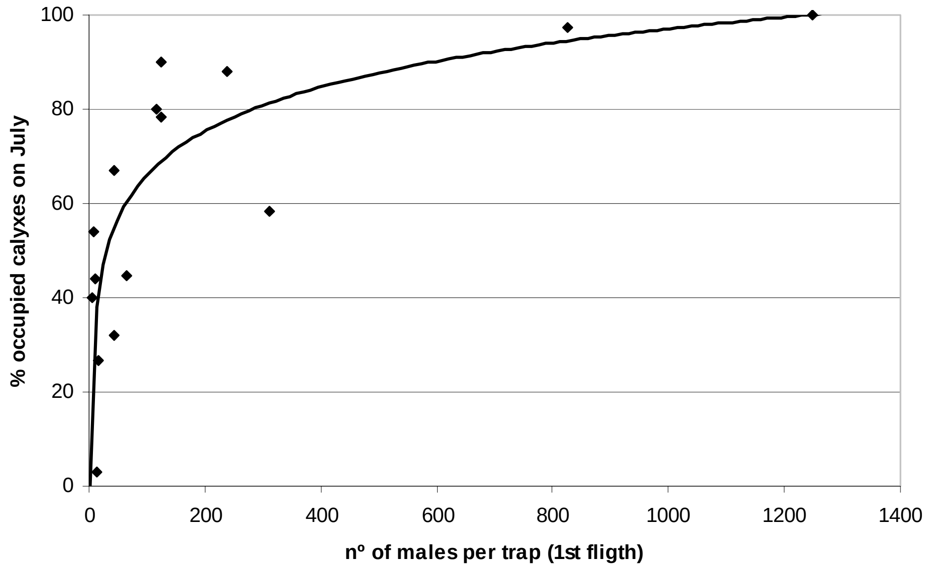

Figure 1. Relationship between nº of males per trap in the first flight and the percentage of occupied calyxes by *P. citri* on July (y=13,06 ln(x)+3,98; R=0,79; n=15; P=0,000)

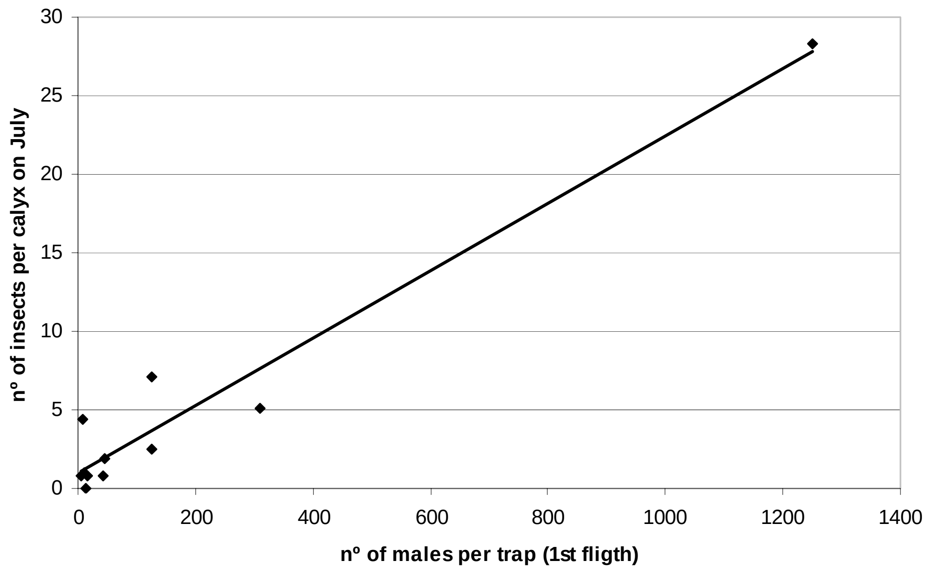

Figure 2. Relationship between nº of males per trap in the first flight and the number of mealybugs per calyx on July (y=0,0215 x+0,9913; R=0,97; n=11; P<0,0001)

The males' second flight takes place between June and July. This flight is, together with the third one, the most abundant of the year. This flight was closely correlated to *P. citri* population observed on the fruit during the first fortnight of August. This relationship concerns not only to the number of insects per fruit, but also to the percentage of attacked fruits (Fig. 3 and 4).

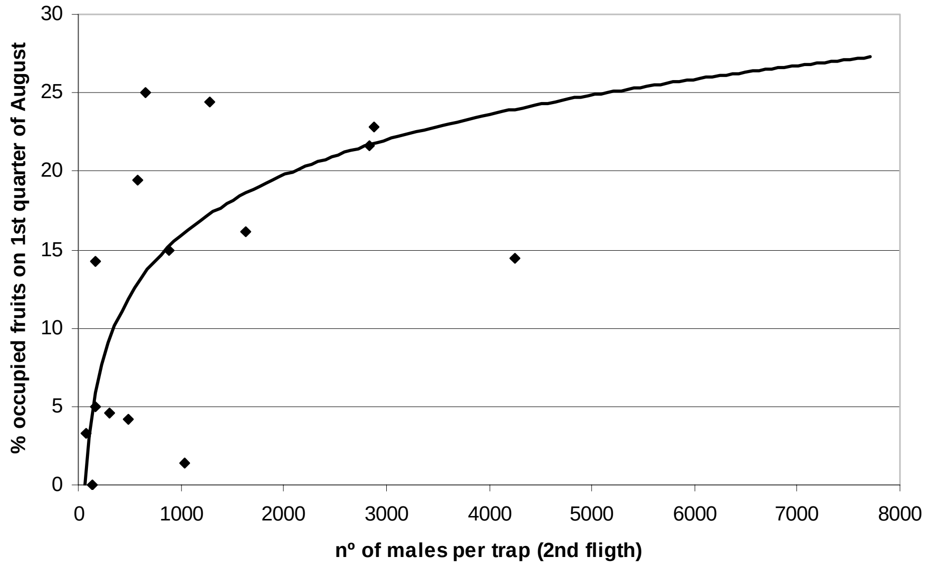

Figure 3. Relationship between nº of males per trap in the second flight and the percentage of occupied calyxes by *P. citri* on the 1st fortnight of August (y=5,56 ln(x)-22.54; R=0,72; n=16;P=0,0018).

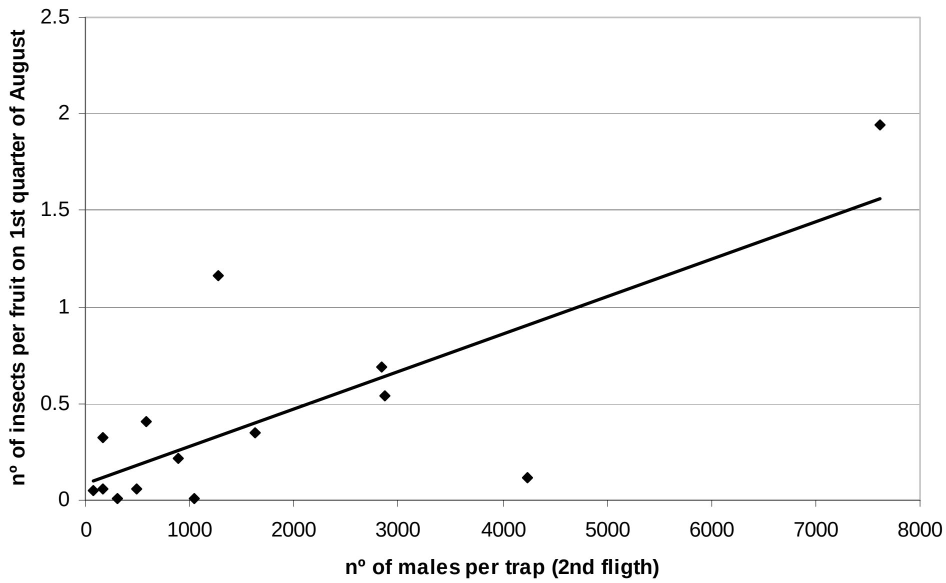

Figure 4. Relationship between nº of males per trap in the second flight and the number of mealybugs per fruit on the 1st fortnight of August (y=0,0002 x+0,0892; R=0,75; n=14; P=0,002).

The males' third flight of *P. citri* takes place during August. The males captured in this flight were correlated to population on fruits in the field in the second fortnight of August and in the second fortnight of September. These relationships between the third male flight and population on fruits occurred for the number of insects and for the percentage of infested fruits (Fig. 5 and 6).

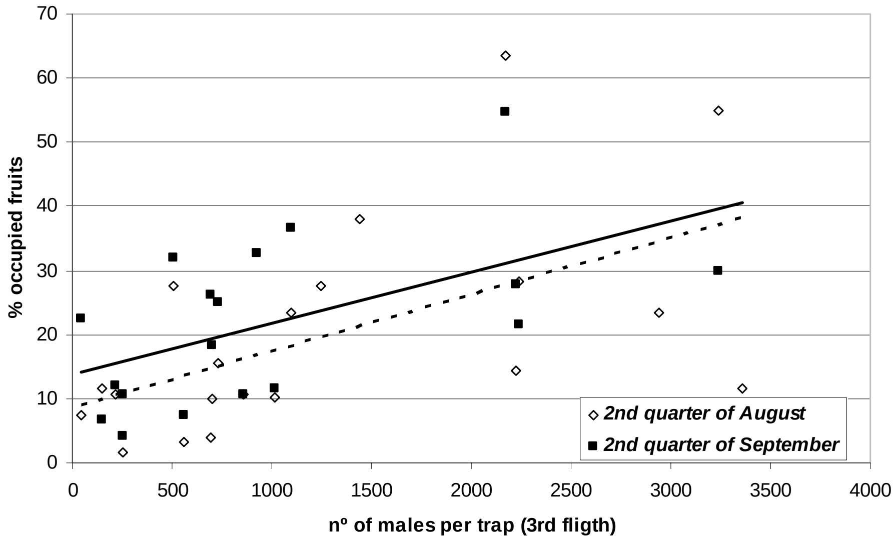

Figure 5. Relationship between nº of males per trap in the third flight and the percentage of occupied fruits by *P. citri* on 2nd fortnight of August (y=0.009x+8,5797; R=0,56; n=20;P=0,01) and 2nd fortnight of September (y=0.008x+13.081; R=0,55; n=18;P=0,02).

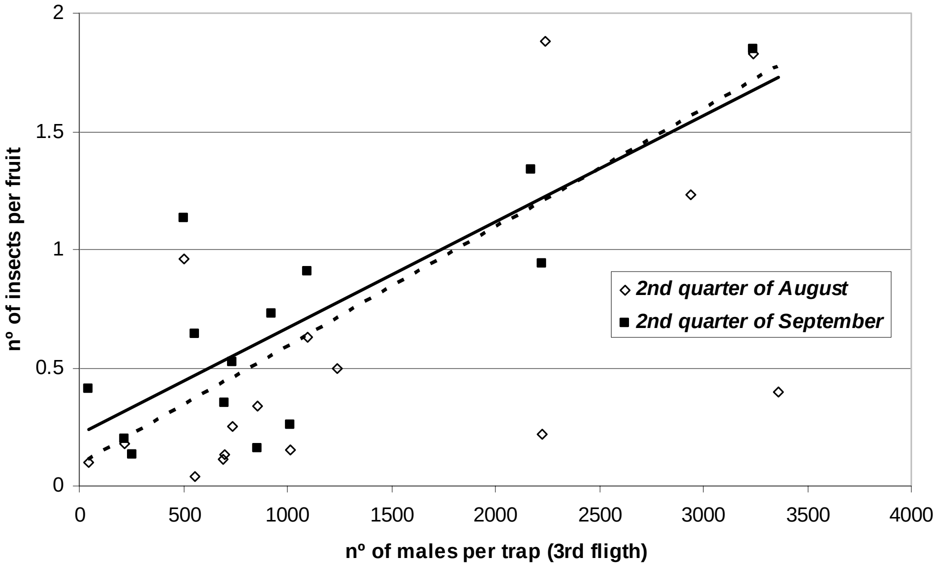

Figure 6. Relationship between nº of males per trap in the third flight and the number of mealubugs epr fruit on 2nd fortnight of August (y=0,0005 x+0,0894; R=0,48; n=17; P=0,05) and 2nd fortnight of September (y=0,0005 x+0,215; R=0,81; n=14; P=0,0005).

The males' fourth flight takes place on September. These captures were related to the population in field on the fruit of the second fortnight of September and first fortnight of December, to the number of insects per fruit and to the percentage of infested fruits (Fig. 7 and 8).

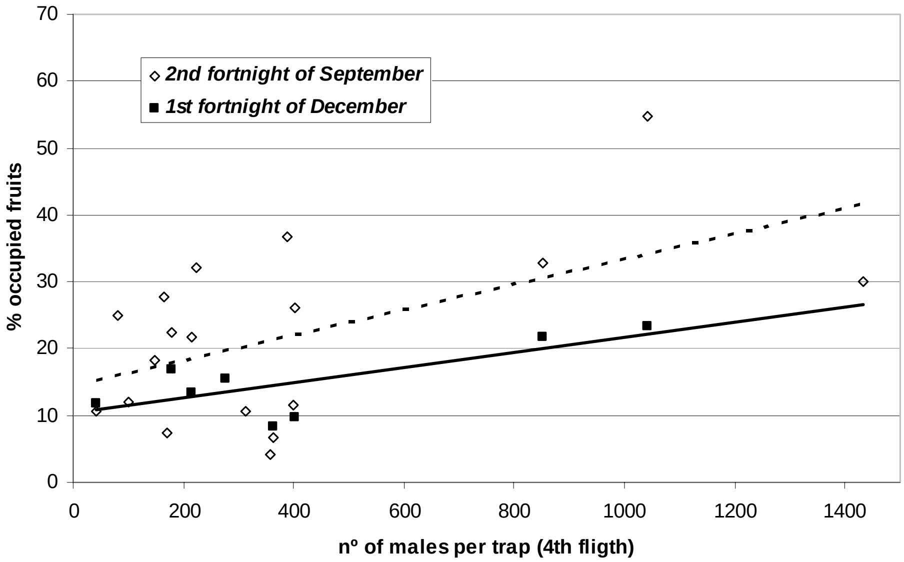

Figure 7. Relationship between nº of males per trap in the forth flight and the percentage of occupied fruits on 2nd fortnight of September (y=0,019x+14,498; R=0,53; n=18; P=0,02) on 1st fortnight of December (y=0,011x+10,271; R=0,53; n=18; P=0,02).

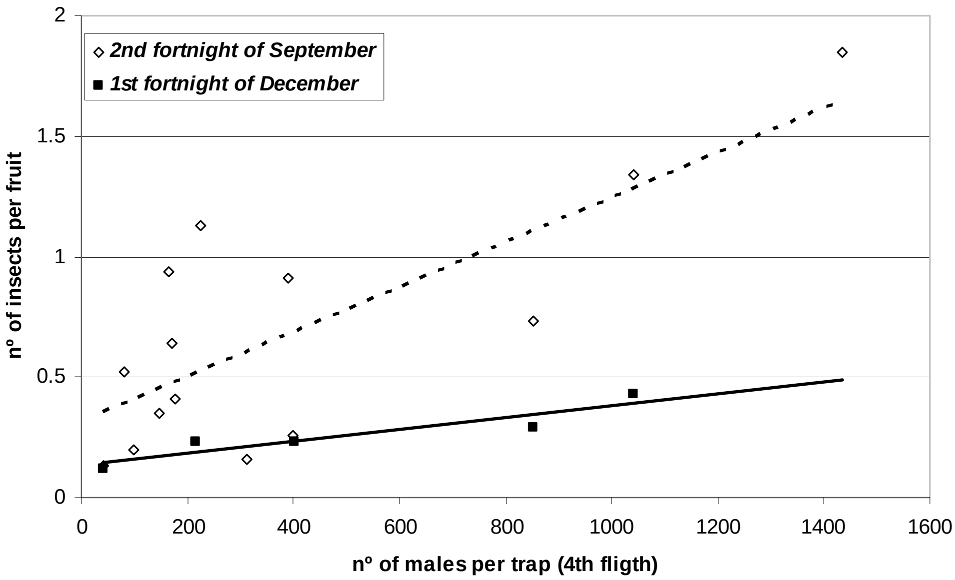

Figure 8. Relationship between nº of males per trap in the fourth flight and the percentage of occupied fruits on 2nd fortnight of September (y=0.0009x+0.31626; R=0,77; n=14;P=0,001) on 1st fortnight of December (y=0.0003x+0.1331; R=0,93; n=5;P=0,02).

In conclusion, the treatment threshold based on the first male flight would be 100 males per trap or 5 males per trap and day, corresponding to 70% of infested calyxes. The treatment threshold based on the second male flight would be 2000 males per trap or 99 males per trap and day, corresponding to 20% of infested fruits in August. The treatment threshold based on the third male flight would be 1250 males per trap or 62 males per trap and day, and 750 males per trap or 37 males per trap and day, corresponding to 20% of infested fruits in August and September respectively. And finally, the treatment threshold based on the fourth male flight would be 300 males per trap or 15 males per trap and day, corresponding to 20% of infested fruits in September (Table 1).

Table 1. Relationship between males of *Planococcus citri* on traps and population infestation on fruits.

Males per trap	**Population under the calyx (70% occupied calyces)**		**Population on fruit (20% infested fruits)**			
	July		**August**		**September**	
	Total males	Maximum (males per trap and day)	Total males	Maximum (males per trap and day)	Total males	Maximum (males per trap and day)
1st flight (May)	100	5				
2nd flight (June – July)			2,000	99		
3rd flight (August)			1,250	62	750	37
4th flight (September)					300	15

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The adoption rate of biological control of *Icerya purchasi* Maskell in Mazandaran, Iran

A. Papzan[1], H. Vahedi[2]
[1] *Extension Dep. Agri. College, Razi Uni., Kermanshah, Iran; h_papzan@yahoo.com*
[2] *Plant Protection Dep. Agri. College, Razi Uni., Kermanshah, Iran; Hassan_vahedi@yahoo.com*

The biological control of *Icerya purchasi* Maskell, cottony cushion scale, as a citrus pest in the biologically-based IPM has been going on in Mazandaran for a long time, using *Rodolia cardinalis* (Mulsant) as a biological agent. Although this program was initially unsuccessful and eventually improving to control of the target pest, there is no assessment of the adoption of this control method. Random deep interview with citrus growers, as a qualitative method, was the methodology used to estimate adoption rate of this control method, in western part of Mazandaran. In this study, the SPSS analyzed data show only 20% of citrus growers adoption (n=155). The most effective activities which helped to increase Mazandaran growers' adoption rate, on this matter, will be discussed.

Mediterranean Fruit Fly

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Parasitism of *Diachasmimorpha tryoni* (Hymenoptera: Braconidae) on the host *Ceratitis capitata* (Diptera: Tephritidae) under Mediterranean temperatures

Eva Garzón-Luque[1], Francisco Beitia[2], J. Vicente Falcó[3]
[1] *Instituto Agroforestal del Mediterráneo, Universidad Politécnica de Valencia. Camino de Vera,14. Apdo. Of. 46022-Valencia, Spain. e-mail: evgarlu@etsia.upv.es*
[2] *Instituto Valenciano de Investigaciones Agrarias (IVIA), Centro de Protección Vegetal y Biotecnología. Apdo. Of. 46113 Montcada (Valencia), Spain. e-mail: fbeitia@ivia.es*
[3] *Instituto Cavanilles de Biodiversitat, Universidad de Valencia. Apdo. of. 22085, 46071-Valencia, Spain. e-mail: j.vicente.falco@uv.es*

Abstract *Ceratitis capitata* (Wiedemann, 1824) is an endemic citrus pest since the 1930s in the East Coast of Spain, where biological control against the medfly was attempted in those first years without any success. In 2003 the Valencian Institute of Agricultural Research (IVIA) began a project to study new possibilities of use of Hymenoptera parasitoids in order to include them in Integrated Pest Management strategies against the Medfly in the Mediterranean Coast of Spain. With this aim the braconid wasp *Diachasmimorpha tryoni* (Cameron, 1911) was imported from Hawaii and a laboratory rearing of this species is in progress in the IVIA facilities. *D. tryoni* has a high parasitism and emergence rates at 25°C and at 21-25°C. The critical temperature of 30°C prevents the emergence of a new generation of parasitoids, but females are able to parasitize the host. When these high temperatures (25-30°C) are applied only for a few hours the parasitoid development is completed and adult emergence occurs. These results can explain the potential adaptability and survivorship of the parasitoid in the Mediterranean high temperatures when field releases are carried out to control the Medfly summer populations. Consequently, parasitism can be successful in the warmer months of the Mediterranean Spanish area.

Key words: *Ceratitis capitata*, *Diachasmimorpha tryoni*, Medfly, parasitoid, biological control, temperature, parasitism rate, emergence rate.

Introduction

Diachasmimorpha tryoni was imported from Hawaii in 2002 by the Valencian Institute of Agricultural Research in order to attempt the classical biological control against *Ceratitis capitata* in Spain (Falcó *et al.*, 2003). This parasitoid is being used in several successful control programs in Central and South America. In Spain it was imported in 1932, but difficulties in transport and rearing processes made the field control program to fail.

The braconid *Diachasmimorpha tryoni* is a solitary koinobiont parasitoid that begins its life cycle laying the eggs on the third instar larvae of the Medfly, then the parasitoid complete its larval and pupae development inside its host. Finally a new parasitoid adult emerge from the puparium. Several authors have discussed some aspects of the biology of *D.tryoni* including references to temperature and rearing parameters (Ramadan *et al.*, 1989; Wong *et al.*,1990; Hurtrel *et al.*, 2001; Falcó *et al.*, 2003) but it is necessary to know thermal requirements of parasitoid and host as well as its effects on parasitism in order to understand the best conditions for release actions as a part of control programs in the Mediterranean area.

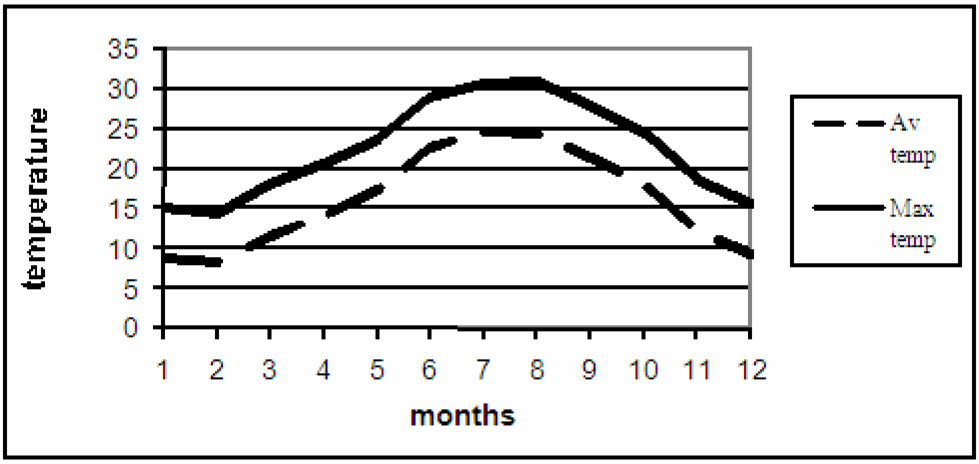

Figure 1: Temperatures of the Citrus area of Valencia (Source: National Institute of Meteorology (INM))

In the Mediterranean east coast of Spain the average temperature is 20-25ºC along four months in summer but the average of maximun temperatures can reach up to 30ºC (Figure 1).

A lack of information about parasitism of *D. tryoni* on the Medfly at 30ºC has been detected as well as the evaluation and comparison of the effect of potentially high Mediterranean temperatures on emergence and parasitism rates. These data can help to assess how temperature affects the possibilities for field releases of *D.tryoni* as a potential biological control agent of the Medfly in the Spanish Mediterranean area along spring and summer periods.

Material and methods

Test temperatures

The tempeatures of the assays were the following ones: 21-25ºC range when the parasitoid is active, and the Medfly attacks the citrus fruits, 25ºC optimal temperature to development and emergence rates for the parasitoid (Hurtrel *et al*., 2001), 30ºC maximal temperature for the development of the parasitoid progeny (Hurtrel *et al*., 2001), and 25-30ºC variable temperature to confirm the parasitoid survivorship subjected to critical high temperature for four hours.

Mass rearing

The Medfly and parasitoid are stablished under rearing conditions in the laboratory of Entomology at the IVIA. The rearing of *Ceratitis capitata* is based in the method proposed by Albajes and Santiago-Álvarez (1980). The laboratory conditions are: fotoperiod of 16:8 (L:D), relative humidity of 75±5% and temperature of 26±1ºC in the light period and 21±1ºC in the darkness period.

The parasitoid rearing was initiated in 2002 with the population that was imported from the laboratory of the U.S Pacific Basin Agricultural Research Center (USDA-ARS- Hawaii). The third instar larvae of he Medfly are offered to adults of *D. tryoni* for 24 hours, then the host larvae are collected and continue the development forming the puparia. 18-20 days after parasitism the new generation of *D. tryoni* emerges from the host puparia (Falcó *et al*., 2003).

Methodology
The experiments were based on solitary parasitoid females previously mated. 20 Medfly larvae were offered to parasite during 24 hours in parasitism cages, daily along its life. Each day the potential parasitized larvae were moved to emergence boxes.

After development period, all the Medfly and parasitoids emerged as well as their pupariums were noted. Closed puparia were dissected to check inside them the presence of preimaginal instars or not emerged adults both of parasitoids and flies. Also the superparasitism cases were evident. The parameters that are studied were the following: Total emergence rate, Daily emergence rate, Total parasitism rate, Daily parasitism rate.

Statistical analyses
The statistical analyses at 3 levels of temperature (21-25ºC, 25ºC and 25-30ºC) were made to the studied biological parameters with the statistical package S.A.S 9.1. Emergence and parasitism have binomial distribution so a logistic regresion with the statistical package SAS was performed. These parameters are compared between temperatures with contrasts and with a significant level of 0.05.

Results and discussion

The parasitism rate is evaluated with puparia from which emerge adult parasitoids and with closed puparia including preimaginal instars and not emerged adults of the parasitoid (Figures 2 and 3). The results are shown in the graphic below. At 25ºC and 21-25ºC there is the higher percentage of parasitism (38.40±2.51% and 38.37±3.05% respectively). These temperatures are included in the range of temperatures of a high activity of the Medfly.

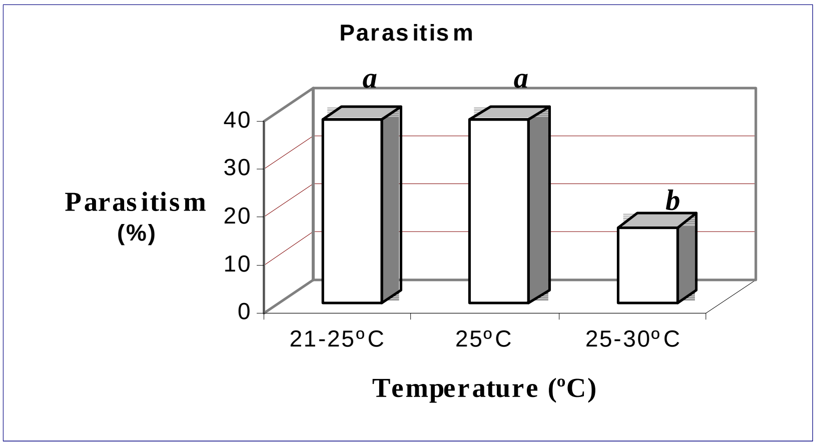

Figure 2: Parasitism rates of *D. tryoni* at different temperatures.

The daily parasitism (Figure 3) is irregular until the second week of the female age when it begins to decrease.At 25-30º the line tendency is different with a 50% of parasitism peak in the middle of female life and posterior very low parasitism; but this parasitism rate and the emergence (Figures 4 and 5) could assure the parasitoid population at the higher variable temperatures.

The highest total emergence rate is reached at 25ºC (28,74 ±1,81) but there are not statistical differences with 21-25ºC. The emergence of females is higher at the lowest variable temperatures but it shows similar statistical differences as before. The emergence at 25-30ºC is clearly statistical different to other tested temperatures.

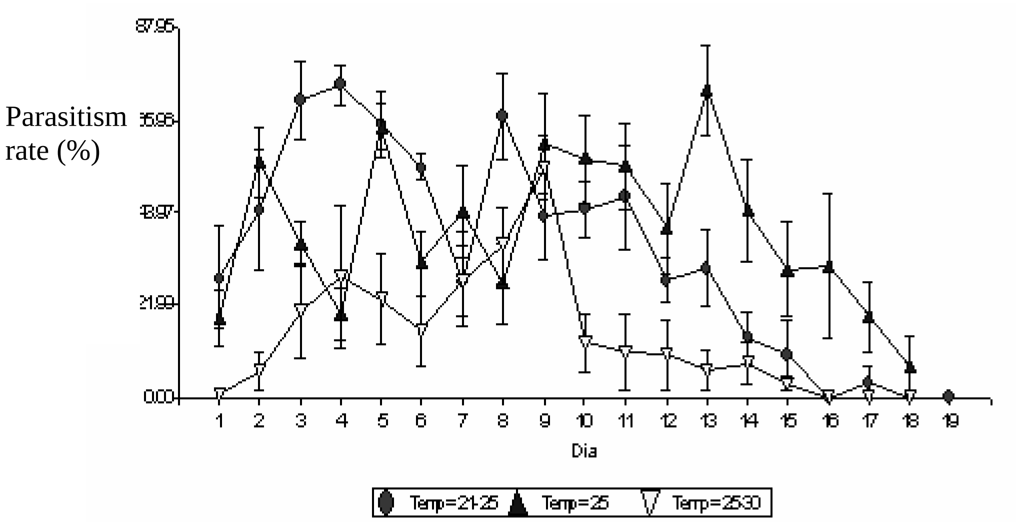

Figure 3: Daily parasitism of *D. tryoni* at different temperatures.

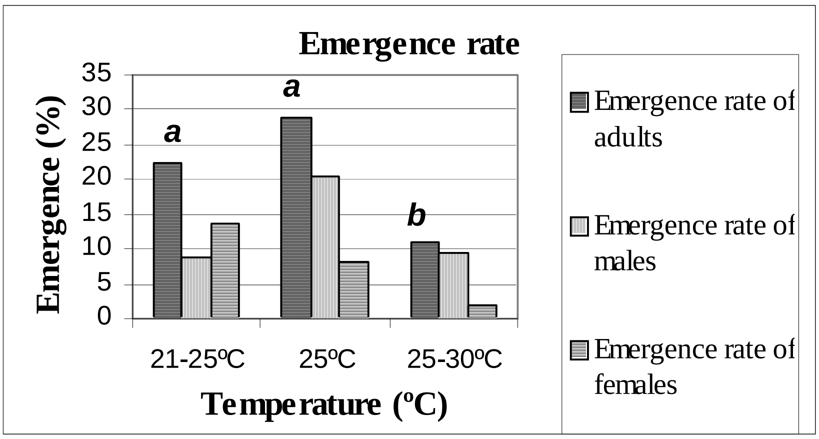

Figure 4: Emergence rate of *D. tryoni* at different temperatures.

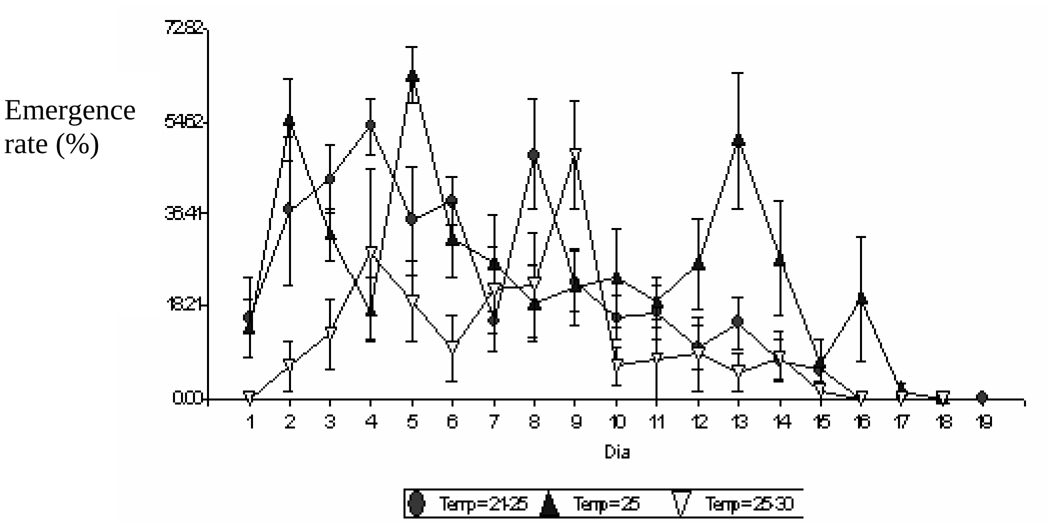

Figure 5: Daily emergence of *D. tryoni* at different temperatures.

The daily emergence (Figure 5) shows the same response at the different temperatures in relation with the daily parasitism rates.

The tested temperature of 30ºC is the maximal temperature that prevents the emergence of the parasitoid *Diachasmimorpha tryoni* (Hurtrel *et al*., 2001). Parasitism was tested at this temperature but, according to literature, no parasitoid emergence ocurred. In this test a total of 1066 closed puparia has been dissected to check the parasitoid preimaginal instars or not emerged adults inside them. 52% of the examined closed puparia include at least one first instar larvae of the parasitoid. So although there is no emergence of the progeny but the female is actively searching for the host and parasitism exists at 30ºC. It could contribute to reduce Medfly population even at this maximal temperature.

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Parasitism of *Spalangia cameroni* (Hymenoptera, Pteromalidae), an idiobiont parasitoid on pupae of *Ceratitis capitata* (Diptera, Tephritidae)

Marta Pérez-Hinarejos, Francisco Beitia
Instituto Valenciano de Investigaciones Agrarias, Unidad Asociada de Entomología IVIA-CIB/CSIC, Apartado Oficial, 46113 Moncada (Valencia), Spain

Abstract: *Spalangia cameroni* Perkins, 1910 is a pteromalid hymenopteran, well known as a pupal parasitoid of flies belonging to different taxonomic families in the order Diptera, as Muscidae, Sarcophagidae and Anthomyiidae. This species is being used as a biological control agent against the housefly (*Musca domestica*) and the stable fly (*Stomoxys calcitrans*). In the family Tephritidae it was known as a parasitoid of *Anastrepha suspensa*, *Dacus cucurbitae* and *D. passiflorae,* but recently it has been described as a parasitoid of the Mediterranean Fruit Fly, *Ceratitis capitata*, in the Valencian Community (Spain). Due to the importance of that fruit fly species as a serious pest on citrus and fruit trees, it has been started the rearing and the biological study of *S. cameroni* in laboratory conditions, in order to know its ability to be used in the biological control of the medfly. Biological and parasitic parameters of the insect, as adult longevity, female fecundity and fertility, influence of host age and temperature on parasitism and female ability in searching the host buried in the ground, are being analysed.

Key words: *Ceratitis capitata*, pupal ecto-parasitoid, *Spalangia cameroni*, parasitic activity

Introduction

In the year 2003, a population of the parasitoid *Spalangia cameroni* Perkins, 1910 (Hymenoptera, Pteromalidae) was found parasitizing pupae of *Ceratitis capitata* (Wiedemann) in field: adults of the insect were obtained in the laboratory emerging from pupae of the medfly found in the field (Falcó et al., 2004; 2006). This was the first record in the world of *S. cameroni* as a parasitoid of *C. capitata*. It is a generalist pupal ectoparasitoid of several taxonomic families in the order Diptera, including Tephritidae: *Anastrepha suspensa* (Loew), *Dacus* sp., *D. cucurbitae* Coquillett and *D. passiflorae* Froggatt (Noyes, 2005), and also now *C. capitata*.

S. cameroni is being used in the biological control of some dipteran species as *Musca domestica* L. (the house fly) and *Stomoxys calcitrans* (L.) (the stable fly), which are a serious problem in animal farms in several countries like Denmark, USA, Australia, Costa Rica and Colombia (Steenberg *et al*., 2001; Geden y Hogsette, 2006). In our laboratory, in the last years, we have studied the biological parameters of *S. cameroni* on pupae of *C. capitata* in order to know possibilities in using this species as a biological control agent of the Medfly.

Material and methods

Laboratory rearing of S. cameroni

To keep a rearing of *S. cameroni* in laboratory conditions (Figure 1), we have developed a very simple method consisting in offering, twice a week, pupae of *C. capitata* (from our laboratory rearing) to adults of the parasitoid confined in a plastic cage (30x20x20 cm) with ventilation and a supply of water and honey to adults, in a climatic chamber (Light 16 h - 24±1ºC, 60-70% RH and Dark 8 h – 21±1ºC, 70-80% RH).

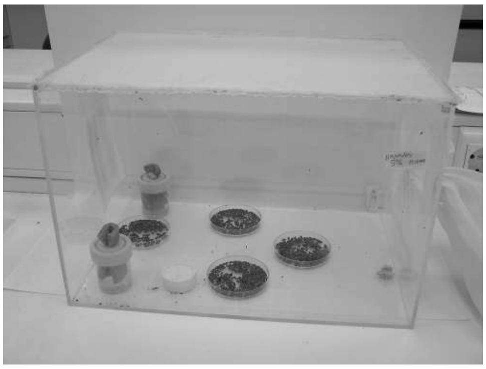

Figure 1. Laboratory rearing cage of *S. cameroni*

Parasitized pupae in plastic Petri dishes were kept in the same climatic chamber to evolve until parasitoid adult emergence.

Experiments on parasitism

Several bioassays have been carried out, in the same climatic chamber mentioned above, in order to know: adult longevity, female fecundity and fertility, killing activity on pupae of *C. capitata* and sex-ratio. Couples of *S. cameroni* were isolated on plastic cages (Figure 2) with a supply of water and honey and ten host pupae per day until female death.

Figure 2. Cages used in bioassays with couples of *S. cameroni*.

Other bioassays were developed to know the effect of temperature on the parasitic activity of the parasitoid. In a climatic chamber, with 60-70% RH and a photoperiod of 16:8 (L:D), several constant temperatures were studied: 15, 20, 25, 30, 35 and 40 ºC. Couples of *S. cameroni* (eight days-old) were confined in plastic cages (the same as in the previous experiments) and females allowed to oviposit during a period of 5 days. Two different experiments were performed: one to know fecundity of females and the other to detect fertility (emergence of adults).

The influence of host age on the parasitism of *S. cameroni* was also examined. A bioassay was performed comparing the effect of old and young pupae of *C. capitata* on parasitism. Experiments of choice and no-choice for the two types of host pupae were developed, using isolated couples of *S. cameroni* in plastic cages (Figure 2) and counting the fecundity and fertility of females, during a period of 5 days, on pupae of 1-3 days-old (young pupae) and pupae of 6-8 days-old (old pupae).

Finally, we have studied the ability of the females of *S. cameroni* in finding and parasitizing buried pupae of *C. capitata*, as it will be the real situation for the parasitoid in the field. For that, several bioassays have been developed with isolated couples in plastic cages (Figure 2) and putting pupae of *C. capitata* in plastic Petri dishes but buried 4 cm in soil.

Results and discussion

The rearing system described above has allowed us to keep a laboratory population of the parasitoid for more than 40 generations.

In Table 1, results on the parasitic activity of *S. cameroni* are shown. It has to be pointed out that the action of females of the parasitoid in killing pupae of the medfly is as important as the female fertility, as it had previously been described by Gerling & Legner (1968) on *Musca domestica,* and this is an interesting characteristic of the parasitoid to be considered in the control of the pest. And another important aspect is the sex ratio in the progeny, which is favourable to females.

Table 1. Data on parasitism of *S. cameroni* on pupae of *C. capitata*.

Adult longevity	18-20 days
α Fecundity	22-24 eggs/α
α Fertility	13-15 individuals/α
Killed pupae	12-15 pupae/α
Sex ratio	70% α

Results on parasitic activity (fecundity and fertility) of *S. cameroni* at different constant temperatures are shown in Table 2. At 20, 25 and 30 ºC females are able of put eggs on pupae of *C. capitata* and there is a complete immature development and emergence of adults of the parasitoid. However, at 15 and 35 ºC we found egg-laying on pupae but no adult emergence was detected. And finally, there was neither egg-laying nor adult emergence at 40 ºC. So these results indicates that *S cameroni* could parasitize *C. capitata* in our Mediterranean climatic conditions.

Table 2. Results on parasitism of *S. cameroni* at different constant temperatures.

Temperature	**Parasitic Activity**
20, 25 & 30 ºC	Egg-laying & adult emergence
15 & 35 ºC	Egg-laying & no adult emergence
40 ºC	Nothing

The bioassay on the effect of host age on parasitism showed that there was a slight preference for old-host pupae (choice test) but no significant differences were found and *S. cameroni* females parasitize old-host pupae as well young-host pupae in non-choice test.

Finally, in bioassays on parasitic activity on buried pupae, we found that females can find and parasitize the pupae buried in soil, but more experiments must be done in order to compare parasitic activity of *S. cameroni* on buried and not buried pupae. Nowadays more research is being developed on the parasitism of *S. cameroni* on *C. capitata* to know real possibilities in using this parasitoid as a biological control agent of medfly populations in our country.

Acknowledgements

Authors want to thank Sandra Santiago and Ignacio Tarazona for their collaboration in the maintenance of the laboratory rearing of *Spalangia cameroni*.

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Importance of ground-dwelling predators on controlling *Ceratitis capitata* in Spanish citrus orchards

C. Monzó[1], B. Sabater[1], J.L. García, A. Urbaneja[1], P. Castañera[2]
Unidad Asociada de Entomología IVIA (Instituto Valenciano Investigaciones Agrarias) - CIB (Centro Investigaciones Biológicas) del CSIC
[1] Centro de Protección Vegetal y Biotecnología (IVIA), Ctra. de Moncada a Náquera km. 4.5; 46113 Moncada, Valencia, Spain
[2] Departamento Biología de Plantas, CIB CSIC. C/ Ramiro de Maeztu 9, 28040 Madrid, Spain

There is little information on the role of the predaceous ground arthropods in citrus crops in Spain. In the present work we report on the activity-density of the predominant ground predators belonging to Araneae, Dermaptera, Staphylinidae and Carabidae. Four citrus groves in Valencia (Spain) were monitored by pitfall trapping across the diagonal in each orchard from August 2003 to April 2007. For the most abundant predators within Araneae (*Pardosa cribata* Simon), Dermaptera (*Forficula auricularia* L.) and Carabidae [(*Pseudophonus rufipes* (DeGeer)], it was assessed the capacity to prey on the *Ceratitis capitata* (Wiedemann) stages that can be found on the citrus ground (third instar larvae, pupae and teneral adults). Moreover, functional response parameters against this prey were also obtained. Finally, prey-detection tools (molecular markers and immuno-assays) are being developed to establish the role of those predators in the control of *C. capitata* under field conditions.

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Study of mass trapping devices to control Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), (Diptera: Tephritidae)

J. Domínguez Ruiz, F. Alfaro Lassala, V. Navarro Llopis, J. Primo Millo.
Centro de Ecología Química Agrícola-Instituto Agroforestal del Mediterráneo. Universidad Politécnica de Valencia. Camino de Vera s/n. Edificio 6C, 5ª planta. 46022 Valencia, Spain

Currently, the mass trapping technique against *Ceratitis capitata* (Wiedemann) is increasing notably as a control method in Spain. There are many studies that test different lures and traps for monitoring programs or mass trapping technique. In this work we have tested the efficacy of new developed traps and lures in citrus orchards (*Citrus reticulata* Blanco). Trials were conducted in Valencia, Spain. Six different types of traps were tested with Biolure (three component lure, ammonium acetate, trimethylamine and putrescine) and 5 traps with trimedlure (TML) dispensers. Moreover, a new mixture of n-methyl pyrrolidine with ammonium acetate has been tested. Results show important differences between type of traps and dispensers. In addition, proportion of males and females depending of trap type has been studied.

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pp. 136-141

Status of Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), and its control in Turkey

Naime Z. Elekçioğlu[1], Nedim Uygun[2], Refik Bozbuğa[1]
[1] *Plant Protection Research Institute, Ministry of Agriculture, 01321, Adana,Turkey*
[2] *Department of Plant Protection, University of Çukurova, 01330, Adana,Turkey*

Abstract*:* Turkey has a long history in biological and integrated control of citrus pests. Mediterranean fruit fly, *Ceratitis capitata* Wied. (Diptera: Tephritidae), is the only pest that causes significant damages in all citrus varieties (except lemon) when not chemically controlled. This pest is of main importance and increased its damage (population) over the last few years. The main reason for the increase of *C. capitata* damage is the alternative hosts of the pest, increasingly cultivated in or close to citrus orchards. The pest population increases on these fruit trees and moves to citrus in late summer or autumn.

The use of traps for monitoring the pest population and determining the proper time to spray and the attractants used during the spraying is important in the control of the pest. The only application recommended for this pest is bait spraying in Turkey. For the development of some more adequate control strategies, directed towards the integrated pest management, a better knowledge is necessary of the dynamics of the pest population. In this perspective, the determination of the population dynamics of *C. capitata* was done by pheromone traps in a Washington Navel orange orchard during 2005-2007. The traps were controlled weekly. The population was at its maximum level during June. We made comparison experiments of two attractants (Ziray and 94/5 coded attractant) by bait spraying method. The bait spray was applied on a 1-2m^2 area on south-east side of the tree and was a combination of attractants and an organic phosphate pesticide (Malathion) in 10-day intervals. Ziray gave better results than 94/5. In the presented paper, studies to determine the best attractant against the pest and the population dynamics of *C. capitata* in east Mediterranean region are reported.

Key words: Citrus, *Ceratitis capitata*, Attractant, Bait spray.

Introduction

About 75 percent of the citrus production in Turkey occurs in the east Mediterranean region centered around Adana province. Area under production has expanded rapidly in the recent years. This expansion is expected to continue over the next years. Turkey has a long history in biological and integrated control of citrus pests (Uygun et al.,1992; 1995 b). Due to the great success of non-chemical control methods, the use of pesticides in citrus was significantly reduced in the last years. However, *Ceratitis capitata* is the only pest that causes significant damages at all citrus varieties (except lemon) when not chemically controlled. It is considered in Turkey as one of the most important factors limiting fruit export markets. It is of main importance especially over the last 3 years. The only application recommended for the pest is bait spraying which is acceptable with IPM strategies. Up to now, at the studies in Turkey, different attractants developed for to use in *C. capitata* control were used with insecticides and the effective ones were detected (Zümreoğlu et al., 1987). According to this method native production attractant, Ziray or export attractants were used by mixing with Malathion 25 WP. Some complaints were being heard about the low attractness of Ziray which is used mostly against *C. capitata*. So some studies were done to find out an alternative attractant against Ziray and as a result native attractant with the code number 94/5 was found more effective than Ziray in the laboratory (Aydoğdu, 1998). This attractant was the combination of proteins (Sorghum, melas) and chemicals (Aminoacids, higroscobic inorganic materials and acids).

The objective of this study was to compare the effectiveness of two protein hydrolysates on *C. capitata*. Trials were conducted to define an effective bait for the development of bait spray techniques against the pest. Also the flight activity of *C. capitata* is reported.

Material and methods

Comparison of the efficacy of two attractants

The biological effectiveness of the attractants, combined with Malathion, were studied during 2003-2004 in Dörtyol (Hatay) by bait spraying tecnique. To determine the adult hatching, Jackson traps were hanged on the trees. Traps were laid out in a randomized block design with 3 rows containing 4 traps per row. Fifty trees were considered as a plot and the experiment were conducted with 4 replicates. According to the adults catched at the traps and the time of maturation of the fruits, the sprayings had been started. The bait spray (combination of attractants and an organic phosphate pesticide (Malathion)) was applied on a 1-2m^2 area on south-east side of the tree with 10-day intervals until harvest. Traps were checked 3, 7 and 10 days after the spray, counting and removing all *C. capitata* adults. At the date of trap control, infection were counted at randomly selected 100 fruits at each plot. Also two kg of fruits were counted as infected or uninfected.

The counting for main evaluation was carried out by determining fruit infection at the beginning of harvesting period. For this aim, from the trees with uninfected fruits in the middle of a plot belong to each replication, randomly gathered 100 fruits were separated as uninfected and infected by *C. capitata*. After that, total uninfected and infected fruit numbers were determined by comparing these numbers to estimated total fruit numbers of the trees. Total uninfected and infected numbers were determined by adding the numbers obtained from the fallen fruits during the trial.

The percentage effects of insecticides were calculated using Abbott equation to the uninfected and infected fruit percentages. The differences among insecticides and their dosages were statistically determined by using the angel values of the percentage effects of the insecticides in Variance Analysis (Duncan Test).

Also, evaluating adult numbers of the pest caught in traps on counting dates, adult population dynamics were observed in this orchard.

Population dynamics of Ceratitis capitata

The flight activity of adult *C. capitata* was studied by pheromone traps (Jackson traps). Trimedlure was the pheromone source at the traps. Four traps were placed in a 30 years old Washington orange orchard in Plant Protection Research Institute, Adana. This orchard was consisted of more than 1200 trees and was about 3 ha in size. These traps were adjusted at a hight of 1.5-2.5 m at the south-east side of the trees. The traps were controlled weekly at spring-autumn and twice a month in the winter during 2005-2007 and the number of medfly adults was recorded. This orchard was sprayed in autumn by Malathion %25 WP (400 gr+1000 gr hydrolyzed protein (Ziray at a concentration of 5%)/10 lt water) by 10 day intervals during 2005-2006 and by Spinosad (Success 0.24 CB-1000ml/10 lt water) in 2007.

Results and discussion

Comparison of the efficacy of two attractions

The results of the studies contributed to determine the biological effectiveness of two baits against *C. capitata* and their percentual efficacy is shown in Table 1. As shown from the table Malathion+Ziray and Malathion+94/5 gave an efficiency of 96.51%, 96.82% in 2003 and 2004 and 88.35%, 87.71% in 2003 and 2004, respectively. Also, the infected fruit percentage was 0.64% and 2.12% at Ziray and 94/5 blocks in 2003. In 2004 the infected fruit percentage was 0.58% and 2.13% at Ziray and 94/5 blocks. However it was higher with 19.22% and 16.35% both of the years at the control block. The block sprayed with Ziray gave the highest effect and took place at different statistical group (a) than the block with 94/5.

The number of adults captured at the traps and infection rate of 100 fruits at the spray and control dates are shown in Table 2. From Table 2 it could be observed that at the control dates after sprayings the individuals captured at the traps and the infection rate was higher at the control plot than the sprayed plots both of years. When we comparise the sprayed plots between each other it can be shown that the plot sprayed with Malathion+94/5 had higher adult number and infection rate than the plot sprayed with Malathion+Ziray.

Population dynamics of Ceratitis capitata

The population dynamics of *C. capitata* adults during 2005-2007 in Adana is shown in Figure 1. The highest number of *C. capitata* was counted in June both in 2006 and 2007. The population was low in spring months during the study. The highest number of adult was counted in June in 2006 and 2007 (avg. 819 adults/trap, avg. 452 adults/trap, respectively). It is thought that the main reason for this increase of the pest population at the beginning of summer is the alternative hosts of the pest such as peach, nectarine, pomegranate, pear, apricot trees, etc., cultivated close to citrus orchards. Usually, the pest is not controlled on these trees. The pest population increases on these fruit trees and moves to citrus in late summer or autumn. From the middle of August the sprayings are started at citrus so the pest population is decreased. During the winter months no adults were captured at the traps.

Conclusion

The results of counting the infection rate and captures at the traps showed that Ziray is a better attractant than 94/5. Because 94/5 gave better results at laboratory trials (Aydoğdu, 1998), it is concluded that the combinations of this attractant with different insecticides (commercial preparations) must be experimented at the field.

A considerable amount of research has been done over the past 30 years on IPM for citrus in Turkey. The Plant Protection Department of Çukurova University in Adana has led an IPM programme consisting of both cultural and chemical control directed at citrus. Around half of all citrus production in Turkey is subjected to some cultural and biological control, with a similar amount under purely chemical control for pests. Cover spray using Malathion has been used in at most of the citrus production for *C. capitata* control. Growers determine when to spray based mainly on the time of the year and state of the fruit. There is no mass trapping for the pest, but some growers do rely on monitoring traps.

In addition to training studies of the growers, different insecticides must be registered against the pest and more extensive ecological studies would need to be conducted for better control of the pest in Turkey.

Table 1. Percentual efficiency of attractives and the capture and infection of *Ceratitis capitata* in Dörtyol (Hatay) in 2003-2004.

Characters	Rep.	No. of fruit counted														Infected fruit (%)		Effect (%)	
		Fallen				During Harvest				Total				Total fruit					
		Uninfected		Infected		Uninfected		Infected		Uninfected		Infected							
		2003	2004	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004
Malathion +Ziray	**1**	10	15	4	5	1529	1624	8	5	1539	1639	12	10	1551	1649	0.77	0.61	95.90	96.75
	2	8	7	4	4	1579	1825	4	4	1587	1832	8	8	1595	1840	0.50	0.43	97.54	97.84
	3	7	9	2	3	1862	1324	10	8	1869	1333	12	11	1881	1344	0.64	0.81	97.33	95.41
	4	7	11	1	2	1799	1865	11	7	1806	1876	12	9	1818	1885	0.66	0.41	95.30	97.30
	Avg.															**0.64**	**0.58**	**96.51a***	**96.82a***
Malathion +94/5	**1**	11	12	13	10	1891	1895	24	29	1902	1907	37	39	1939	1946	1.75	2.00	89.35	88.24
	2	17	14	8	11	1822	1818	27	22	1839	1832	35	33	1874	1865	2.04	1.80	90.97	83.51
	3	10	9	11	13	1617	1611	21	18	1627	1620	32	31	1659	1651	2.18	1.87	89.40	91.47
	4	12	13	9	7	1510	1516	35	38	1522	1529	44	15	1546	1574	2.54	2.85	83.68	87.61
	Avg.															**2.12**	**2.13**	**88.35 b**	**87.71b**
Control	**1**	14	19	49	53	1619	1624	329	336	1633	1643	378	389	2011	2032	18.79	19.14	-	-
	2	5	7	76	71	1191	1198	222	228	1196	1205	298	299	1494	2480	19.94	25.08	-	-
	3	10	13	61	58	1321	1327	223	217	1331	1340	284	275	1615	2719	17.58	10.11	-	-
	4	11	14	60	64	1523	1526	266	259	1534	1340	326	323	1860	2923	20.59	11.05	-	-
	Avg.															**19.22**	**16.35**	-	-

* Means in columns followed by the same letter are statistically different according to Duncan test (p=0.05)

Table 2. The number of adults captured at the traps and fruit infection rate (%) at the spray and control dates.

No of spray	Control dates after spray	Malathion+Ziray				Malathion+94/5				Control			
		No of adults		Infection (%)*		No of adults		Infection (%)*		No of adults		Infection (%)*	
1. Spray		2003	2004	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004
	3. day	18	1	4	4	30	8	4	3	23	38	5	4
	7. day	4	10	3	2	12	10	3	1	19	15	17	20
	10. day	5	6	2	1	8	15	2	3	11	8	15	18
2. Spray	3. day	4	11	2	2	6	12	4	4	14	15	29	31
	7. day	4	7	1	3	6	9	2	2	9	11	25	22
	10. day	2	2	0	1	4	3	0	1	8	4	26	28
3. Spray	3. day	4	13	1	1	3	10	3	2	6	19	22	20
	7. day	4	9	2	1	5	12	2	1	5	28	19	16
	10. day	5	8	1	0	3	21	1	2	3	28	16	17
4. Spray	3. day	1	16	0	0	2	31	0	1	0	48	9	12
	7. day	1	7	0	0	3	19	0	0	1	41	8	10
Total		**52**	**90**	**16**	**15**	**82**	**150**	**21**	**20**	**99**	**255**	**191**	**198**
Harvest													

*Avg. of 4 replicates

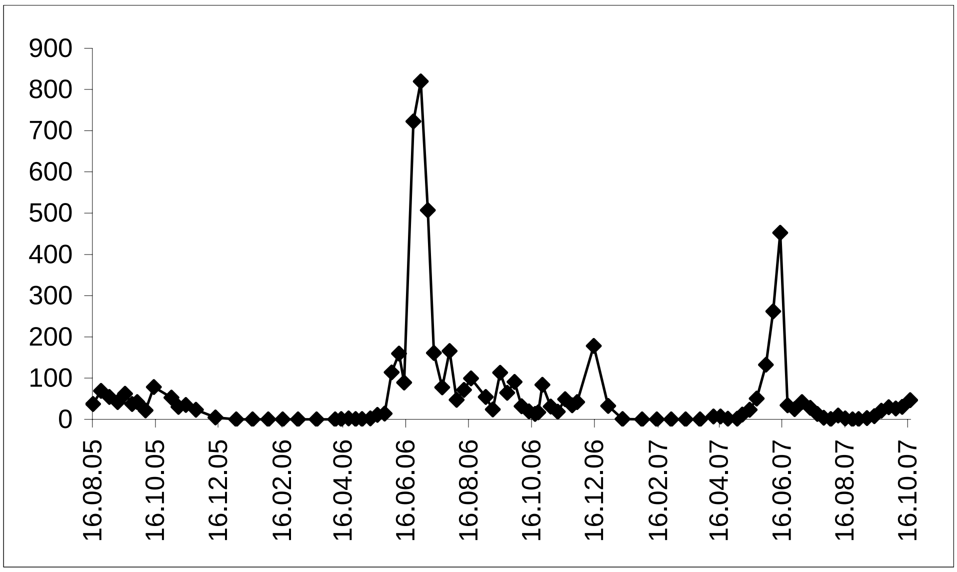

Figure 1. The population dynamics of *Ceratitis capitata* adults during 2005-2007 in Adana.

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Field experiments towards the development of a strategy for the control of the MedFly (*Ceratitis capitata*) using Match Medfly RB03 (Syngenta) in Citrus orchards

A. Mazih[1], S. Eltazi[2], I. Srairi[2], S. Sahil[3], H. Bouguiri[4], M. Miloudi[4], Y. Moubaraki[1], Y. Bourachidi[1]
[1] *Institut Agronomique et Vétérinaire Hassan II, Agadir, Morocco; mazih@iavcha.ac.ma*
[2] *Domaines Abbes Kabbage-Souss, Agadir, Morocco*
[3] *DPVCTRF, Agadir, Morocco*
[4] *Syngenta Agro*

Field trials in citrus orchards, in Souss valley (Morocco), were carried out in order to assess the efficacy of Match Medfly RB03 (Match Fly -Kill or ADRESS) device (Syngenta Agro) to control medfly (*Ceratitis capitata*). Twenty to twenty five devices/ha containing an insect grow regulator (lufenuron gel), baited with male and female attractants, were hanged on trees at least 6 to 8 weeks before fruits ripening, until the end of harvest. Lufenuron ingestion leads to the autosterilization of wild adult Medfly in the field. Small and medium-sized field trials (1 to 5 ha each) have shown a very good level of fruit damage prevention. The efficacy of the method was measured by monitoring Medfly populations, and fruit damage sampling, in treated blocks, compared with those of the control blocks, where bait sprays spot treatments were applied. According to our findings, it appears that the efficacy of Match Fly -Kill RB03 to manage fruit fly problem is at least the same as the conventional sprays used by the grower. Large-scale field trials are under way in order to confirm these results.

Evaluation of mass trapping using M3 bait-station to control Medfly in Citrus orchards

S. Eltazi[1], A. Mazih[2], I. Srairi[1], Y. Bourachidi[2]
[1]*Domaines Abbes Kabbage-Souss, AGADIR, Morocco*
[2]*Institut Agronomique et Vétérinaire Hassan II, AGADIR, Morocco; mazih@iavcha.ac.ma*

The ready-to-use M3 bait station for the control of fruit fly species attacking citrus was tested. The bait stations (1/tree) were placed in a 2 ha Nules clementine orchard (800 trees), in Souss valley (Morocco), approximately 4 weeks before colour break. The fruit fly populations were monitored using MaghrebMed traps baited with trimedlure. Fruit infestation levels were determined at harvest. The M3 bait station effectively controlled fruit fly. Only a single application during the fruiting season was necessary, without any spray treatment, while 2 insecticide sprays were done in the control block. The level of adult's catches remained under the threshold, and no infested fruits were recorded at harvest.

Control in Citrus Fruit Crops
IOBC/wprs Bulletin Vol. 38, 2008
pp. 144-149

Improvement of *Ceratitis capitata* mass-trapping strategies on citrus in north-eastern Spain

Campos Rivela, J. M., Martínez-Ferrer, M. T., Fibla Queralt, J. M.
IRTA Amposta Ctra. de Balada, Km 1 E- 43870 Amposta (Tarragona) Spain

Abstract: In Spanish citrus orchards, *C. capitata* mass trapping strategies are based on 45-50 traps per hectare density, homogeneously placed across the fields. In order to protect early and mid season varieties from the *C. capitata* attack, traps are hung about 1.5 months before harvest. Enhance of mass trapping methods in this study was focused on studying the moment of trap hanging on the trees and their spatial distribution in the field. In 2 ha groves, Tephri-trap traps baited with Tri-pack attractant were used for these trials. In two early citrus varieties groves (Marisol mandarin and Navelina orange) located in Tarragona and Valencia provinces (Spain), two different strategies were conducted in order to study the effect of advancing the trap placement, July instead of August. We attempted to reduce *C. capitata* populations during summer and to achieve a low pest presence when fruits are ripening.

The effect of trap distribution, perimeter and regular, across the orchards was tested in two early mandarin varieties groves (Loretina and Marisol) and one mid season variety (Clemenules) in Tarragona. No differences were found between setting traps dates. Percentage of attacked fruits ranged between 0.5 and 0.75% at harvest in early varieties (even chemical treatments had to be applied), and achieving a 0% fruit damage in the mid season variety. No significant differences in the percentage of attacked fruits between the regular and perimeter position of the traps were found. Further studies should be carried out in order to study those strategies in larger areas.

Key words: *Ceratitis capitata*, mass trapping

Introduction

Ceratitis capitata is one of the most important pests worldwide attacking more than 200 fruit species (Christenson and Foote, 1960). Currently it is accepted that *C. capitata* control must be conducted by combining several strategies including chemical sprayings, biological control, and techniques like chemosterilant or sterile-insect, and with mass trapping. In Spanish citrus, problems are mainly focused on early citrus varieties like Marisol, Loretina, Satsuma, as mandarin, and Navelina as orange. Then again, the abundant presence of other host trees surrounding or within the citrus orchards in our region threatens the *C. capitata* control (Israely *et al*., 1997). *Ceratitis capitata* shows complex spatial and temporal patterns, that are and driven by a multitude of ecological parameters (Papadopoulos *et al*., 2003).

Mass trapping strategies consist of capturing *C. capitata* populations in the field by placing numerous traps baited with attractant and thus preventing the fruit to be attacked by the pest. In this experiment, our aim was to study the effect of advancing the moment when traps are placed in the orchards, and the effect of two different trap distributions: regular across the orchard and only on its perimeter.

Material and methods

The trials were conducted in the south of Tarragona province (Spain). We used Tephri-trap® baited with Tripack®, a three component food-based synthetic attractant, with DDVP as toxicant. In all cases plots were 1 ha large and 45 traps per ha density were used.

In the first experiment Marisol mandarin and Navelina orange were used. Two different plots were assessed to study the effect of advancing 1.5 months the moment when traps are placed in the orchard in order to reduce the *C. capitata* populations. In Plot 1 the traps were placed 3 months prior harvest, and in Plot 2 the traps were placed 1.5 months later. Thus, that means that the attractant must be replaced in Plot 1 since it only lasts 1.5 months.

The second experiment consisted of comparing two different traps distribution in the field. In the regular distribution, traps were placed in the plots in order to keep the same distance among them, and thus, traps were placed each five rows per seven trees. In the perimeter distribution, traps were placed only on the border of the plot, surrounding it. Three different varieties were assessed, two early-season mandarin (Marisol and Loretina) and one mid-season (Clemenules). Two plots per orchard were performed.

Ceratitis capitata populations were estimated by checking weekly three traps within the plots and counting the number and sex of adults captured. During the mass trapping period all traps in the plots were checked. The variable used was the number of females per trap and day Also the fruit damage was sampled by sampling 10 to 30 trees per plot and 10 to 20 fruits per tree. Fruit ripening was monitored by measuring the rind colour index with a Minolta Colormeter®, and by obtaining the Brix degrees-acid ratio. Orange or mandarin fruits are suggested to be harvested when the colour index reaches 15 and the Brix-acid ratio is about 7.

When necessary, according to captures of *C. capitata* per trap and day, the percentage of attacked fruits and the maturity index, sprayings with the following pesticides were performed to support mass trapping:

<u>Total sprays</u> (1000 l/ha):
Malathion 50 and 90% p/v EC, Fosmet 50% WP

<u>Bait sprays</u> (180 l/ha):
Fenthion 50% p/v EC , Malathion 50 and 90% p/v EC
Lambda-cyhalothrin 2,5% WG
Hydrolyzed protein 30% p/v SL

Data were analyzed statistically by analysis of variance (PROC GLM, SAS Institute 1998). If necessary, data were arcsine or root square-transformed before the analysis. Means were compared using Duncan's multiple range test with a 95% significance level.

Results and discussion

In the first experiment in the Marisol variety, *C. capitata* captures per trap and day were similar in both plots, always under 0.7 female per trap and day (Fig 1). No spraying was applied in this variety.

The colour index was sampled for two months and increased from -27 to -12, and the Brix acid ratio was about 6 in mid October. Therefore, according to both parameters the harvest was conducted between the 13th and the 27th of October. The percentage of attacked fruits by *C. capitata* in October was always under 1% (Table 1) in both plots and with no significant differences between the two strategies. Therefore, no effect by advancing the traps hanging in the field was showed, since hanging them 1.5 months before seems enough to achieve a satisfactory control.

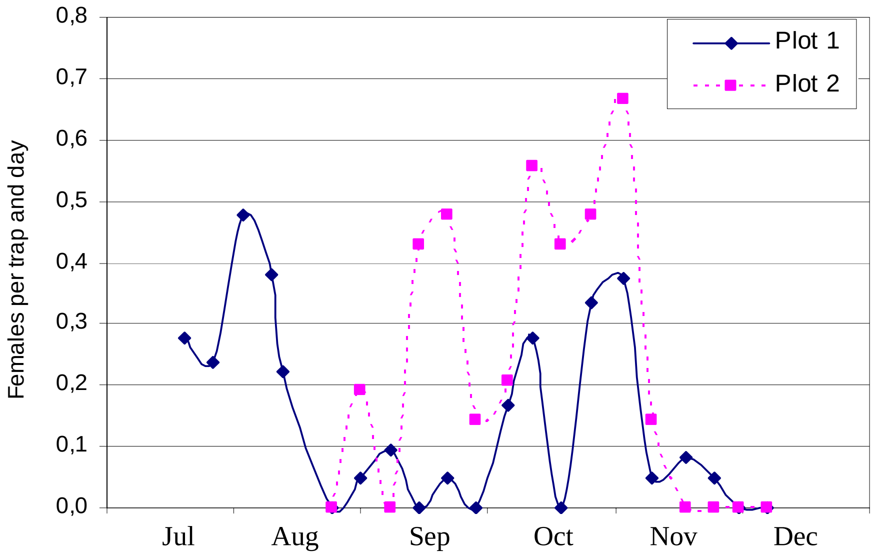

Figure 1. *Ceratitis capitata* dynamics with two different mass trapping strategies in Clementine Marisol variety orchard in Tarragona (Spain). (Plot 1: Traps were placed 3 months before harvest. Plot 2: Traps were placed 1.5 months before harvest). (The arrows represent the chemical treatments).

Table 1. Percentage of attacked fruits by *C. capitata* in Marisol variety in traps placed 3 months (Plot 1) and 1.5 months (Plot 2) prior to harvest.

Plot \ Date	8 oct	13 oct	27 oct
Plot 1	1 ± 0.6 a	0.00 ± 0.00 a	0.82 ± 0.38 a
Plot 2	0.22 ± 0.22 a	0.18 ± 0.18 a	0.5 ± 0.32 a

Means within a given column followed by the same letters did not differ significantly. Duncan's Multiple Range Test $P < 0.05$

Treatments were conducted systematically in the Navelina variety. Although during August *C. capitata* dynamics showed high populations, later when in November the Navelina variety reaches the maturity index, the *C. capitata* populations were, but for one week, always under one female per trap and day. At harvest, during the start of November, when Brix-acid ratio was 7.0 ± 0.21 and the colour index was -9.9 ± 0.37, *C. capitata* populations were even lower.

According to the results in both varieties, placing traps 3 months prior harvest did not improve the mass trapping system and even increased its cost, by having to replace the attractant on the traps.

In the second experiment in Loretina variety, mass trapping started at the end of August, and during that period the *C. capitata* populations ranged in both plots between 1 and 4 females per trap and day. Those populations were high enough to carry out several treatments. At the end of the mass trapping period, due to both treatments and mass trapping, *C. capitata* populations were under one female per trap and day.

Colour index and internal maturity parameters reached harvest levels during the first week in October. As can be seen in Table 2, no significant differences were found between percentages of attacked fruits in both plots that ranged from 2.0 to 5.5%.

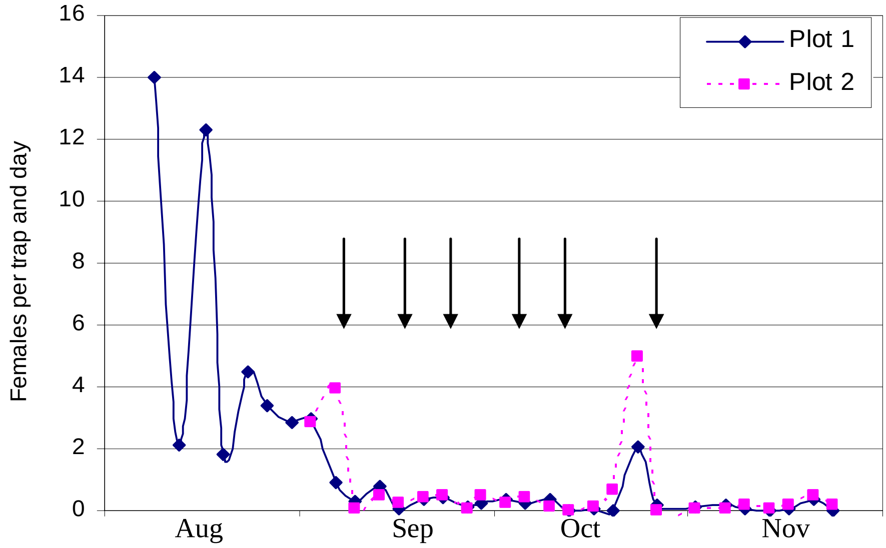

Figure 2. *Ceratitis capitata* dynamics with two different mass trapping strategies in an orange Navelina variety orchard in Tarragona (Spain). (Plot 1: Traps were placed 3 months before harvest. Plot 2: Traps were placed 1.5 months before harvest). (The arrows represent the chemical treatments).

According to the results in both varieties, placing traps 3 months prior harvest did not improve the mass trapping system and even increased its cost, by having to replace the attractant on the traps.

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Colour index and internal maturity parameters reached harvest levels during the first week in October. As can be seen in Table 2, no significant differences were found between percentages of attacked fruits in both plots that ranged from 2.0 to 5.5%.

In the second experiment in the Marisol variety, *C. capitata* populations during the mass trapping period ranged between 1 and 4 females per trap and day in both plots. *C. capitata* populations declined from the start of mass trapping, and also after treatments during early October. The percentage of attacked fruits ranged from 2.7 to 5.8%, with no significant differences between plots (Table 2).

In Clemenules variety, it is shown how in mid-season varieties, due to both November low temperatures and mass trapping technique, the *C. capitata* populations were very low during the whole experimental period. Captures were even lower when fruit was harvested in the regular and the perimeter distribution plots. No fruit attacked by *C. capitata* was observed in the field at harvest. In fact, colour index was 2.78 and the Brix acid ratio was 11.96, which reveal that the fruit could have been harvested almost two weeks before.

The two different trap distributions, regular and perimeter, offered the same level of the *C. capitata* control. Mass trapping strategy offers enough control in mid-season varieties, since they ripen during November, when *C. capitata* populations have declined due to the low temperatures. In early-season varieties, when maturity parameters achieve levels that make fruit susceptible to be attacked by *C. capitata*, it is necessary to perform several chemical sprayings. Along with mass trapping strategies the number of sprayings can be reduced by half. In orchards larger than 1 ha, results are expected to be better.

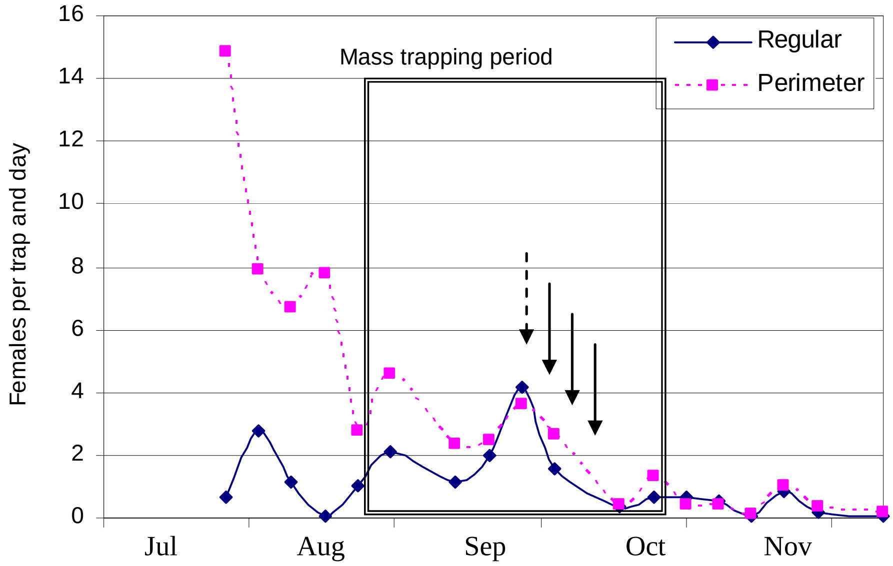

Figure 3. *Ceratitis capitata* dynamics in two different mass trapping distributions in a Loretina variety orchard in Tarragona (Spain). (The arrows represent the chemical treatments)

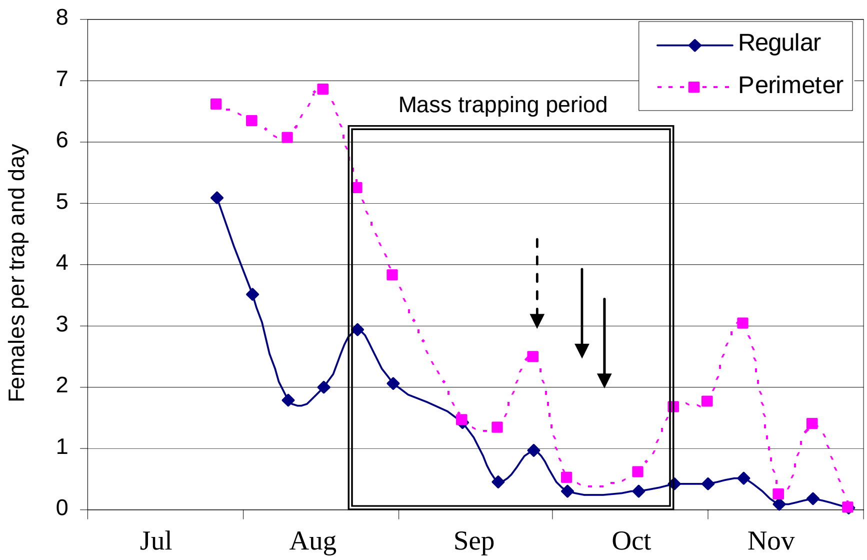

Figure 4. *Ceratitis capitata* dynamics in two different mass trapping distributions in a Marisol variety in Tarragona (Spain). (The arrows represent the chemical treatments)

Table 2. Percentage of attacked fruits with regular or perimeter traps distribution in two different early mandarin varieties.

	Loretina		**Marisol**	
Date	**Regular**	**Perimeter**	**Regular**	**Perimeter**
27 September	2.00 ± 1.30 a	2.90 ± 0.60 a	3.80 ± 0.85 a	3.50 ± 1.00 a
3 October	2.50 ± 0.73 a	3.50 ± 0.72 a	5.91 ± 0.89 a	3.60 ± 0.69 a
10 October	4.50 ± 0.75 a	5.51 ± 0.82 a	5.80 ± 0.83 a	4.69 ± 0.79 a
18 October	1.70 ± 0.44 a	3.10 ± 0.65 a	4.00 ± 0.71 a	3.40 ± 0.71 a

Means within a given row followed by the same letters did not differ significantly. Duncan's Multiple Range Test $P < 0.05$

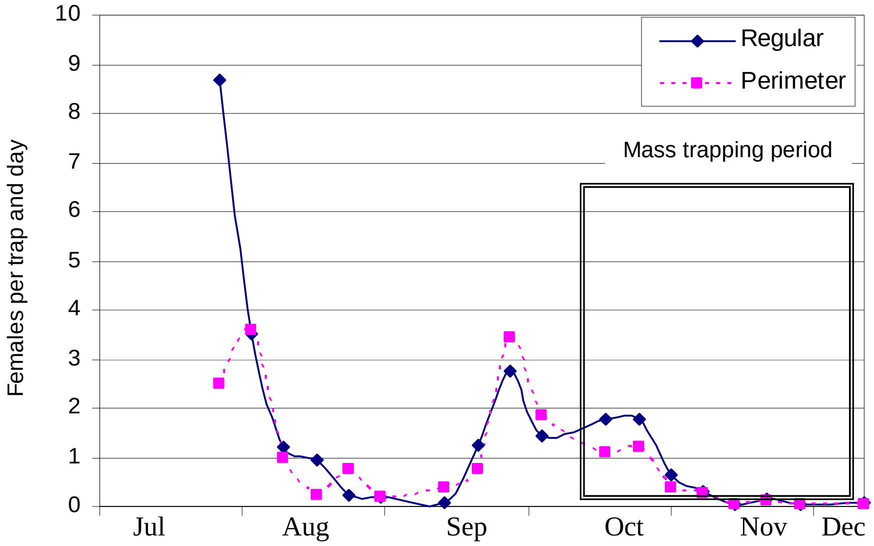

Figure 5. *Ceratitis capitata* dynamics in two different mass trapping distributions in a Clemenules variety in Tarragona (Spain).

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Control in Citrus Fruit Crops
IOBC/wprs Bulletin Vol. 38, 2008
pp. 150-156

Integrated control of Mediterranean fruit fly *Ceratitis capitata* (Wied.) by mass trapping with an enzymatic hydrolyzed protein

J.M. Llorens[1], E. Matamoros[2], A. Lucas[3], C. Marín[4] and N. Sierras[4]
[1] *Servicio de Sanidad Vegetal de Alicante, Generalitat Valenciana, c/ Profesor Manuel Sala, nº2, 03003 Alicante, Spain*
[2] *Servicio de Sanidad Vegetal de las Tierras del Ebro, Generalitat de Catalunya, Ctra. Valencia, nº108, 43520 Tarragona, Spain*
[3] *Servicio de Sanidad Vegetal Región de Murcia, c/Mayor, s/n, 30150 Murcia, Spain*
[4] *I+D Fisiología Vegetal, Bioiberica, S.A, Ctra. N-II, km. 680,6. 08389 Palafolls, Spain*

Abstract: Field trials were conducted on susceptible mandarin trees (*Citrus reticulata* cv. Beatriz and Oronules) and fig trees (*Ficus carica* cv. Colar) in Tarragona, Alicante and Murcia (Spain) to assess the effectiveness of a specifically developed enzymatic hydrolyzed protein (Cera Trap®) to control the Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae). The efficacy of Cera Trap (CT) was compared with standard farm control strategies (mass trapping or chemical treatments), assessing medfly captures and fruit damage (infested fruits on trees, at the ground and in the warehouse). The efficacy field trials showed that, 1) capture levels on CT plots were similar to those obtained by the standard farm control strategies, and, 2) fruit damage was lower on CT treated trees than under the farm standard control strategies. The CT hydrolyzed protein successfully controls the pest population, decreases medfly fruit damage and provides long-term control (from fruit ripening until harvest) reducing pesticide application to a minimum or even making it completely unnecessary.

Key words: *Ceratitis capitata*, mass trapping, liquid protein, attractant

Introduction

The Mediterranean fruit fly *Ceratitis capitata* is one of the most destructive agricultural pests worldwide. It is native of sub-Saharan Africa and has a long history of invasion success. In the Mediterranean area medfly is one of the most difficult pests to handle. It is extremely polyphagous, it breeds a high number of generations per year and its attacks occur close to harvest time. Various control techniques have been developed, like mass trapping, sterile male release and the use of repellent products, in order to control medfly with the minimum amount of pesticide residue. Medfly control by mass trapping in the main *Citrus* producing areas in Spain, is mostly carried out by means of government funds. Several mass trapping methods are used in Spain. Their main characteristics are the need to complement it with the use insecticide and the difficulty in handling. Trials conducted on a large scale with expensive chemical male sterilization have not yet obtained relevant efficacy results either.

This study aimed at evaluating the efficacy of an enzymatic hydrolyzed protein (Cera Trap) against the Mediterranean fruit fly (medfly) *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae) in comparison with standard farm control strategies (mass trapping or chemical treatments). The composition of the evaluated product (Cera Trap) is free of pesticides in its formulation and none is required in the traps. The medfly is strongly attracted, enters into the traps baited with the hydrolysed protein and, being unable to escape, drowns in the liquid and dies. This paper reports on trials undertaken to control established pest population. Three different field trials carried out in diverse crops in Spain are analysed.

Material and methods

Field trials were conducted in three study sites (Tarragona, Murcia and Alicante) with Mediterranean climate in Spain during 2005, 2006 and 2007. Different management of experimental sites were as follows:

Tarragona field trial

Field trial were carried out in Santa Barbara, Delta del Ebro, Tarragona (Spain) on a susceptible mandarin orchard (*Citrus reticulata* c.v Beatriz), with 606 trees per hectare (plant age: 5 years). The main crops of the area are *Citrus*, olives and rice. To evaluate the efficacy of the different treatments, an irregular plot of 1.5 ha approximately on size was used for each treatment. The following treatments were compared in this study: 1) standard mass trapping system with 50 traps per hectare 2) Cera Trap (CT) with 70 traps per hectare and 3) CT with 100 traps per hectare. Traps were hung on the sunny part of the tree at a height of 1.7 m approximately. CT traps were hung empty and filled with 300 ml of the hydrolyzed protein product with a knapsack sprayer without the nozzle (no pressure is needed). The first mass trapping system was installed on September 5, 2005. The second and third mass trapping systems were installed on August 19, 2005, and refilled on September 6 and 23, with 200 ml and 150 ml of CT per trap, respectively.

To monitor the flight activity of *C. capitata*, control traps were placed in each plot on September 20. The number of flies captured per trap was recorded weekly. Damaged fruit remaining on trees and ground was identified by examining sampled fruit during the ripening period until harvest. Once a week, damaged fruit (trees and ground) were recorded from ten trees randomly selected from each plot. To assess fruit damage at harvest, 500 fruits per treatment were collected and brought to the warehouse. Seven days after the harvest punctured fruit were recorded, collected, and removed. Two weeks after the harvest punctured fruit were reported again.

Murcia field trial

Field trial were carried out in San Javier, Campo de Cartagena, Murcia (Spain) on a susceptible mandarin orchard (*Citrus reticulata* c.v Oronules), with 416 trees per hectare. The main crops of the area are *Citrus* and vegetables. To evaluate the efficacy of the different mass trapping systems, an irregular plot of 1.5 ha approximately on size was used for each treatment. In this field trial the following treatments were compared: 1) standard chemical treatment (Spinosad 48% p/v + hydrolized protein 30% p/v; 30 ml/hl + 600 ml/hl; applied rate 80 l/ha); 2) standard chemical treatment (Malathion 90% p/v + hydrolized protein 30% p/v; 600 ml/hl + 600 ml/hl; applied rate 80 l/ha) and 3) CT mass trapping system with 100 traps per hectare. The two standard chemical treatments 1 and 2 were applied with a manually operated knapsack sprayer (Model Maruyama MD 07 with a 1.5 diameter flat nozzle and 100 kPa of pressure). Traps from treatment 3 were hung on the sunny part of the tree at a height of 1.7 m approximately. The traps are hung empty and charged with 300 ml of the hydrolyzed protein product with a knapsack sprayer without the nozzle The first and second treatment were applied on August 25, 2005. Six additional applications of spinosad (chemical treatment 1) and malathion (chemical treatment 2) were carried out on September 1, 9, 20, 30 and October 7 and 15, 2005. Temperature, humidity and wind data were collected during the application dates. Treatment 3 was located on August 16, 2005 and refilled on September 2 and 20, 2005 with 200 ml and 150 ml of CT per trap respectively.

On August 18, to monitor the flight activity of *C. capitata*, Tri-pack control traps were placed in each plot. The number of flies captured per trap was recorded weekly. Damaged fruit remaining on trees and ground was identified by examining sampled fruit during the maturation period until harvest. Once a week, damaged fruit (trees and ground) were recorded from ten trees randomly selected from each plot. To assess fruit damage at harvest, 500 fruits per treatment were collected and brought to the warehouse. Seven days after the harvest punctured fruit were recorded, collected, and removed. Two weeks after the harvest punctured fruit were reported again.

Alicante field trial

Field trial were carried out during two consecutive years (2006 and 2007) in Albatera, Alicante, Valencia (Spain) on a susceptible fig orchard (*Ficus carica* c.v Colar), with 236 trees per hectare. The main crops of the area are *Citrus*, fig trees, pome granate tree, table grapes and vegetables. To evaluate the efficacy of the different treatments, an irregular plot of 11 ha approximately on size was used for each treatment. The following treatments were compared: 1) standard chemical treatment (Lambda cihalotrin 10% p/v + hydrolized protein 30% p/v; 125 ml/hl + 600 ml/hl; applied rate 50 l/ha) and 2) CT mass trapping system with 118 traps per hectare. The first treatment was applied with a motor pump (1.5 nozzle diameter and 150 kPa of pressure). The first treatment was applied on June 12, 2006. Six additional applications of lambda cihalotrin were carried out on June 20, 29 and July 6, 14, 20 and 28, 2006. Treatment 2, traps with 300 ml of hydrolysed protein per trap were hung on May 21, 2006 and refilled on June 18, July 14 and on August 11, 2006 with 200 ml, 150 ml and 300 ml of CT per trap respectively.

On July 20, to monitor the flight activity of *C. capitata*, six Nadel traps baited with Trimedlure and six Tephry traps with Tri-pack were placed in each plot. The number of flies captured per trap was recorded weekly.

During 2007 the same protocol was followed. The standard chemical treatment (Malathion 50% p/v + hydrolized protein 30% p/v; 600 ml/hl + 600 ml/hl; applied rate 80 l/ha) was applied on July 1, 2007. Six additional applications of malathion were carried out on June 20, 29 and July 6, 14, 20 and 28, 2006. Treatment 2, traps with 300 ml of hydrolysed protein per trap were hung on May 21, 2006 and refilled on June 18, July 14 and on August 11, 2006 with 200 ml, 150 ml and 300 ml of CT per trap respectively. Damaged fruit remaining on trees and ground was identified by examining sampled fruit during the harvest period.

Results and discussion

Tarragona field trial

A high population of *Ceratitis capitata* level was present during all the fruit maturation period. Daily medfly captures per trap by the monitoring system is given by figure 1. The results show that Cera Trap (CT) 67 traps/ha. and specially CT 100 traps/ha maintain a low population level in comparison with the standard mass trapping. On September 28 and October 5 two treatments with Malathion (Malathion 50% p/v; 300 ml/hl and 500 ml/hl; applied rate 1250 l/ha) were applied on the entire study orchard for prevention and due the high pest pressure in the standard mass trapping treated plot.

The number of punctured fruits (tree and ground) over the study period is given by figure 2. The most efficient was CT 100 traps per hectare, followed by CT 67 traps per hectare, both of which performed better than the STD mass trapping system. Differences among treatments in both total number of tree and ground punctured fruit during fruit ripening, were substantial. CT mass trapping system, with 67 traps per hectare and 100 traps per hectare, show an almost complete protection on fruit against infestations of the medfly, whereas the standard mass trapping system seems to fail to protect fruits.

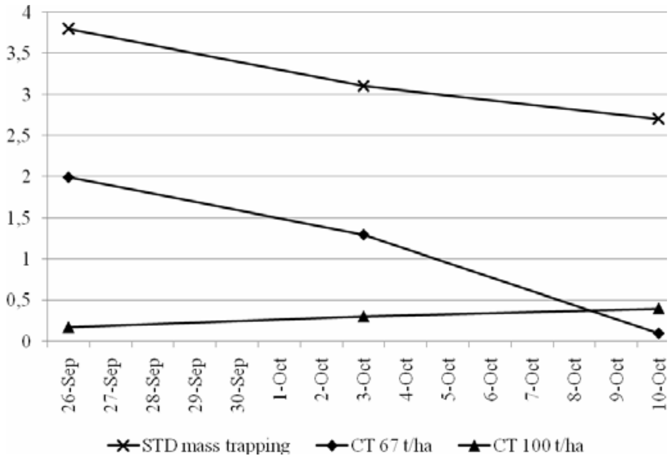

Figure 1. Captures by the monitoring system (number of medfly/trap/day) located on the 20 September.

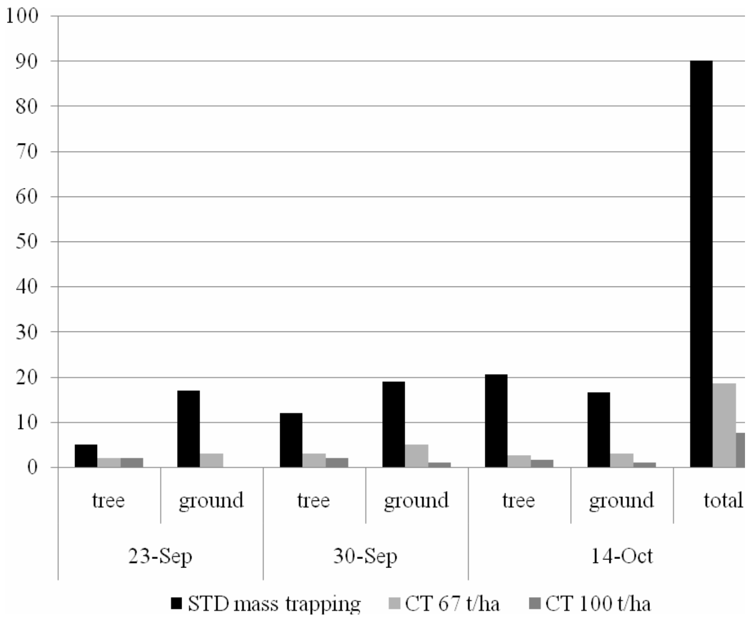

Figure 2. Number of damaged fruit on trees and ground (10 trees randomly sampled/plot).

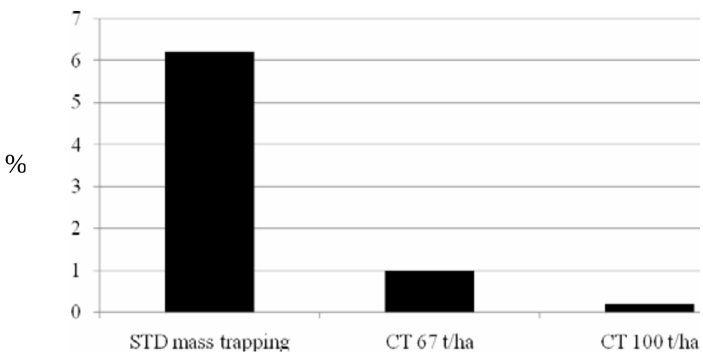

Figure 3. Percentage of damaged fruit in warehouse after 15 days of the harvest date (evaluated sample size 500 fruits/plot).

The average number of punctured fruit in warehouse during the studied period is given by figure 3. The standard mass trapping system shows a little efficacy with regard to fruit damage. The CT 67 traps per hectare treatment show an acceptable efficacy whereas the CT 100 traps per hectare show an almost complete protection on fruit against infestations of the medfly (<1% damaged fruit). The poor efficacy of the standard mass trapping system may be due the high *C. capitata* populations present during the study period.

Murcia field trial

In early September, the different treatments evaluated in this field trial (chemical standard treatment 1, spinosad; chemical standard treatment 2, malathion and Cera Trap 100 traps/ha) showed no substantial differences in daily medfly captures per trap (figure 4), and remained relatively stable after September 15 till the end of the trial. However, the mass trapping plot no insecticide application was necessary.

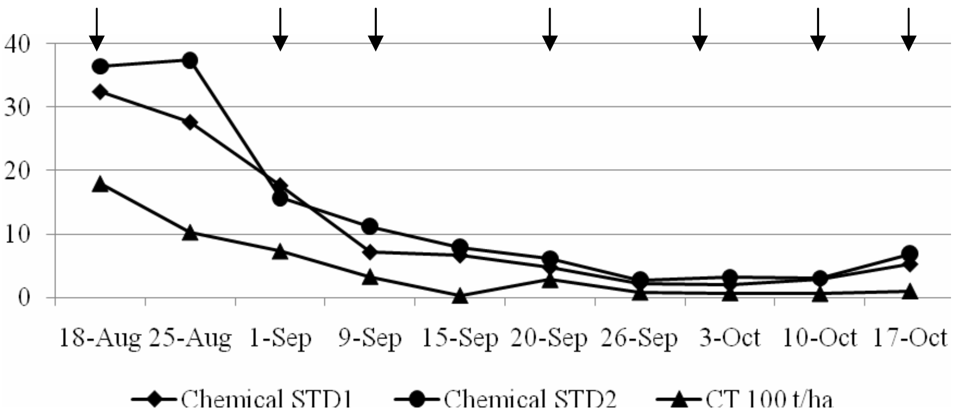

Figure 4. Captures with the monitoring system (number of medfly / trap /day). Black arrows show the pesticides application dates. No insecticide treatment was applied in CT plot.

The total number of punctured fruit, result from six recounts during the ripening period, is given by figure 5. Differences among treatments in the total number of punctured fruit during fruit ripening, on tree and ground, instead, were significant. The CT 100 traps/hectare treatment showed the best performance, as in Tarragona field trial, with at least three times less punctured fruit on trees and ground than the other treatments.

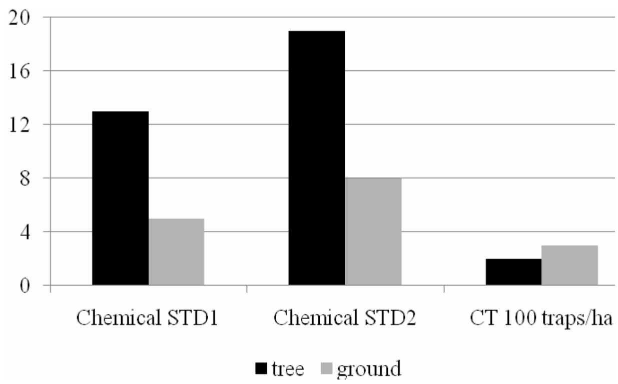

Figure 5. Number of damaged fruit on trees and ground (10 trees randomly sampled/plot).

The average numbers of punctured fruit in warehouse over the studied period are given by figure 6. In this case, all three treatments show an almost complete protection on fruit against infestations of the medfly (< 1% damaged fruit). However, the malathion-based product seems to show the lowest efficacy with regard to fruit damage.

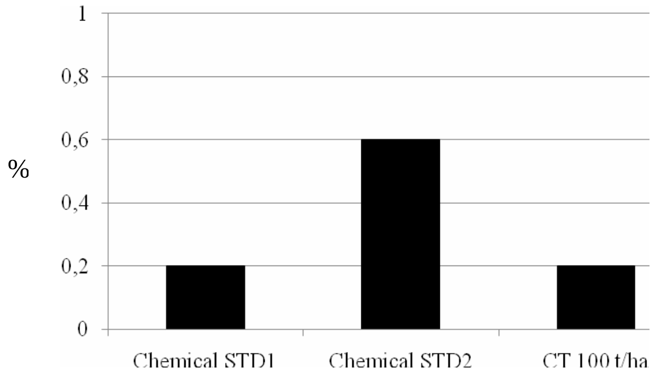

Figure 6. Percentage of damaged fruit in warehouse after 15 days of the harvest date (evaluated sample size 500 fruits/plot).

Alicante field trial

In field trial carried out on Alicante 2006, both treatments evaluated (chemical standard treatment and Cera Trap mass trapping 118 traps per hectare) are able to control the medfly pest during the studied period (from ripening till harvest). Consequently, in any of the studied plots during the recollection period, the *C. capitata* population was able to settle. In the mass trapping plot no insecticide application was necessary.

After the harvest period, when the chemical treatments are finished (July 28), the pest population remains controlled in the CT controlled plot, being from seven to ten times lower in this plot than in chemically standard treated plot. On September 28 the daily number of medfly captures per trap falls dramatically due a hard rainfall, giving an anomalous value. The results of the mean of medfly captures/trap/day are given by figure 7.

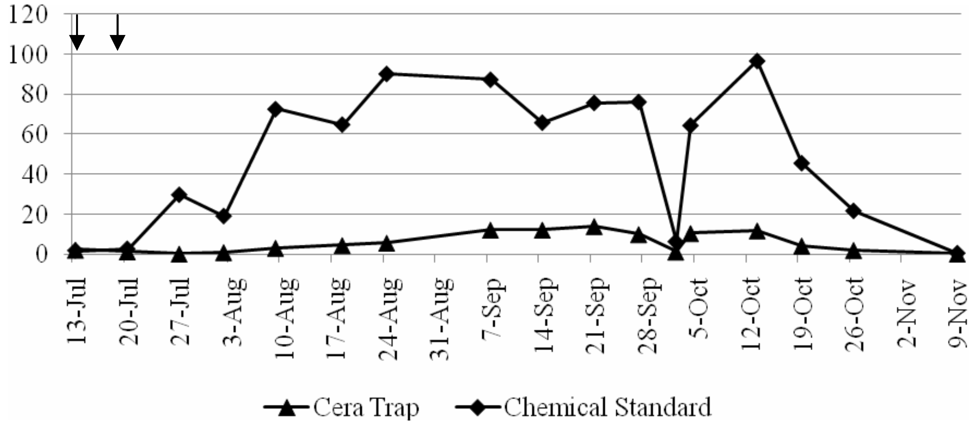

Figure 7. Mean of medfly captures/trap/day (2006). Black arrows show the pesticides application dates (only in the chemically standard plot).

The results of the field trial carried out on Alicante 2007 are given by figure 8. The period between July 3 and July 31 corresponds to the second harvest period. As in 2006, the pest population remains controlled only in the CT controled plot, being from seven to ten times lower in this plot than in the chemically standard treated plot.

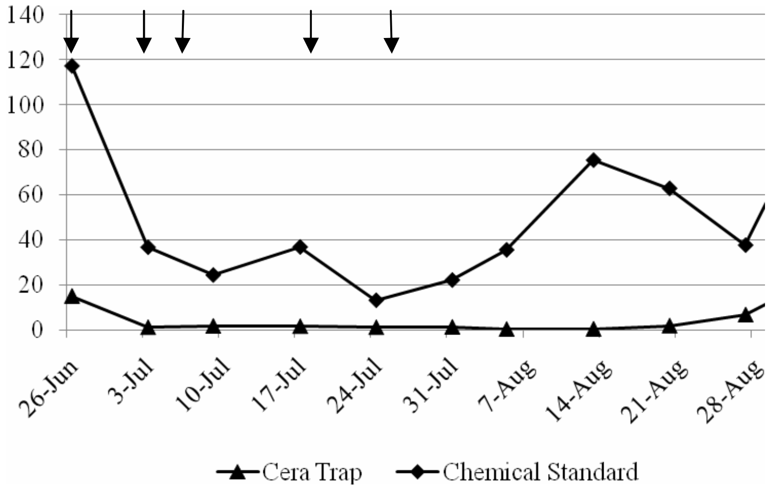

Figure 8. Mean of medfly captures/trap/day (2007). Black arrows show the pesticides application dates (only in the chemically standard plot).

Conclusions

The results of our study suggest that the hydrolyzed protein evaluated CT is an effective system against *Ceratitis capitata*, as it is able to reduce pesticide application to a minimum or even makes it completely unnecessary.

In conclusion, Cera Trap successfully controls the pest population, decreases medfly fruit damage and therefore gives a good opportunity to the IPM strategies as well as organic citrus and fruit harvests. Furthermore Cera Trap provides a long term control from fruit ripening until harvest.

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The use of Biofeed devices in Israel's agriculture aimed for export

Nimrod Israely
Biofeed Environmentally Friendly Pest Control, Nili 71930, Israel; nisraely@biofeed.co.il

To endure the increasing demands of exported fruits one must assure that exported fruits are free of chemical residues as well as from quarantine pests such as *Ceratitis capitata* (Wied.). Unfortunately, *C. capitata* infests its hosts shortly before harvest. Currently, weekly insecticide spraying is the common control method. To avoid its undesired side effects other control methods such as SIT, mass trapping and poisonous feeding stations are being evaluated. The Biofeed is a feeding station control device, which is currently being evaluated in Israel. It is widely used in Israel to control *C. capitata* and *Bactrocera oleae* (different baits), mainly for export produce. Using Biofeed, farmers have reduced their overall use of pesticides by 50 to 90 percent. Furthermore, 70 percent of farmers had no need for supplementary spraying to control *C. capitata*. During 2006 and 2007 the Israeli Ministry of Environmental Protection has actively supported the use of Biofeed by farmers around Israel, promoting the use of environmentally friendly control methods. In general, the control quality achieved by farmers using the Biofeed was as good (or better) as conventional spraying. Field studies teach us that about 30 percent of wiled males are attracted to the Biofeed. Often, when infestation is found within treated area by SIT, the area is sprayed by insecticides killing the natural population along with the sterile males. Low rate control of males by a complimentary control method may be an advantage, should Biofeed be used in conjunction with SIT. We believe that if Biofeed is chosen to be used in conjunction with SIT it can be further improved for a lower male attraction, hence, create an increasing quantitative advantage for the sterile male flies over wild females. It is also possible that the sterile males will be less attracted to the Biofeed compared to wild flies since they probably have lower need for proteins. Preliminary experiments of SIT and Biofeed are currently being conducted in Israel.

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Preliminary evaluation of GF-120 to control of *Ceratitis capitata* (Wiedemann) (Diptera, Tephritidae) in commercial citrus orchards

D. Rinaldi[1], M.E. Porto[2], E. Tescari[2], G.E. Cocuzza[3]
[1] *AgriGeos, via G. Bruno 136, 95131 CATANIA, Italy; rinaldi@agrigeos.com*
[2] *DowAgroSciences, viale Masini 36, 40126 BOLOGNA, Italy*
[3] *Dipartimento di Scienze e Tecnologie Fitosanitarie, sez. Entomologia, Università degli Studi, via S. Sofia 100, 95123 CATANIA, Italy*

GF-120 fruit fly bait, a formulated product containing spinosad (0.24 g/l), having both an attractant and feeding stimulant function, was evaluated against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). The efficacy of the product was compared with malathion (440 g/l of active ingredient) + hydrolized protein (300 g/l), an effective mixture widely applied to control the pest. An untreated control supplied information about the Medfly pressure. Trials were carried out in two different Navel orange orchards in the years 2004 and 2005 and in a mandarin orange orchard in the year 2005. The test sites were located in Ispica (Sicily), an area in which the Medfly represents the most important pest in citrus. For all the trials, GF-120 was applied at 1 l/ha, suspended and mixed in 20 l of water. The solution was sprayed by a single anti-drift nozzle, producing drops of 4-6 mm in diameter, that were band-sprayed on the southern side of the canopy; only one row every four was sprayed. The reference, diluted in 350 l/ha of water, was delivered by means of the farm air blast sprayer equipped with 4+4 flat fan nozzles, and a foliar broadcast application was performed. Experiments were considered as a completely randomized design; due to the high cost of crop destruction, 1 ha/treatment was used with four replications inside. Differences between the treatments were determined by checking, at harvest time, 300 fruit per plot; data were statistically evaluated as percentage of damaged fruits.

In the trial carried out in 2004 on navel orange (4 applications at 7/9-day intervals), treatments resulted statistically different from the untreated, showing a lower percentage of damaged fruits (GF-120 2.83%, malathion + h.p. 4.25%, untreated 34.92%), but they did not resulted statistically different between them. Similar results were recorded in 2005 (5 applications at 7/8-day intervals), where no difference was possible to detect between treatments (1.8% of damaged fruits in GF-120 and 3% in malathion + h.p.); both of them showed a significant difference compared to the untreated check (33.2%). Also in the trial on mandarin orange (6 applications at 7/8-day intervals), treatments did not result statistically different between them (GF-120 4.6%, malathion + h.p. 9.9%), but different from untreated (37.7%). Results achieved in all the trials indicate that GF-120 is a promising product to control *C. capitata*. Percentage of damaged fruits in the plots treated with GF-120 and malathion + h.p. were similar. Nevertheless, compared with the latter, the foliar surface treated with GF-120 is strongly reduced, as well as the impact on the environment, since only 0.24 g a.i. per ha is distributed.

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New results with the ADRESS® bait station system based on lufenuron to control the Mediterranean Fruitfly, *Ceratitis capitata* Wiedemann

S.W. Skillman[1], R. Liguori[2], A. Lopez[3], E. Mas[3], A. Morcos[4], J. Pedras[5]
[1] Syngenta Crop Protection AG, Postfach, 4002 Basel, Switzerland
Syngenta Group Companies: [2] Italy, [3] Spain, [4] Egypt, [5] Portugal

Results of field trials in different countries show how ADRESS® bait stations, containing a gel-bait laced with lufenuron, protect different fruit crops by preventing the eggs of Mediterranean fruit flies from hatching. The system works well on high populations and effects last for at least one year. Key benefits compared to standard bait sprays for fruit growers who have heavy problems with Medfly were perceived to be the safety and IPM compatibility of the system and the high level of efficacy combined with the long lasting effect.

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Mass trapping of *Ceratitis capitata* Wied., with Tephi-Trap and Tripack MFL: optimising the control strategy

M.E. Wong[1], J. Olivero[2], A.L. Márquez[2], F. Montoro[3], N. Rivera[3], E.J. García[3]
[1] *Centro de Investigación y Formación Agraria "Campanillas", IFAPA, Málaga, Spain*
[2] *Dept. Biología Animal, Universidad de Málaga, Spain*
[3] *Dept. Sanidad Vegetal de Málaga , Consejería de Agricultura y Pesca, Junta de Andalucía, Spain*

We compared the efficacy of three different designs for a mass trapping device to control the Mediterranean fruit fly, *Ceratitis capitata*. We worked in three orange orchards, each one located in a different citrus area of Malaga province. Every orchard was 5 ha extensive, and had early citrus varieties whose periods of sensibility to the Mediterranean fruit fly occur simultaneously. We used a different mass trapping design in every orchard, all of them using Tephy-Trap fly-catchers lured with the MFL Tri-Pack:

- Standard: 50 fly-catchers per ha. All traps were installed equidistant in July.
- Density reduction: 40 fly-catchers per ha. 20% of the traps were installed equidistant in March, and the 80% left in July.
- Perimeter arrangement: 50 fly-catchers per ha. 50% of the traps were installed in March, in trees located next to the borders of the orchard, and the 50% left in July, equidistant, within the orchard.

We monitored the evolution of *C. capitata* weekly, using Kenotrap and Nadel fly-catchers lured with the MFL Tri-Pack. The data obtained were processed with analysis of variance and with the Scheffé and LSD mean comparison tests. The design with the highest control efficacy was the perimeter arrangement, followed by the density reduction, in despite of both cases implied a density trap reduction, at least in the central area of the orchard, with respect to the standard method. We think that the earlier starting date of the most efficacious devices might be helpful to stabilize the fruit fly populations before it gets damaging for the crop.

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The importance of dispersal surveys on the behavioural knowledge of Mediterranean Fruit Fly sterile males (*Ceratitis capitata* Wiedemann) (Diptera: Tephritidae) released at an orchard at Biscoitos and in the Angra urban area, Terceira Island, Azores

Horta Lopes, D.J. [1]; Pimentel R. [1]; Dantas, L. [2]; Zorman, M. [3]; Macedo, N. [1]; Figueiredo, A. [1]; Mumford, J.D. [4] & Mexia, M.M. [5]

[1] *Universidade dos Açores, Centro de Biotecnologia dos Açores, Departamento de Ciências Agrárias, Secção de Protecção de Plantas, 9701-851 Terra chã, Açores, Portugal, dlopes@notes.angra.uac.pt*

[2] *Programa Madeira-Med, Estrada Eng. Abel Vieira, 262, 9135-260 Camacha, Madeira, Portugal*

[3] *Faculty of Agriculture, Vrbanska 30 , SI-2000 Maribor, Slovenia*

[4] *Centre for Environmental Policy, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL57PY, United Kingdom*

[5] *Universidade Técnica de Lisboa, Instituto Superior de Agronomia, Departamento de Protecção de Plantas e Fitoecologia, Tapada da Ajuda, Lisboa, Portugal*

Abstract: Biotechnical control could be a more practical and ecological means against pests compared to the alternative of using chemical products. With this point of view the sterile insect technique (SIT) using sterilized males of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) produced at the Madeira-Med programme facilities could be applied on Terceira Island. Therefore, in 2007 two dispersal tests were conducted to evaluate the sterile male dispersion over two areas on Terceira Island, one in an apple orchard (Biscoitos) and another in an urban area (backyards of Angra city). These tests were made in September 2007 with a release of 75 and 150 thousand flies at the Biscoitos and Angra areas, respectively. The dispersal test areas were first projected in computer images using ArcGIS 8 software and placed in using a Garmin GPS. In ArcGIS 8 the release points were projected in a line crossing an inner circle with 7 points spaced at 50 meter intervals and two concentric circles of 30 traps at 100 and 200 meters from the central release point that was plotted from the field after the *C. capitata* sterile adult male release. Adults (wild and sterile) captured in these traps were collected 24h, 72h and 8 days after the release. The major goal was to know the dispersal behaviour of the sterile males in the orchard and urban environments. In this test the wild males captured in the two concentric trap circles (at 100 and 200 meters) were analysed. In both tests the sterile adult males showed a distribution after release similar to the wild ones and covered all the area very quickly and stayed there for almost a week competing with wild *C. capitata* adult males. The results obtained showed a good dispersal capability of the sterile flies produced on Madeira Island in the Terceira Island climatic conditions and that the use of SIT can be a possibility to limit the Mediterranean fruit fly in Terceira and the Azores.

Key words: Medfly**,** *Ceratitis capitata***,** SIT, GIS, dispersal, Azores

Introduction

The Azores Islands are an archipelago situated in the Atlantic Ocean, between America and Europe, comprising nine islands covering 2.352 km^2, distributed in three groups: the Oriental group (Santa Maria, São Miguel), the Central group (Terceira, Graciosa, São Jorge, Pico, Faial) and the Occidental (Flores, Corvo). This work was one part of a wider integrated investigation carried out in the INTERFRUTA project, an interregional cooperation project that includes Azores, Madeira and Canary Islands, financed by FEDER under the EC Program INTERREG III-B (Lopes, 2005a).

This project had as one goal to demonstrate the use of GIS, especially ESRI software, ArcView 3.2., as a tool that can be useful to know *C. capitata* Wiedemann (Diptera: Tephritidae) adult dispersion and also to know the damage level that appears in commercial fruit production areas (Nunes *et al*., 2004; Lopes *et al.*, 2005b, 2005c; Pimentel *et al*, 2005). *C. capitata* is an important pest throughout the Mediterranean area. In the Azores Islands, as in Madeira, it is also a great menace to fruit tree production (Carvalho & Aguiar, 1997). Monitoring *C. capitata* adult dispersion is very important because it is a polyphagous pest causing severe losses on many different hosts.

Some preliminary tests were conducted on *C. capitata* adult behaviour and its dispersal capabilities. The areas which are more affected by this pest were determined, and its population dynamics and its seasonal presence in the different groves of the island were examined, based on expectations from earlier studies (Bodenheimer, 1951; Leonardo, 2002). The period of greatest fruit damage and losses, and the most seriously affected kinds of fruit were identified. Two sexual competiveness tests made in cages were carried out to evaluate the performance and the sexual compatibility of sterile male flies produced in Madeira against wild Mediterranean fruit fly males and females from Terceira Island. Following these studies, it was necessary to determinate what form of release of flies was the most appropriate for Terceira Island orchard conditions and release trials were begun in 2005. In 2007 a similar study was also made in the urban area of Angra do Heroísmo, the major city of Terceira Island.

Four dispersal tests were done from 2005 to 2007: on July 16th, 2005, on July 19th, and two in 2007, on September 4th and 5th, 2007. Tests were conducted to evaluate the sterile male dispersal over four different areas (three rural and one urban) in Terceira Island. These tests were made in an effort to conclude if the SIT application on Terceira could contribute to the eradication or suppression of the Mediterranean fruit fly population as one major tool of introducing the applied integrated plant protection methods against this key pest of Terceira fruit production areas.

Material and methods

1st and 2nd dispersal tests

Working closely with the technicians from the Madeira-Med programme, sterile males of Mediterranean fruit fly produced in Madeira facilities were imported as pupae and two initial dispersal tests were conducted, one in 2005 at Bicas rural area and another in 2006 in an apple orchard at Biscoitos. These tests were to demonstrate the kind of dispersal that proved to be more efficient under Terceira Island conditions, one based on a single dispersal point or distribution of several points of dispersal in a single strait line. These two tests were firstly projected on a computer using ArcGIS 8 software and placed in the field setting using a Garmin GPS. After the release two concentric circles of traps were projected to place in the field using AcrGIS 8, using 30 traps each at 100 and 200 meters from the central release point. The traps used in all these tests were Delta traps (Jackson type) with a specific sexual pheromone (trimedlure) that attracts only *C. capitata* males.

The release points were also projected in this software and they featured a line crossing the inner circle with 7 points spaced at 50 meter intervals. The trap canvasses were made 24h, 72h and 8 days after the release. The males released at the centre were marked with red colour and those released at the other 7 points were marked with green colour. After collecting the adults from the two trap circles (100 and 200 meters) those coloured were identified using an ultra violet light that scans all the surface glue coated base of all the traps.

In the first dispersal test at Bicas about 114 thousand sterile males were released. The Bicas farm is characterized by being a miscellaneous orchard with citrus, chestnut, bananas, and other small fruit trees. The second dispersal test took place at an orchard at Biscoitos. The selected area is mainly occupied by apple trees and was conducted in 2006 with a release of 100 thousand sterile males.

3rd and 4th dispersal tests

From Madeira-Med programme facilities sterile males of Mediterranean fruit fly were imported as pupae and two dispersal tests were carried out in the late summer of 2007, one in the apple groves at Biscoitos and another in the urban area of Angra do Heroísmo, the major city of Terceira Island.

The major goal of these two tests was to know and understand the dispersal behaviour of the sterile males after their release in the apple groves area and in the backyards that normally have some citrus trees, loquats and figs to know the performance of the sterile adults after their release in these environments. In these tests the release of the flies was from several points (7, spaced at 50 meter intervals) in a straight line. These two tests were also projected on computer using ArcGIS 8 software and placed in the field using a Garmin GPS. After the release were traps were placed in the field using the AcrGIS 8, two concentric circles of 30 traps in each at 100 and 200 meters from the central release point of the seven used (Fig. 1). The traps used were Delta traps (Jackson type) with a specific sexual pheromone (trimedlure) that attracts only *C. capitata* males. These seven release points were also projected in this software and they featured a line crossing the inner circle with 7 points spaced at 50 meter intervals. The traps were collected 24h, 72h and 8 days after the sterile adult male release. The males released were marked with red colour to easily be detected when passing the ultraviolet light on the glue coated trap base. In the first dispersal test at Biscoitos about 75 thousand sterile males were released and in the Angra urban area 150 thousand were released.

Results and discussion

The applicability of the GIS software in all these dispersal tests was extremely important allied with the utilization of the computer program ArcGis connected with the Garmin GPS to analyze all field data obtained, which permitted us to understand the dispersal behavior of the sterile adult males released in all the areas studied.

1st dispersal test

The data from the first dispersal test that took place in 2005 in Bicas and where 114 thousand sterile males were liberated, it was only possible to recapture 4.2% of the released sterile males. From these 2.4% had green colour and 0.05% red. 24 hours after the release most of the two circles of traps registered green fly captures and only one trap from the internal ring captured 2 red flies (Fig. 2, 3 and 6).

From the data obtained in the traps the three-dimensional GIS map shows the adults after release appear to look for a shelter and only fly and disperse in the low altitude areas. 72 hours after all the traps in the two rings continue to capture flies from the 7 points of dispersal but the number of those that were released from the central point increased, and it was possible to capture red flies in the two rings. Eight days after dispersal the amount of green flies was still the same and the amount of the red increased, especially in the outer ring.

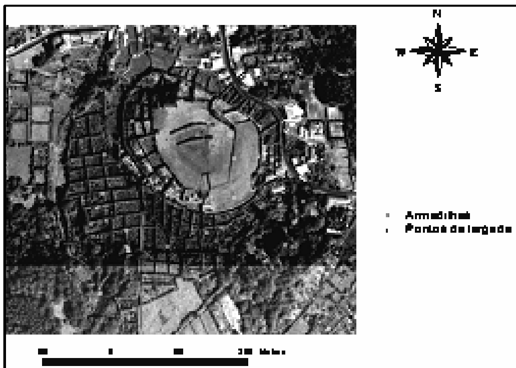

Fig. 1 – GIS map with the dispersal points and the two circles at Bicas grove in 2005

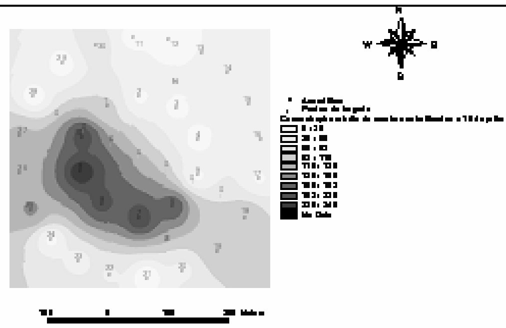

Fig. 2 – GIS map of *C. capitata* adult sterile males 24 h after their release from 7 points at Bicas grove in 2005

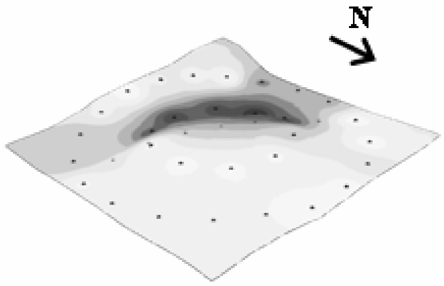

Fig. 3 – GIS three dimensional map of *C. capitata* adult sterile males 24 h after their release from 7 points at Bicas grove in 2005

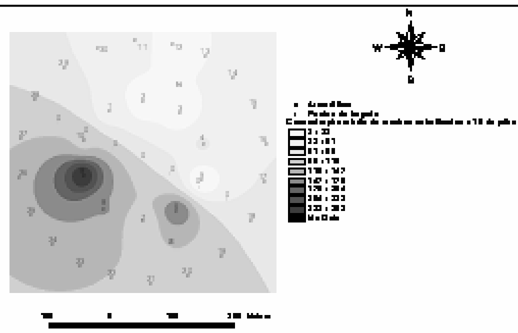

Fig. 4 – GIS map of *C. capitata* adult sterile males 72 h after their release from the 7 points at Bicas grove in 2005

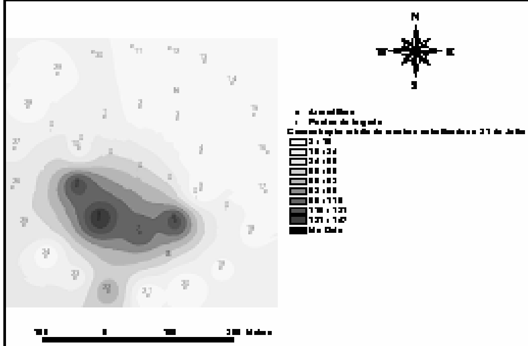

Fig. 5 – GIS map of *C. capitata* adult sterile males 8 days after their release from the 7 points at Bicas grove in 2005

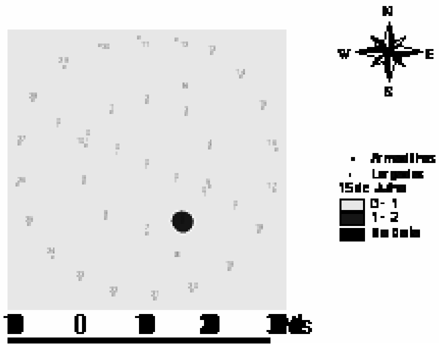

Fig. 6 – GIS map of *C. capitata* adult sterile males 24 h after their release from the central point at Bicas grove in 2005

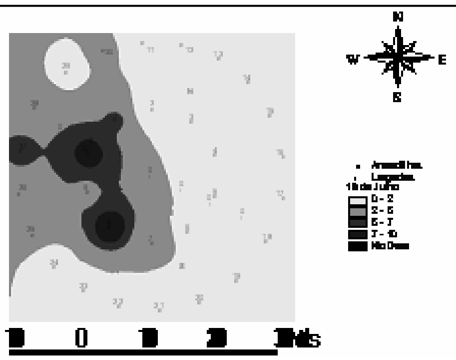

Fig. 7 – GIS map of *C. capitata* adult sterile males 72 h after their release from the central point at Bicas grove in 2005

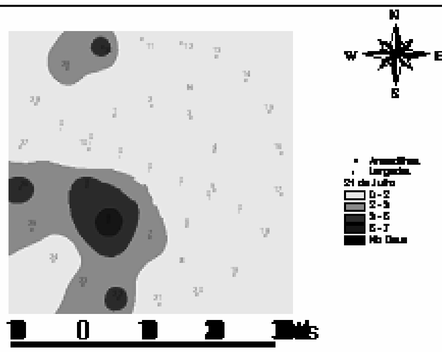

Fig. 8 – GIS map of *C. capitata* adult sterile males 8 days after their release from the central point at Bicas grove in 2005

Analysing all the data obtained from this dispersal test we can conclude that 24 hours after the release the green sterilised adult males from the 7 points covered all the orchard area and those released from the central point only achieved that eight days after. The results point to the fact that the seven points of release seem to be the better technique to achieve a fast and better dispersal in these conditions (Lopes *et al.*, 2005b; 2005c; Pimentel, 2005a; 2005b).

2nd dispersal test

The second dispersal test took place in 2006 at the northern part of Terceira Island, in Biscoitos apple groves. In an apple orchard 100 thousand sterile males were released, once more imported from the Madeira-Med program facilities. Once more the goal of this dispersal test was to study the dispersal behavior of the sterile males after release from several points and from one single point. From this single point were released 50 thousand "red" males and from the others six points were liberated 8,333 per point (Fig.9) (Lopes, 2006; 2007). The sterile males were imported as pupa from Madeira but did not emerge properly and some of them were not in good condition. That fact had some repercussion on the results obtained in the field after their release. Nevertheless, 24h and 72h (Fig. 10 and 11) after release some dispersal movement was detected in the sterile adult males that were released from the several points because some of them (50 adults) were captured in the external circle of traps (Fig.11). Those released at the central point 24 hours after showed only a little fly movement to the North of the release point and they were captured at lower levels in the traps, showing some difficulty in terms of dispersion (Fig. 12). As a result it was decided to make another dispersal test in the same area in 2007.

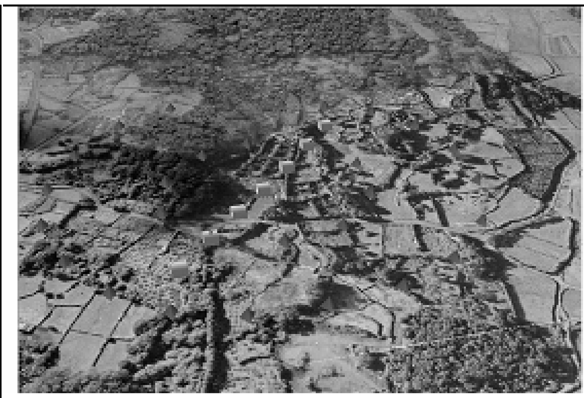

Fig. 9 – GIS map with the dispersal points and the two trap circles at Biscoitos apple grove in 2006

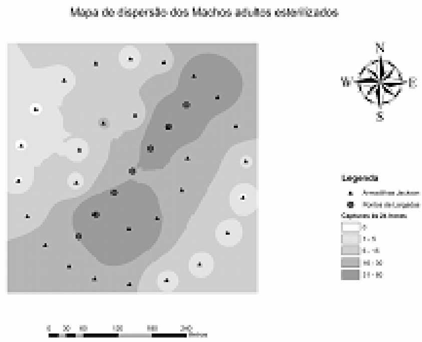

Fig. 10 – GIS map of *C. capitata* adult sterile males 24 h after their release from the 7 points at Biscoitos apple grove in 2006

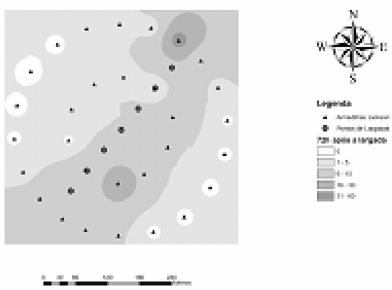

Fig. 11 – GIS map of *C. capitata* adult sterile males 72 h after their release from the 7 points at Biscoitos apple grove in 2006

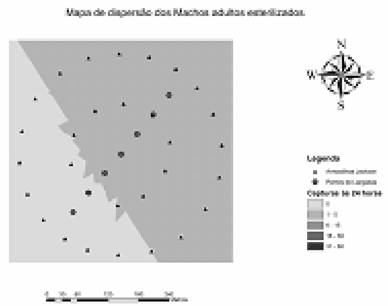

Fig. 12 – GIS map of *C. capitata* adult sterile males 24 h after their release from the central point at Biscoitos apple grove in 2006

Fig. 13 – GIS map with the dispersal points and the two trap circles at Biscoitos apple grove in 2007

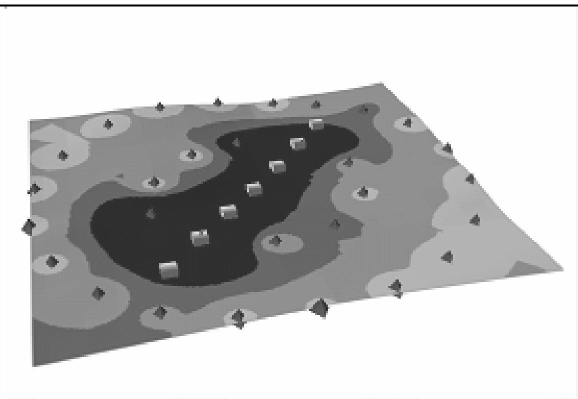

Fig. 14 – GIS map of *C. capitata* adult sterile males 24 h after their release at Biscoitos apple grove in 2007

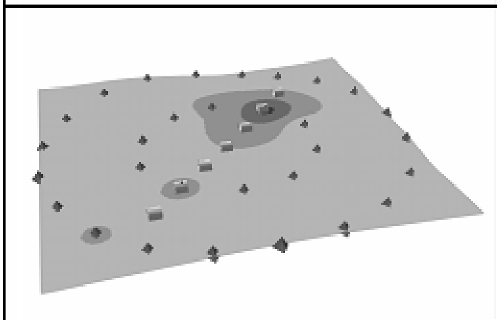

Fig. 15 – GIS map of *C. capitata* adult sterile males 72 h after their release at Biscoitos apple grove in 2007

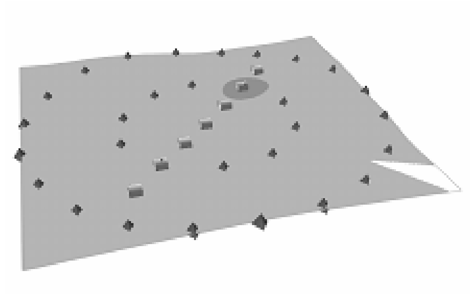

Fig. 16 – GIS map of *C. capitata* adult sterile males 8 days after their release at Biscoitos apple grove in 2007

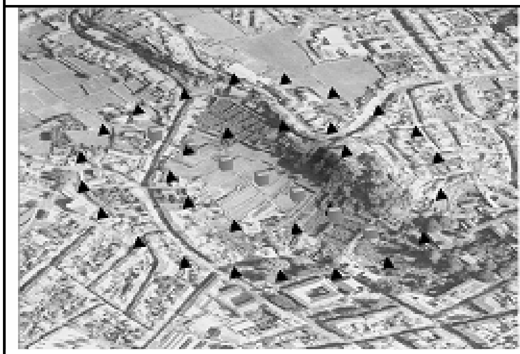

Fig. 17 – GIS map with the dispersal points and the two trap circles at Angra in 2007

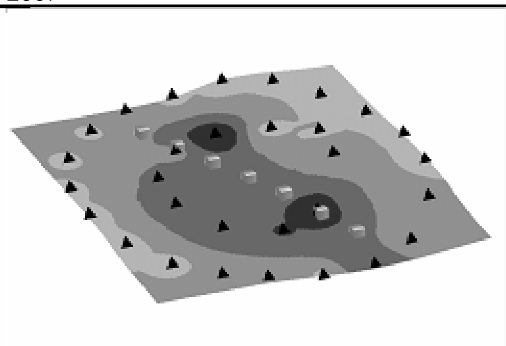

Fig. 18 – GIS map of *C. capitata* adult sterile males 24 h after their release at Angra in 2007

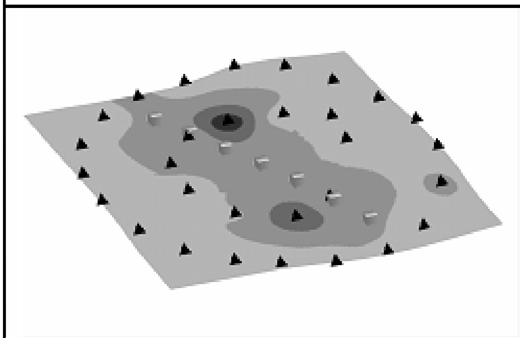

Fig. 19 – GIS map of *C. capitata* adult sterile males 72 h after their release at Angra in 2007

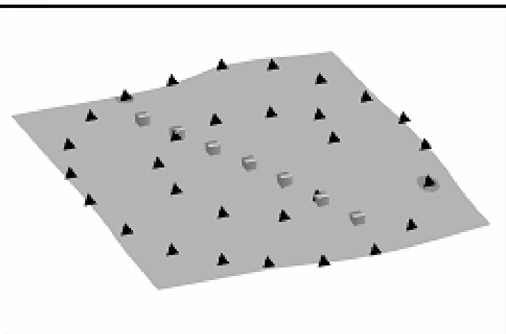

Fig. 20 – GIS map of *C. capitata* adult sterile males 8 days after their release at Angra in 2007

3rd dispersal test

The third dispersal test took place in 2007 at the northern part of Terceira Island, in the same Biscoitos apple grove used in 2006. In that apple orchard were liberated 75 thousand sterile males from seven points in a straight line (Fig.13). These *C. capitata* sterile males were once more imported from the Madeira-Med program facilities. The goal of this dispersal test was to study the dispersal behavior of the sterile males after release in that apple grove situated in the fruit production area (Lopes, 2006; 2007). After 24h and 72hours (Fig. 14 and 15) almost all the adults sterile males were well distributed in all the orchard area and surroundings and only a few of them stayed near the original release point. From these results we can conclude that the flies released have an excellent behaviour that permitted their fast and efficient spread over the entire apple grove because some of this population quickly reached the exterior circle of traps at 200 meters (Fig. 14 and 15).

4th dispersal test

The fourth dispersal test took place in 2007 in the urban area of the major city of Terceira Island, Angra do Heroismo, more precisely in the backyards of the urban area houses where 150 thousand sterile males were released, again imported from the Madeira-Med program facilities. The goal of this dispersal test was to study the dispersal behavior of the sterile males after release in the urban area. These sterile adult males were also released from seven points in straight line (Fig.17).

After 24h and 72hours (Fig. 18 and 19) almost all the adult sterile males were well distributed in all the area and stayed there even a week after the release. From these results we can conclude that the flies released have an excellent behaviour that permitted their fast and efficient spread over the entire urban area because most of the sterile males quickly reached the exterior circle of traps at 200 meters (less than 24 hours after their release) (Fig. 18).

Even eight days after their release the sterile males were in the surroundings and inside the dispersal area (Fig. 20). It is important to note that there is a perfect area match between the *C. capitata* wild male population and those sterile males that were released (Fig. 18, 19, 21 and 22). That fact indicates that in this area there was likely to be competition for the Mediterranean fruit fly females between the sterile and wild males.

All four dispersal tests gave a major contribution to know the dispersal behavior of *C. capitata* sterile males in Terceira and permitted us to conclude that SIT could work in Terceira island conditions. With the use of these biotechnical means to fight against the Mediterranean fruit fly it is possible to achieve a greater goal of total implementation of integrated pest management instead of the application of traditional plant protection measures in Terceira Island, Açores.

Acknowledgements

This work was financially supported by the Azores Regional Government; In addition, INTERREG III-B European Community Program funded all the investigation done in the INTERFRUTA I and II Projects; Our thanks are expressed to all of the partners and their investigators who worked in the INTERFRUTA I and II projects, especially those from the Madeira-Med programme from Madeira; We also thank the fruit producers on Terceira who cooperated and allowed us to work in their orchards; To the Serviço de Desenvolvimento Agrário da Terceira (SDAT) for all the support of their technicians, particularly Eng.ª Maria Luísa Ornelas for all the field work done; To the Terceira Fruit Producers Cooperative (FRUTER) and in a special way to their technicians, Maria da Conceição Filipe Carvalho, António Domingues and Mónica Melo, for their collaboration in the dispersal of the sterilised males in the study areas; To the two the fruit producer owners of the orchards from Biscoitos and

Bicas that gave all their collaboration that made it possible to achieve these project goals. This work was financially supported by the EC Program INTERREG III-B and resulted from the INTERFRUTA I (MAC/3.1/A1) and INTERFRUTA II (05/MAC/3.1/A4) projects.

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Effectiveness of clays and copper products in the control of *Ceratitis capitata* (Wiedemann) in organic orange orchards

V. Caleca, G. Lo Verde, M. Palumbo Piccionello & R. Rizzo

Dipartimento S.En.Fi.Mi.Zo., Sezione di Entomologia, Acarologia e Zoologia, Università degli Studi di Palermo, Viale delle Scienze, 90128 Palermo, Italy, E-mail: caleca@unipa.it.

Abstract: Medfly, *Ceratitis capitata* (Wiedemann), is the key pest of early ripening citrus cultivars. Its control in organic groves is usually difficult, due to the almost complete lack of permitted effective insecticides.The research was carried out in 2005 and 2006, to evaluate in the field the repellent and antiovipositional action of clays and copper products to *C. capitata.* Tested products are known to limit another tephritid, *Bactrocera oleae* (Gmelin). Trials were carried out in an organic orange orchard located at Castelvetrano (Trapani Province, Sicily). Kaolin, copper hydroxide and copper oxychloride (in 2005), and kaolin, bentonite and copper hydroxide (in 2006) were tested and compared with an unsprayed plot. In both years, data on infested fruits were collected at the harvest, recording the presence of medfly punctures. Total infestation on kaolin treated fruits (29% in 2005, 62% in 2006) was significantly lower than copper hydroxide (50% in 2005, 82% in 2006) and control theses (73% in 2005 and 88% in 2006); no difference was found between the last two treatments. No statistically significant differences were found comparing white and blue copper oxychloride with control and kaolin. In 2006 the infestation level on fruits treated with bentonite was 74%, significantly lower than control, but higher than kaolin. In the same year no statistically significant differences among treatments were recorded in fruit drop.

Key words: Medfly, organic agriculture, kaolin, bentonite, copper hydroxide and copper oxychloride

Introduction

Medfly, *Ceratitis capitata* (Wiedemann), is the key pest of early ripening citrus cultivars. Its control in organic groves is usually difficult, due to the almost complete lack of permitted effective insecticides.

Products containing clays and copper were tested in the past (Russo, 1937; Russo and Fenili, 1949; Russo, 1954) and more recently (Prophetou-Athanasiadou et al., 1991; Tsolakis and Ragusa, 2002; Saour and Makee 2004; Belcari et al., 2005; Caleca and Rizzo, 2006) against *B. oleae* obtaining positive results, mostly for their repellent and antiovipositional action.

Mazor and Erez (2004) demonstrated a positive effect of kaolin also in controlling *C. capitata* in nectarines, apples and persimmons. Although Marchini and Wood (1983) tested a repellent action of copper sulphate towards medfly ovipositing females, no field work on the effectiveness of copper products against this tephritid has been realised.

Along the two years of the research we tested the effectiveness of some products containing clays (kaolin or bentonite), copper hydroxide and copper oxichloride in an organic orange orchard.

Material and methods

Trials were carried out in an organic orange orchard consisting of 250 trees (cv. *Navelina*) located at Castelvetrano (Trapani Province, Sicily). Medfly population was monitored using three traps baited with trimedlure; caught males were counted every week in 2005, and every two weeks in 2006.

As shown in table 1, in 2005 kaolin, copper hydroxide and copper oxychloride (white and blue formulation) were tested and compared with an unsprayed plot (control); a single spray was applied. In 2006 kaolin, bentonite and copper hydroxide were tested and compared with an unsprayed plot (control); two sprays were performed.

In both years, data on infested fruits were collected on 10 trees of each plot. At the harvest, from 60 to 100 fruits per tree were examined, recording the presence of medfly punctures; in 2005 harvest occurred on 27th November; in 2006 on 15th, 24th and 30th of November. In 2006 all fruits on each sampled tree were counted at the beginning of the trial and fruits dropped because of medfly attack were counted every 14 days.

Data on total infestation and dropped fruits were statistically analysed using 1-way ANOVA, followed by Tukey post-hoc test ($p<0.05$). Data on temperature and rainfall were kindly provided by SIAS (Servizio Informativo Agrometeorologico Siciliano of the Sicilian Region); they are from Castelvetrano Seggio SIAS weather station.

Table 1. Tested products and their doses in 2005 and 2006.

Commercial product	Composition	Dose per treatment (kg/ hl of water)	2005	2006
Surround WP	95% kaolin	5	x	x
Coprantol Ultramicron	35% copper as hydroxide	0.3 (copper= 0.11)	x	x
Cuprobenton	15% copper as oxychloride and sulphate + 70% bentonite	0.8 (copper= 0.12; bentonite= 0.6)	x	
Blue Cuprobenton	As above, but blue coloured	As above	x	
Bentonite AG/W8	100% bentonite	5		x
Unsprayed			x	x

Results and discussion

In 2005 (Fig. 1) the highest captures, 46 and 48 males per trap were recorded on 8th and 22nd October respectively; in November captures gradually decreased below 20 males per trap reaching a minimum of 4 captures on 19th November. The only day of October with heavy rain (34 mm) was 26th.

In 2006 (Fig. 2) the trend of medfly captures was quite different in comparison with the previous year.The highest captures, 27 and 23 males per trap, were recorded on 4th and 20th September respectively; the number of caught males decreased to 0.3 males per trap on 18th November; after this low level, captures increased again reaching again 18 males per trap on 18th November. As consequence of the rainy period recorded from 13th to 20th October (81 mm of rainfall) the second treatment was performed on 22nd October.

Figure 1. Trend of medfly captures, air temperature and rainfall in 2005.

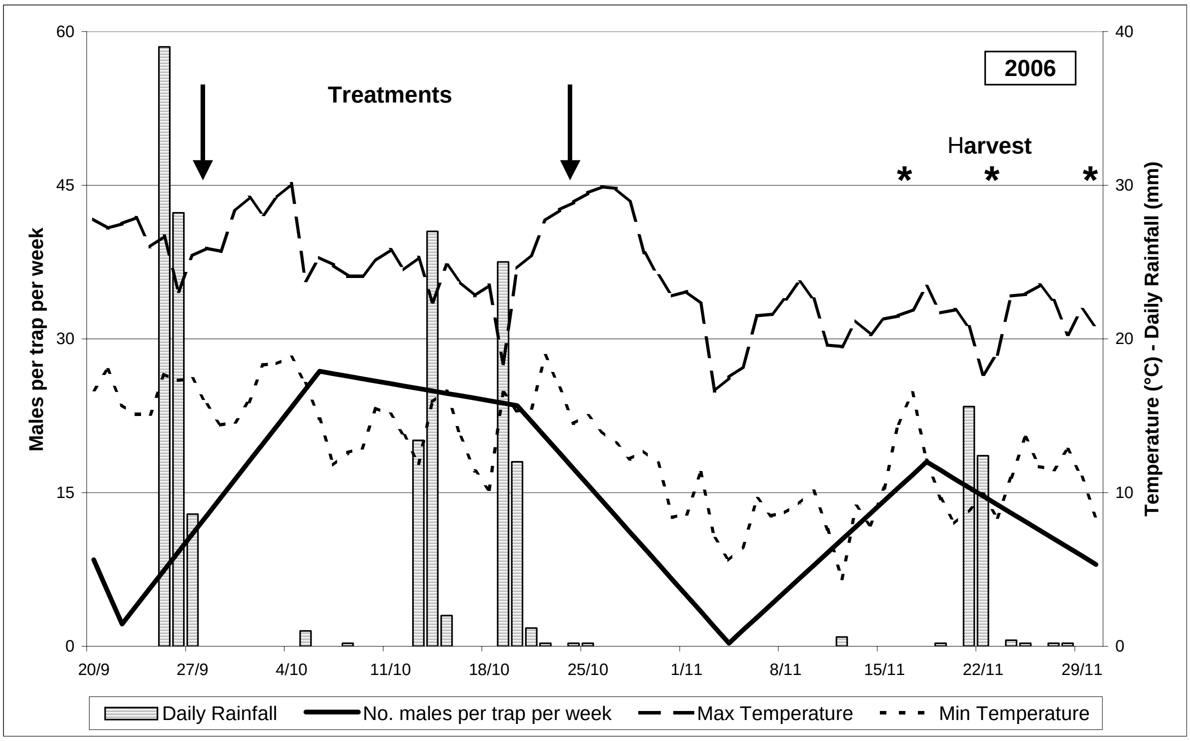

Figure 2. Trend of medfly captures, air temperature and rainfall in 2006.

As shown in Figs. 3, 4, and in Tab. 2 total infestation on kaolin treated fruits (29% in 2005, 62% in 2006) was significantly lower than copper hydroxide (50% in 2005, 82% in 2006) and control theses (73% in 2005 and 88% in 2006); no difference was found between these two products and white and blue copper oxychloride, whose intermediate values did not show significant differences when compared with control and kaolin. In 2006 the infestation level on fruits treated with bentonite was 74%, significantly lower than control, but higher than kaolin thesis (Figs 3,4). In the same year no statistically significant differences among treatments were recorded in fruit drop (Tab. 2).

Table 2. Infestation due to *C. capitata* recorded in Navelina oranges in 2005 and 2006 (mean % ± standard error, different letters within each column denote statistically significant differences; 1-way ANOVA followed by Tukey post-hoc test; $p < 0.05$).

Theses	**2005**	**2006**	
	Punctured oranges at harvest	**Punctured oranges at harvest**	**Fruit drop due to *C. capitata***
Surround WP	19 ± 4.2 b	60 ± 2.2 c	2.6 ± 0.89 a
Coprantol Ultramicron	40 ± 5.2 a	78 ± 2.2 a	3.9 ± 0.89 a
Cuprobenton	27 ± 4.6 ab	-	-
Blue Cuprobenton	32 ± 4.7 ab	-	-
Bentonite AG/W8	-	70 ± 2.4 b	4.5 ± 0.99 a
Untreated	43 ± 5.2 a	83 ± 2.2 a	4.4 ± 0.89 a

Our results demonstrate that tested clays, kaolin and bentonite, reduce punctures on oranges, while no repellent effect of copper hydroxide was recorded on medfly infestation.

The results of bentonite, worse than kaolin, are probably linked to its limited permanence on fruits, since the need of protection for oranges occurs in a rainy period. More sprays, also for kaolin, are necessary to adequately control *C. capitata*.

Surround WP confirms its effectiveness (Mazor and Erez, 2004), but other clay products tested in orange orchards and olive groves are much cheaper than it; attention has to be paid in improving the permanence of clays on fruits and in the evaluation of the economic convenience of more treatments with clays less effective but cheaper than Surround WP.

Powder and powdery products are known for their negative effects on parasitoids of scales (Alexandrakis and Neuenschwander, 1979), but the use of clays in orange orchards during a so limited and rainy period (October and November) should not significantly affect parasitoid fauna.

Clays are very useful tools to control tephritid and other insect, and are environmental friendly, but until now they are not allowed as products for plant protection in European and Swiss organic farming; kaolin is allowed in U.S.A. organic farming.

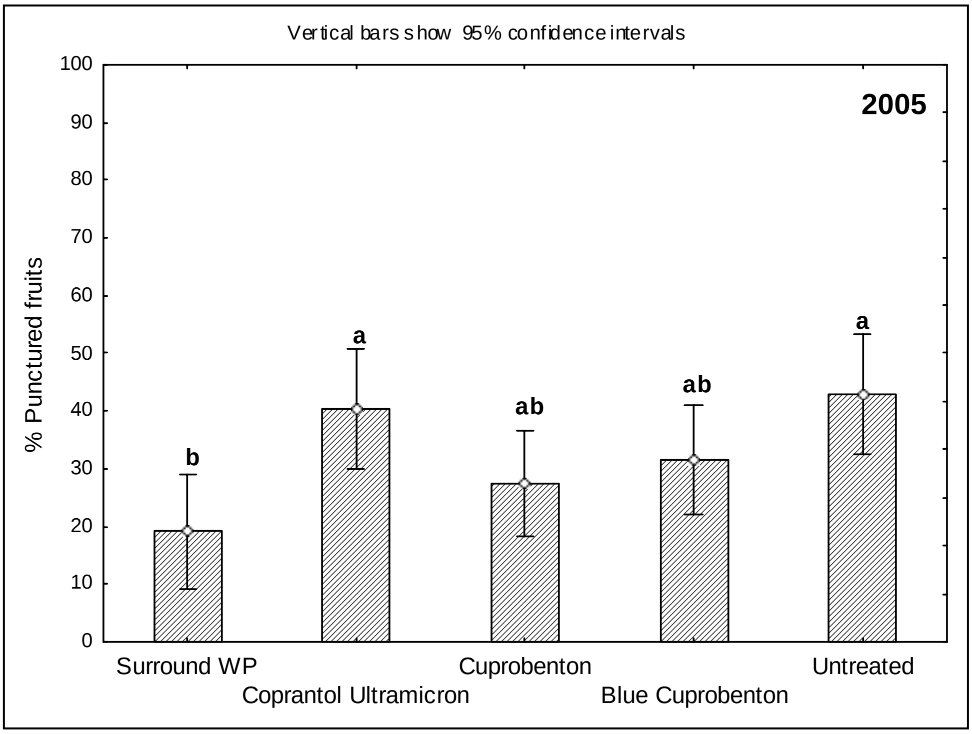

Figure 3. Total infestation on harvested orange fruits in 2005 (different letters denote statistically significant differences).

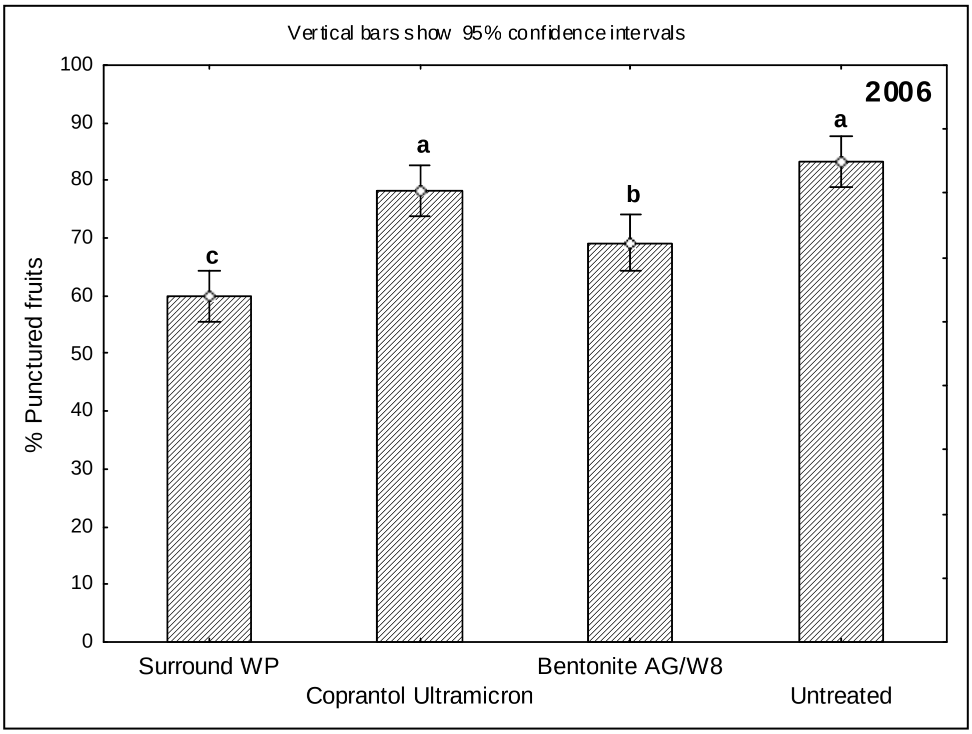

Figure 4. Total infestation on harvested orange fruits in 2006 (different letters denote statistically significant differences).

Acknowledgements

We thank Failla brothers, owners of the orange orchard, Calogero Ciaccio and Giuseppe Fontana for their help in collecting data.

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Characterization of a *Bacillus thuringiensis* strain collection isolated from Spanish citrus agro-ecosystem and evaluation of insecticidal activity on *Ceratitis capitata* (Diptera: Tephritidae)

J.C. Vidal Quist, P. Castañera Domínguez, J. González Cabrera
Unidad Asociada de Entomología IVIA-CIB CSIC. Instituto Valenciano de Investigaciones Agrarias (IVIA). Ctra. De Moncada a Náquera km 5. 46113, Moncada, Valencia, Spain; cvidal@ivia.es

Mediterranean fruit fly is one of the most devastating fruit pests worldwide, current control is mainly based on synthetic insecticides. The environmental impacts they produce, in addition to development of resistance justify the need to implement sustainable control alternatives. *Bacillus thuringiensis* Berliner (Bt) based products lead bioinsecticide market. They have been proven to be active against insects of many orders, including dipterans. However, no active strain against *Ceratitis capitata* Wiedemann has been described to date.

In the present study a collection of 374 Bt strains has been developed from samples collected in citrus agro-ecosystem in Valencian Community (Spain). The collection was characterised by means of phase-contrast microscopy, SDS-PAGE and PCR reaction to detect 20 genes of cry and cyt protein toxins. Groups of genes codifying for toxins active against lepidopteran, coleopteran, nematode and dipteran species were selected.

PCR analysis identified 17 combinations among selected genes, being more abundant those effective against lepidopterans, present in more than half of the strains. Protein electrophoresis revealed 67 different profiles that, in many cases, could be correlated with bacterial morphology and gene composition. Toxicity bioassays against *C. capitata* were carried out for all strains in the collection, registering maximum mortalities of 30%. Additionally bioassays with isolates from other collections (509 strains) were performed, showing similar mortality levels.

Citrus Leaf Miner

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Citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracilariidae) and its parasitoids. Ten years after the implementation of Classical Biological Control in Spain

F. Karamaouna[1], S. Pascual[2], A. Urbaneja[3], J.A. Jacas[2]

[1] *Benaki Phytopathological Institute; Department of Pesticides' Control and Phytopharmacy, Laboratory of Efficacy Evaluation of Pesticides, 8 St. Delta Street, 14561-Kifissia, Athens, Greece*

[2] *Associate Unit UJI-IVIA; Universitat Jaume I; Campus del Riu Sec; E – 12071 – Castelló de la Plana, Spain*

[3] *Associate Unit UJI-IVIA; Institut Valencià d'Investigacions Agràries; Ctra. Montcada-Nàquera km 4.5; E – 46113 – Montcada, Spain*

The citrus leafminer *Phyllocnistis citrella* is a pest which originates from eastern and southern Asia and posed a serious threat to the citrus industry of the Mediterranean region upon its introduction in Spain in 1993 and its subsequent spread. Today, fourteen years after the introduction of the pest and the following implementation of a Classical Biological Control Programme comprising the introduction of six exotic parasitoid species (*Ageniaspis citricola*, *Quadrastichus* sp., *Semielacher petiolatus*, *Galeopsomyia fausta*, *Cirrospilus ingenuus* and *Citrostichus phyllocnistoides*) for the control of the pest, the citrus leafminer does not cause damage of economic importance on mature trees whereas a new balance of the parasitoid complex-pest system has established in the area of release. Monitoring of the incidence and quantification of the impact of both exotic and indigenous parasitoids and predators on the control of the citrus leafminer in mandarin orchards at three different locations in Eastern Spain showed that the exotic parasitoid *Citrostichus phyllocnistoides* is the predominant species, holding the 99.42% of the total in the survey. Indigenous parasitoid species represented minor percentages of the total: *Pnigalio* spp. (0.33%), *Cirrospilus brevis* (0.06%), *Sympiesis gregori* (0.06%), Pteromalidae species (0.11%) and *Diglyphus* sp. (0.03%). The results provide insights into the interactions between the exotic and the indigenous natural enemies of the citrus leafminer and will contribute on the evaluation of the Classical Biological Control Programme.

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Evolution of *Phyllocnistis citrella* Stainton (Lepidoptera, Gracillariidae) and its parasitoids in the last five years in citrus orchards of western Sicily (Italy)

Lo Genco Alessandro, Ciotta Concetta and Lo Pinto Mirella
Dipartimento Scienze Entomologiche, Fitopatologiche, Microbiologiche e Zootecniche (SEnFiMiZo), Università di Palermo, Viale delle Scienze 13, 90128 Palermo, Italy
logenco@virgilio.it, lopinto@unipa.it

Abstract: *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is native to India, China, and other Southeast Asian countries that has spread rapidly since 1993 to all citrus-growing areas of the world. In Italy, the pest was first discovered during the autumn of 1994, in some citrus groves of Sardinia and, subsequently, in Sicily in the summer of 1995 showing a very rapid range expansion in other citrus-growing regions of Italy. Currently, damage to mature trees under typical Mediterranean conditions is considered only esthetical, but *P. citrella* causes economic problems on young trees, nurseries, and overgraftings. Since the first occurrence of the citrus leafminer in Sicily, several indigenous natural enemies have been found attacking the pest, although only few parasitoid species were observed living on this phytophagous in the last years. The aims of this study were to monitor the population dynamics and mortality of *P. citrella*, and its natural enemies with parasitism levels, from 2002 to 2006, in some unsprayed citrus orchards in western Sicily, Italy. Results showed differences on dynamics of stages of *P. citrella* and of its parasitoid complex related to climate effects. Also, the monthly percentage mortality and parasitism are reported. The major percentage of parasitism was imputable to *Citrostichus phyllocnistoides* Narayanan and *Semielacher petiolatus* (Girault).

Key words: CLM dynamics, parasitism, mortality

Introduction

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae: Phyllocnistinae) is a phytophagous native from Southeast Asia. In Italy, the pest was first discovered during the autumn of 1994 in some citrus groves of Sardinia (Benfatto, 1995; Ortu *et al.* 1995) and subsequently in Sicily in the summer of 1995 (Balzani *et al*., 1995; Longo and Siscaro, 1995; Liotta and Manzella, 1995) showing a rapid range expansion in other citrus-growing regions of Italy.

Damage is caused by the larvae, producing serpentine mines on young and tender leaves and shoots. The larvae, contrarily to other miners, do not feed on the foliar parenchyma, but only from the juices that pour from it when the cuticle is separated (Garrido Vivas, 1995). The pest attacks all citrus cultivars but it is an economic problem only on re-grafted plants (Caleca *et al.*, 1995, 1997, 2000), and an aesthetic damage on ornamental citrus (Del Bene & Landi, 1999), on young plants in nurseries (Caleca,2000).

Biological control is a rational approach to reducing damage by citrus leafminer. Since the first occurrence of the citrus leafminer in Sicily, several indigenous natural enemies have been found as attacking the pest (Liotta *et al.*, 1996). Several species of exotic parasitoids were introduced and released in citrus groves of Sicily (Siscaro *et al.* 1997; Mineo and Mineo, 1999). The most abundant species, in the last five years are the exotic ectoparasitoids *C. phyllocnistoides* Narayanan, and *S. petiolatus* (Girault) (Liotta *et al.*, 2003). The aims of this study were to monitor the population dynamics, parasitism levels and mortality of *P.citrella*, from 2002 to 2006, in some unsprayed citrus orchards in western Sicily.

Material and methods

This study was carried out during four consecutive years, from June 2002 to September 2006, in organic citrus orchards, located in areas of Trapani and Palermo (western Sicily, Italy). The orchards were planted to orange, and lemon. No insecticides were applied to the organic citrus orchards. At each location, 200 citrus tender leaves were randomly collected every 15 days, placed in plastic bags, and taken to the laboratory for examination under a stereomicroscope. Numbers of leaves sampled and total number of *P. citrella* (sum of live and dead larvae, pupae, pupal cases) were recorded. Apparent percentage parasitism was calculated. Mortality was calculated by dividing the number of hosts with parasitoid eggs, larva or pupa plus number of hosts killed by a parasitoid or by a predator including the unknown mortality with total number of CLM (living and dead) (Amalin, 2002).

Results and discussion

Population Dynamics

The seasonal trend of citrus leafminer population observed during the 4 years of study was similar. Population of *P. citrella* (Fig.1) began to increase in June-July, and reached its maximum in August, followed by a decline until the first moths of following year. There were no *P. citrella* individuals in April and May.

The comparison of the years 2002 and 2003 revealed that population of *P. citrella* began to infest citrus orchard at the end of spring (late June), peaking at 2.92 immatures/leaf in late August 2002 and 1.42/leaf in late August 2003. By September, the CLM population decreased from 1.29/leaf to 0.01/leaf in January 2003, from 1.01/leaf to 0.01/leaf in March 2004.

In the following years, from 2004-2006, the infestation were observed to increase earlier (early June) compared to previous years (late June), with exception of 2005 (early July).The highest values of infestation were observed in early August, reaching peaks of 1.09/leaf in 2004, 2.26/leaf in 2005 and 2.03/leaf in 2006. By late August, *P. citrella* density began to decline from 0.97/leaf to 0.01/leaf in early March 2005 and from 1.79/leaf to 0.01/leaf in late March 2006.

Parasitism

In early July 2002, the percentage parasitism (Fig.2) of *P. citrella* began to increase from 33.33 %, peaking on November at 48.15%. The maximum percentage parasitism in 2003 was 64.52% in December. In both years, parasitoids were not found from January to June, coinciding with absence of *P. citrella* infestation. In contrast, parasitism in January 2004 was recorded. Parasitism levels were recovered early in the year 2004 (late June) compared to 2003 (July), reaching a peak of 54.21% in December. In 2005, the maximum percentage parasitism was 70.83% in late January and 62.61% in late September. In the following year 2006, the highest percentage of parasitism, 61.51%, was observed in late August. These two years were characterized by the high values of parasitism from January to early March. The major percentage of parasitism was imputable to *Citrostichus phyllocnistoides* Narayanan and *Semielacher petiolatus* (Girault).

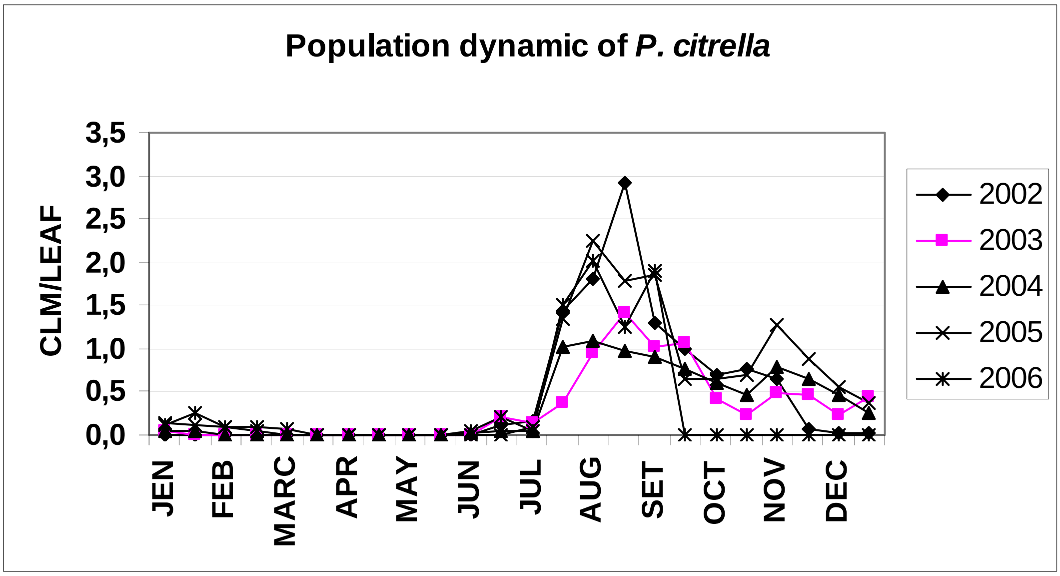

Fig1. Population dynamics of *P. citrella* from 2002 to 2006.

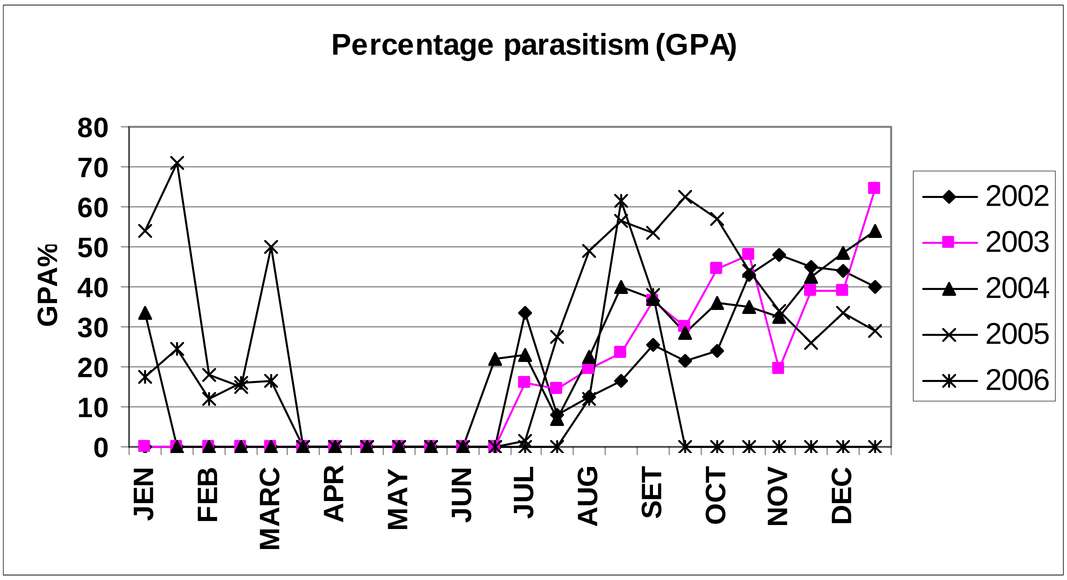

Fig 2. Percentage parasitism (GPA) of *P. citrella* from 2002 to 2006.

Mortality

There were differences in the mortality patterns from 2002 to2003 and 2004 to2006 (Fig3). In 2002 and 2003 *P. citrella* mortality increased from June-July onwards, reaching peaks of 100% (2002) and 93.75% (2003) in late December. In both years, no mortality was observed from January to June.

In the last three years (from 2004 to 2006), mortality was observed in the winter (from January to March), through the summer (June to September), and fall (mid September to December). Mortality peaks were recorded from January to February 2004 (100%), late January 2005 (95.12%) and late March 2006 (100%). CLM mortality due to natural enemies was high during the winter in comparison to the first years. In 2005, CLM mortality was observed later thasn other years (late July), probably because of low mean monthly temperature during the winter (5-6 C°) and spring (<20 C°).

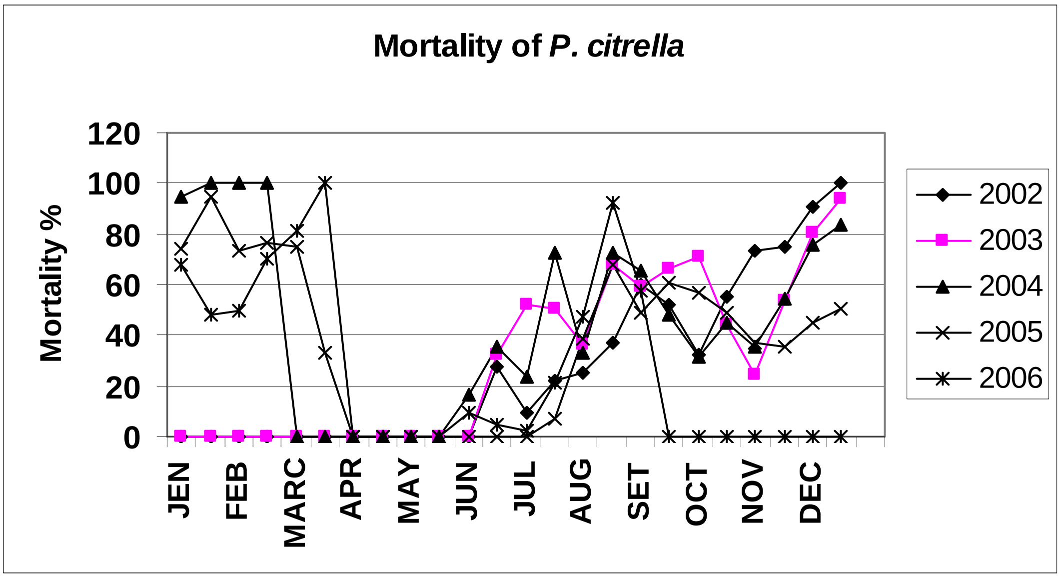

Fig 3. Mortality of P. *citrella* from 2002 to 2006.

Conclusion

After the introduction of *Citrostichus phyllocnistoides* in 1999 (Mineo and Mineo, 1999) and the accidental introduction of *Semielacher petiolatus* in 1998, the citrus leafminer population density decreased compared with the previous years. Ours study confirms that C. *phyllocnistoides* is the predominant parasitoid and one of the main factors responsible for the decline in leafminer population. But other factors such as predators contributed significantly to the overall management of *P. citrella* in the field as shown by the mortality estimates for five years.

Ours study shows that the period without CLM individuals become shorter from year to year and concentrated in two moths April and May, probably due to variation in climatic conditions among years.

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Bio-ecological study of the parasitoid complex of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in Western Algeria

Malika Boualem [1], Claire Villemant [2], Abdallah Berkani [1]
[1] Université Abdelhamid Ibn Badis, Faculté des Sciences et Sciences de l'Ingénieur, Département d'Agronomie, Laboratoire de Protection des végétaux, BP 188-CP Mostaganem, boualemmalika@yahoo.fr. [2] Muséum National d'Histoire Naturelle, UMR 5202 CNRS, CP 50 Entomologie, 45 rue Buffon, 75005 Paris, France.

Abstract: Samplings carried out in 2003-2005, from June to October, in a *Citrus sinensis* orchard of the Mostaganem wilaya (West Algeria) showed that *Phyllocnistis citrella* infestation was important, the percentage of attacked leaves always exceeding 95% in August. Maximum parasitism rates reached consistent values, from 45% in August 2003 to 78% in August 2005, while predation and other mortality factors remained less important. The 3rd larval instar appeared to be the most sensitive to parasitism and the two first ones the most affected by other mortality factors. Seven Hymenoptera Eulophidae parasitoids were recorded during the study period: *Semielacher petiolatus*, *Cirrospilus pictus*, *Cirrospilus vittatus*, *Pnigalio pectinicornis*, *Citrostichus phyllocnistoides*, *Closterocerus formosus* and the hyperparasitoid *Pediobius* sp. Among primary parasitoids, the indigenous *C.pictus* and the introduced *S. petiolatus* are the most efficient enemies. In the frame of CLM integrated control in Algeria, it should be recommended to reinforce the populations of its natural enemies, notably the two last species, in favouring their indigenous hosts and host-plants.

Key words: biological control, parasitoids, *Phyllocnistis citrella*, *Semielacher petiolatus*, *Cirrospilus pictus*, life cycle

Introduction

Phyllocnitis citrella Stainton (CLM) is a citrus pest native to southern Asia which dramatically spread since 1993 and is now widespread around all the major citrus-growing areas of the five continents. First reported in Algeria in 1994 (Berkani et al., 1995), CLM is now considered as one of the most important citrus pests of this country. It attacks all varieties of citrus and some related plant species (Legaspi & French, 2003). Damage are caused by the larvae forming serpentine mines in the leaves, in which they are well protected from insecticide sprays, making them difficult to control (Moreira et al., 2006).

It has been suggested that biological control can become a successful tool for the population regulation of this pest (Diez et al., 2006). Our purpose was to facilitate biological control of CLM, in inventorying its natural enemies and determining their life cycle in laboratory. As in many other areas, a reduction in the pest population has been observed in Algeria in relation to activity of introduced and indigenous natural enemies (Boualem et al., 2007).

Material and methods

Samplings were carried out in 2003-2005, from June to October, in a *Citrus sinensis* orchard of the Mostaganem area. To follow the evolution of *P. citrella* during the three years, ten trees were selected randomly, from which three leaves were taken each week at the four cardinal points from three levels of the plant: high, medium and low. The 360 leaves collected for each sample date were examined under the binocular microscope in order to count the different pre-imaginal instars of the pest as well as number of died and alive individuals. Identification of the various CLM instars referred to Badawy (1969).

For parasite study, 250 infested leaves were collected every weak. The parasitized CLM larvae or pupae were placed in Petri dishes containing the artificial diet developed by Murashigue and Skoog (1962). Parasitoid pupae were then individually reared in small vials. The parasitism, predation and natural mortality rates of the different CLM instars were established and the various hymenopteran parasitic species (7 Eulophidae and 1 Pteromalidae) identified under supervision of G. Delvare (CIRAD, Montpellier) (Boualem et al., 2007).

Results

Infestation rate

CLM infestation was always important, the percentage of attacked leaves reaching in August a maximum of 97% in 2003, 95% in 2004 and 99% in 2005 (Fig. 1).

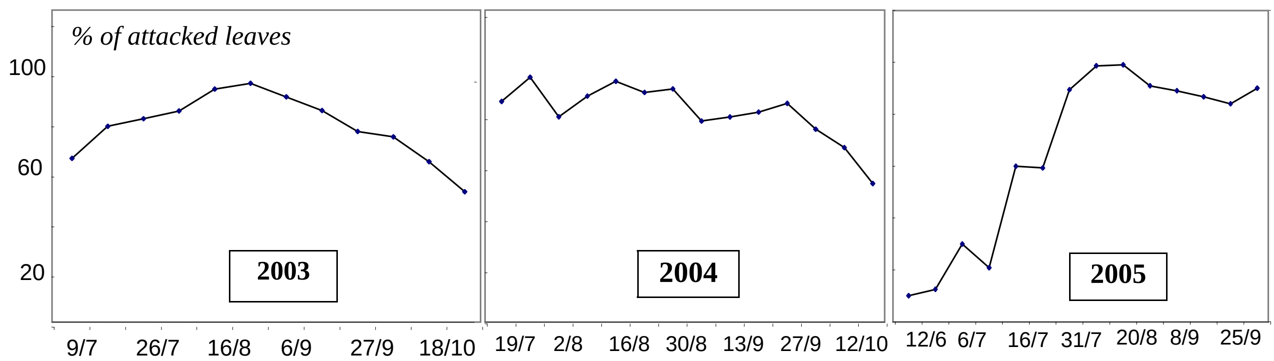

Figure 1: CLM infestation rate from 2003 to 2005 in West-Algeria

Natural mortality

The mortality rates recorded from 2003 to 2005 highlighted the importance of the parasitic activity. Parasitism attained very high levels mainly during August where it largely exceeded 40%. While mortality of unidentified origin notably increased the total CLM natural mortality, with maxima in August 2003 (24.3%), September 2004 (39.8%) and October 2005 (21.8%), predation impact was always very low or even absent (Fig. 2).

Parasitoid impact

Maximum parasitism rates reached consistent values: more than 45% in August 2003, about 60% in August 2004 and 50% in September-October 2004, and till 78% in August 2005 (Fig. 2). Over the three years, the introduced species *S. petiolatus* was the most powerful enemy with a relative abundance of about 64% in 2003, 30% in 2004 and 44% in 2005.

The second most efficient CLM parasitoid was the indigenous species *C. pictus* with a relative abundance reaching 19% in 2003, about 40% in 2004 and 26% in 2005 (Tab. 1). These two species, which showed a clear dominance throughout the study period, thus proved their good aptitude to control CLM populations in Algeria.

C. phyllocnistoides was, during our study, for the first time recorded in Algeria (Boualem et al., 2007). The activity of this allochtonous parasitoid increased gradually during the three study years, reaching a rather interesting relative abundance in 2005 (13.8%).

Table 1: Relative abundance (%) of CLM parasitoids in West-Algeria.
SPE: *Semielacher petiolatus*; CPI: *Cirrospilus pictus*; CPH: *Citrostichus phyllocnistoides*; CFO: *Closterocerus formosus*; PPE: *Pnigalio pectinicornis*; CVI: *Cirrospilus vittatus*; PSP *Pediobius* sp. (hyperparasitoid)

Year	SPE	CPI	CPH	CFO	PPE	CVI	PSP
2003	63,5	19,1	7,8	2,6	2,6	3,5	0,9
2004	30,1	39,8	7,1	5,3	14,1	1,8	1,8
2005	43,6	25,8	13,8	6,0	4,3	0,6	6,0

Parasitoid life cycles

Laboratory rearing, from egg to adult, of the different parasitoid species showed that the shortest development duration was achieved by *S. petiolatus* (12.4 days) and the longest by both *Closterocerus formosus* and the hyperparasitoid *Pediobius* sp. (about 17 days). The development time reached about 14-15 days for three other species (Tab. 2).

Table 2. Development duration of CLM parasitoids in West-Algeria

Parasitoid species	reared individual number	development time (days)
Semielacher petiolatus	139	12.43 ± 3
Cirrospilus pictus	102	14.81 ± 3.39
Citrostichus phyllocnistoides	92	14.77 ± 2.52
Pnigalio pectinicornis	42	14.80 ± 3.07
Closterocerus formosus	44	17 ± 2.94
Pediobius sp.	26	16.96 ± 3.34

The most sensitive CLM instars to parasitism

The 3rd and 4th larval instars were the most sensitive to parasitism with respective mortality rates reaching 34 and 22.2 % of the reared CLM individuals, while no egg parasitoids were obtained during the three years of the study (Fig. 3). The two first larval instars were on the contrary the most affected by predation and other mortality factors.

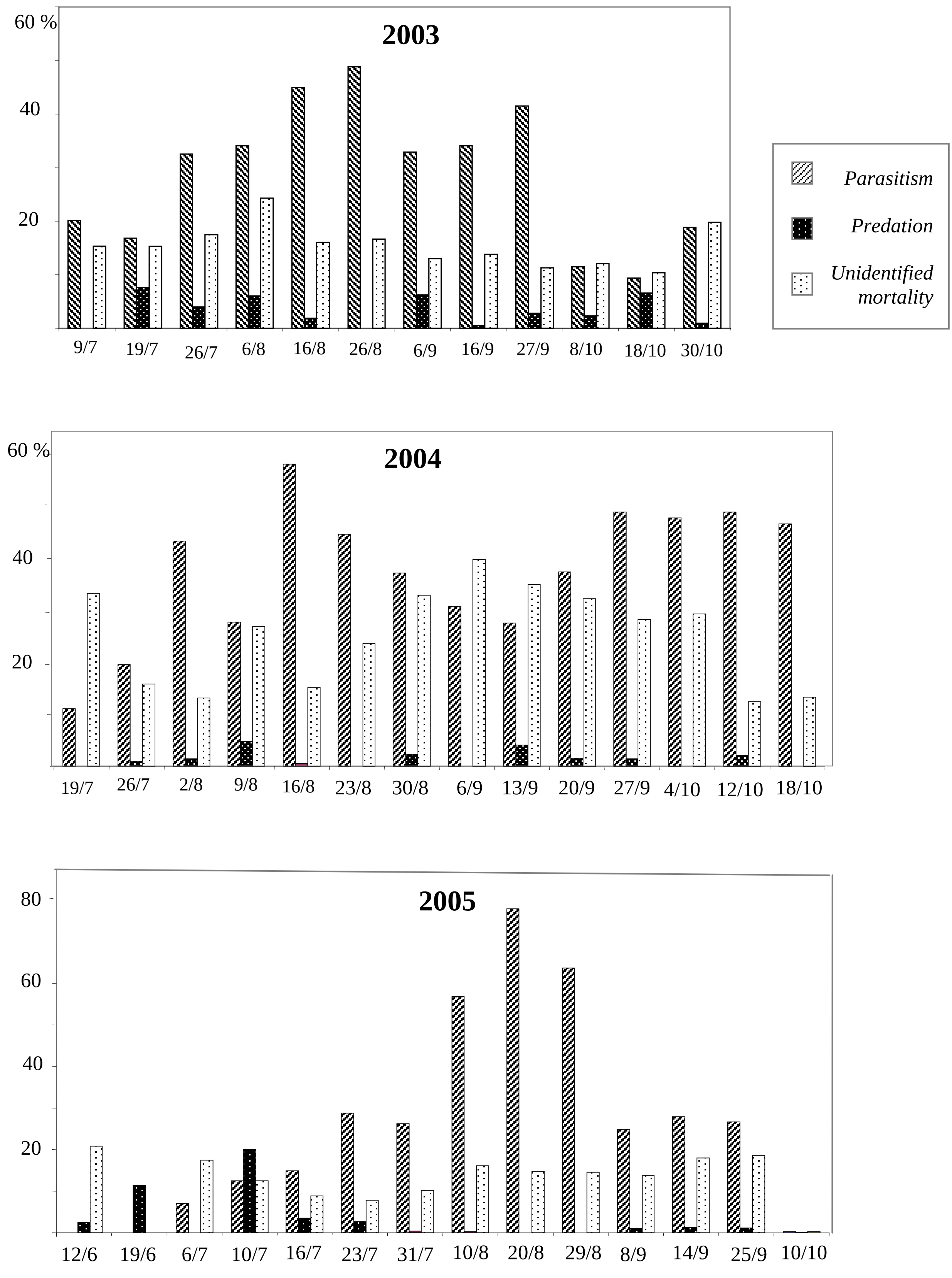

Figure 2. CLM natural mortality rates from 2003 to 2005 in West-Algeria

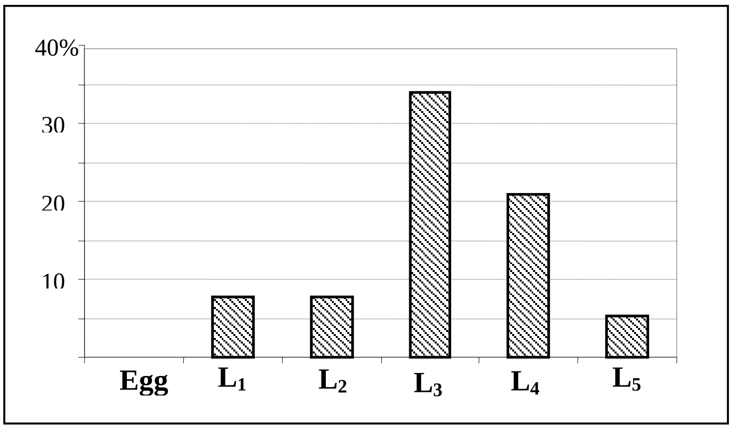

Figure 3. Percentage of parasitized CLM individuals in relation to developmental instars

Discussion

In addition to exotic enemies recently introduced, only three parasitoids were first recorded in Algeria during the studies carried out to find efficient CLM natural enemies: *P. agraules*, *C. vittatus* and *C. pictus* (Berkani et al., 1996; Saharaoui et al., 2001). Our study evidenced three new parasitoid species: *C. phyllocnistoides, C. formosus* and *P. pectinicornis* (Boualem et al., 2007).

The acclimatized *S. petiolatus* proved to be the most efficient antagonist of CLM in our country. Its presence was regular all along the pest activity period, with a relative abundance reaching each year more than 30% of all the recorded parasitoids. *C. pictus* was the most powerful indigenous parasitoid. According to KHEDER et al. (2002), the increasing impact of *S. petiolatus* since its introduction in Tunisia seemed to have induced a population reduction of the other indigenous parasitoids, *C. pictus* excepted. Among the new recorded species, two (*C. formosus* and *P. pectinicornis*) are indigenous while *C. phyllocnistoides* is allochtonous and should have spread from a neighbouring country (Tunisia or Morocco). It was notably introduced to control CLM in Morocco (Rizqi et al., 2003). In many countries, this species is now considered as one of the most efficient antagonist of the pest (Vercher et al., 2003; Garcia-Mari et al, 2004).

Conclusion

Numerous parasitoid species are able to develop on CLM around the world, their host choice more often depending from the host living modalities than from its taxonomic property. In fact, all these parasitoids develop on leaf miner larvae of various hosts ((Massa et al., 2001; Vercher et al., 2003; Boualem et al., 2007). In the scope of the biological control program performed against CLM in western Algeria, it would be convenient to identify these hosts and favour the growth of their host plants in order to reinforce the populations of CLM natural enemies.

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On what scale native plants can enhance biological control? The case of the parasitoid complex of *Phyllonorycter delitella* (Duponchel) on *Quercus* trees and the citrus orchard

M.C. Rizzo, P. Lucido, A. Agrò
SENFIMIZO Department, University of Palermo, viale delle Scienze 13, I-90128 Palermo, Italy; macoriz@unipa.it

Phyllonorycter delitella (Duponchel), leafminer on *Quercus pubescens* s.l., was studied in Sicily from July 2005 to May 2007 in a Mediterranean wood (Bosco di Ficuzza, province of Palermo). The wooded area is 4,000 ha wide and consists mainly of Holm Oaks and Downy Oaks, occasionally mixed to Ash Trees, Cork Oaks, Turkey Oaks, Maples and Pears. Leaves infested by *P. delitella* were collected every two weeks to study biology, ecology and parasitoid complex of the leafminer. Leaves with mines were analysed with a binocular and those showing parasitized larvae were isolated and put into glass tubes till the emergence of adult insects.

On the whole, 226 hymenopteran parasitoids were obtained till now, 75.2% being Eulophidae, 23% Braconidae and 1.8% Cynipoidea hyperparasitoids. Among the Eulophidae, a single female of *Citrostichus phyllocnistoides* (Narayanan) was detected; this species was introduced in Sicily in 1999 for biological control of the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera, Gracillariidae). Previously considered as a specific parasitoid of the citrus leafminer, in the Mediterranean Basin this exotic species has been recorded till now on six alternative hosts on native plants (comprising the new host record *P. delitella*). Similarly, another supposed specific parasitoid of the citrus leafminer, *Semielacher petiolatus* (Girault) (Hymenoptera, Eulophidae), has been recorded on six other hosts since it spontaneously spread in Sicily in 1998 from other Mediterranean regions.

Many studies showed an effective action of both these parasitoids in reducing *P. citrella* populations. However, in a different research no displacing effect could be observed till now on the native parasitoids constituting the parasitoid complexes of their non-target hosts. Parasitization of the two exotic species on the new hosts is indeed always very low, ranging around 1% from the time of their introduction up to now, as the present case confirms (0.4%). However, the occasional exploiting of other leafminer populations allows them to go through the period of scarce availability of the target pest. The new interesting aspect of the present study is the distance of the wooded area from the nearest citrus orchards, which are situated quite far (4.5 km) in a low flat area. Moreover, three of the known hosts of *C. phyllocnistoides*, including *P. delitella*, live in natural habitats. These facts confirm that patches of spontaneous vegetation may contribute to conservation biological control, and underline their role as permanent ecological infrastructures.

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Damages and control of *Phyllocnistis citrella* Stainton (Lepidoptera Gracillariidae) in Sicilian citrus nurseries after 13 years of its arrival

V. Caleca
Università di Palermo, SENFIMIZO Department, Entomology, Acarology and Zoology section, viale delle scienze, 90128 Palermo, Italy

The citrus leafminer, *Phyllocnistis citrella* Stainton, never caused economic loss in citrus fruit production since its first detection in Sardinia (1994) and the rest of Italy (1995), but it is commonly considered harmful to young or newly grafted plants and to ornamental ones. After reaching high infestation levels in 1998 and 1999 (on average 1.7-2 larvae and pupae per leaves 3-5 cm long, from July to November), data recorded in Sicilian nurseries showed a marked decrease of *P. citrella* infestation on untreated plants from 2000 onwards (on average 0.3-1.1 larvae and pupae per leaves 3-5 cm long, from July to November). The reduction of the citrus leafminer population is mainly due, also in the nurseries, to the effective action of *Semielacher petiolatus* (Girault) and *Citrostichus phyllocnistoides* (Narayanan), two exotic Eulofid parasitoids introduced in Sicily in 1998-2000.

Studies on injury levels carried out from 1998 to 2004 in Sicilian nurseries showed that only under particular conditions growth and development of young plants (even few months old seedlings), were significantly affected by the attack of *P.citrella.* The approximate economic injury level for rootstocks resulted 3-3.5 larvae and pupae per leaves 3-5 cm long; as this threshold was exceeded only in few weeks of 1998 and 1999, interventions addressed to citrus leafminer control are nowadays not required in this kind of plants. On the contrary, citrus plants requiring high aesthetical standards, like ornamental and grafted plants, resulted heavily injured, and the aesthetical economic injury level is close to 0.4 larvae and pupae per leaves 3-5 cm long. Since 2000 we observed that infestation level between June and November was below this threshold for an increasing number of weeks year by year, suggesting the possible reduction of treatments, previously performed without any sampling every 7-10 days. So a sequential sampling programme, based on a previously developed model, was tested in two nurseries. Results allowed a reduction of a third of insecticide treatments. Other tests were carried out on products permitted in organic farming, mineral oils and azadirachtin, recording a quite good level of control of *P. citrella.* Control of the citrus leafminer in Sicilian nurseries is still performed by almost weekly insecticide treatments, without any preliminary sampling and with the consequent frequent outbreaks of tetranychid mites. Application of our results on a large scale could improve *P. citrella* control in nurseries, reduce costs and decrease its environmental impact.

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The control of Citrus leaf miner *Phyllocnistis citrella* Stainton with bioinsecticides

Tatjana Perović[1], Snježana Hrnčić[2]
[1] *Biotechnical Institute, Centre of Subtropical Cultures, Bjelisi bb, 85000 Bar, Montenegro;*
[2] *Biotechnical Institute, Centre of Plant Protection, Kralja Nikole bb, 81000 Podgorica, Montenegro;*

Abstract: The aim of this paper was to evaluate the efficacy of some bioinsecticides for the control of Citrus Leaf Miner. The investigations were carried out in nursery of Centre of Subtropical Cultures in Bar (Montenegro), during 2006 and 2007. Four and six month old plants of the mandarin Unshiu cv. Cawano Wase were used. The foliar spraying was applied with two insecticides: Success™ and Oikos™. Efficacy of insecticides was evaluated 7, 14 and 21 days after application and assessment was according to the Abott formula, based on the number of a live larvae and pupae per 100 leaves per sample. Results showed high efficiency of insecticides based on spinosad and azadirachtin in the control of *Phyllocnistis citrella* seven days after treatment. After that efficiency rapidly decreases.

Key words: *Phyllocnistis citrella,* citrus leaf miner, bioinsecticides, spinosad, azadirachtin

Introduction

Phyllocnistis citrella is the most important citrus pest in Montenegro, specialy in nurseries and young plantations. For its efficient control large number of foliar treatment using chemicals from diferent chemical groups, as neonicotinoides, abamectin, insect grow regulator etc., is needed in the course of a year.

The modern tendency in plant protection takes into account the development of new techniques and methods able to enhance all those biotical factors of natural control that are more selective and that influence as little as possible the ecosystem's balances.

In this context there was considerable experimental activity with the purpose to evaluate the effect of use of the natural substances on reducing the citrus leaf miner infestation.

In this work the effect of compounds of natural origin on *Ph. citrella* infestation in 2006 and 2007 was investigated.

Material and methods

The research was carried out in the nursery of Centre of Subtropical Cultures in Bar (Montenegro). Four and six month old nursery plants of the mandarin Unshiu cv. Cawano Wase were used. The experimental programme involved the identification of three plots, two were treated with bioinsecticides while the remaining one acted as untreated control.

The first sections was treated with bioinsecticides based on spinosad, whose active principle (spinosin A and spinosin D) represented fermention products of the actinomycetes *Saccharopolyspora spinosa,* present in the ground and active for ingestion and contact on different species of insects including leaf miners in various crops. Spinosad is not a plant systemic, but penetrate leaves. It acts through activation of the acetylcholine nervous system through nicotinic receptors, and effects on the GABA, too (Thompson et al., 2000). Based on its good ecotoxicological profile EPA registered spinosad as a reduced-risk material and it is also approved for organic certification in the EU.

The second section received a treatment based on azadirachtin that is the principal active ingredient of neem tree, *Azadirachta indica*, extracts. It is activ on ingestion and contact on white fly, leaf miners and other different pests. It is sistemic. As ecdison antagonist it acts through inhibition of hitin and disrupt insect moulting. Neem extracts show repellent and antifeeding effect. Maximum of efficacy on first faze of insect growth. Since 2000 it is also approved for organic agriculture (Capella et al., 2000).

The Table 1 shows the insecticides and the quantities applied in the trials.

Table 1. Review of insecticides used in trial

Insecticide	*Active ingredient*	*Concentration of chem. (ml/plant)*
Success	Spinosad	0.1
Oikos*	Azadirachtin	0.15

* + mineral oil 0.5%

All the trials were carried out in five replicates, 10 nursery plants presenting one replicate. Manual sprayer 12 l in capacity up to dropping was used. The effect of the applied insecticides was recorded on the 7^{th}, 14^{th} and 21^{st} day after the treatment (DAT). The efficacy was determined in compliance with Abbott method based on the number of a live larvae and pupae on 100 sample leaves.

Results and discussion

Results of the two years investigations on effect of compounds of natural origin on *Ph. citrella* infestation were expressed as efficacy of insecticides and presented in Tables 2 and 3.

In the first year of investigation on 100 sample leaves, on the day of a treatment, 2.0 larvae's and 0.19 pupae's /leaf in average, were detected.

On the day of a treatment in the second year, on the same size sample, 2.16 larvae's and 0.66 pupae's/ leaf in average were detected.

Tab.2. Insecticide efficacy for *Ph. citrella* control (Bar, September 8^{th}, 2006)

		7 DAT			14 DAT			21 DAT		
Insecticides	Conc. of chem (%)	Number of larvae	Number of pupae	Efficacy (%)	Number of larvae	Number of pupae	Efficacy (%)	Number of larvae	Number of pupae	Efficacy (%)
Success	0.1	2	0	98.52	84	0	68.1	312	70	18.7
Oikos*	0.15	6	0	95.7	112	0	57.41	312	65	19.78
Control		126	14	-	208	55	-	288	182	-

* + mineral oil 0.5%

According to the stated data, it may be inferred that in both study years Success had the highest efficacy (98.5%) seven days after the treatment. The efficacy of the mentioned chemical rapidly decreased after 14 days, ranging from 66.2 to 68.1%. Higher decrease in the efficacy was registered 21 days after the treatment, below 20%. Dates of the high efficacy in

Ph. citrella control using chemicals based on spinosad were reported by Salas et al. (2004) and Stansly & Fulcher (1995). In the trials carried out in Argentina by Salas et al. (2004) spinosad gave similar efficacy to abamectin. Stansly & Fulcher (1995) examined efficacy of different concentration of spinosad and noted its high efficacy for citrus leaf miner control in dependence of applied rate.

Tab.3. Insecticide efficacy for *Ph. citrella* control (Bar, July 3rd, 2007)

		7 DAT			14 DAT			21 DAT		
Insecticides	Conc. of chem.(%)	Number of larvae	Number of pupae	Efficacy (%)	Number of larvae	Number of pupae	Efficacy (%)	Number of larvae	Number of pupae	Efficacy (%)
Success	0.1	3	0	98.34	193	2	66.2	521	97	7.06
Oikos*	0.15	34	0	81.21	300	3	47.48	498	123	6.61
Control		127	54	-	531	46	-	364	301	-

* + mineral oil 0.5%

Oikos showed high efficacy which seven days after the treatment, was ranged from 81.2 to 95.7%. After 14 days the efficacy was unsatisfactory, decrease below 58%. There are many data about using azadirachtin in control of citrus leaf miner. However, different tests carried out in Florida, India and Italy showed heterogeneous effects. The trials carried out in Italy by Conti D. et al. (1997, 1998) and Conti F. et al. (2004) revealed acceptable efficacy of azadirachtin. Jayanthi & Verghese (2004) reported that azadirachtin can be used as follow-up sprays under heavy infestation and as prophylactic sprays during new flush emergence. Saravan & Savithri (2005) noted less damage on 7, 10 and 15 days after treatment in azadirachtin plot. In the study of Stansly & Fulcher (1994; 1995) chemical based on azadirachtin demonstrate low efficacy in citrus leaf miner control.

Spinosad and azadirachtin are new products of natural origin, which can be adopted for controlling citrus leaf miner. Results were quite satisfactory, although there is need to continue experimental trials by means of more effective techniques for employing this useful product.

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pp. 195-198

Control trials of the Citrus Leaf Miner *Phyllocnistis citrella* Stainton (Lepidoptera, Gracillariidae, Phyllocnistinae) in nurseries

Tatjana Perović [1], Snježana Hrnčić[2]
[1] *Biotechnical Institute, Center of subtropical cultures, Bjeliši bb, 85000 Bar, Montenegro*
[2] *Biotechnical Institute, Center of plant protection, trg Kralja Nikole bb, 81000 Podgorica, Montenegro*

Abstract: Our research was orientated primarily towards to determinate the efficiency and duration of effects of insecticides from the neonicotinoid group for the control of citrus leaf miner *Phyllocnistis citrella.* The trials were conducted in nursery of Center of subtropical Cultures in Bar, on the three and four month old plants of mandarin Unshiu cv. Kawano Wase. After the miner attack had been identified in the nursery the following insecticides were applied as soil drench: Confidor 200 SL (a.i. imidacloprid), Calypso 480 SC (a.i. thialoprid), Mospilan 20 SP (a.i. acetamiprid) and Actara 25 WG (a.i. thiametoxam). The research demonstrated that these insecticides provide efficient control of the pest in the course of several weeks.

Key words: citrus leaf miner, nursery plants, insecticides, soil drenching.

Introduction

The citrus leaf miner *Phyllocnistis citrella* Stainton is the newest member of the citrus entomofauna in our country. It attacks all varieties from the Citrus, Poncirus and Fortunella genera, damaging young leaves, shoots and fruits. The symptoms of attack are clear and easy to spot. The attacked leaves and shoots have characteristic snake-like twisted, silverfish white and satin shine mines. The attacked leaves deform, curl and sometimes fall off.

The biggest damage *Phyllocnistis citrella* does is in nurseries. The damaged young plants remainder in growth, and because of the deformed leaves, mines and necrotic zones they lose on the market values.

An ongoing production of the new flush in the nurseries requires a continuing protection from this pest, most frequently from June to November. Foliar applied pesticides provide efficient protection during 14 days, which requires a large number of treatments during a year.

Owing to the highlighted systemic effect the pesticides from the neonicotinoid group can be applied trough soil too.

The aim of this research was to determine the most efficient pesticides and dosages, which will mitigate the destruction of the citrus leaf miner in the nurseries.

Material and methods

The trials were conducted in Center of Subtropical Cultures in Bar during 2002 and 2004. The nursery plants of mandarin Unshiu cv. Kawano Wase, four and three months old were used. Each plant was in a container with 16cm diameter and 25cm height.

After the attack of *Ph. citrella* had been identified in the nursery the plants were drenched with the insecticide solution in 100 cm^3 of water. The nursery plants were optimally drenched on the day before the treatment. The drenching was then done three times a week.

The Table 1 shows the insecticides and the quantities applied in the trials. Untreated nursery plants were taken as a control.

All the trials were cared out in six replicates, one nursery plant presenting one replicate. The treatment was done on August 9th in 2002 and July 2nd in 2004.

The effect of the applied insecticides was recorded in the period of 90 days after the treatment (DAT). Further following of the insecticides efficiency was not possible due to the lack of growth. The efficiency of the insecticides was determined in compliance with Abbott method based on the number of a live larvae and pupae on 60 sample leaves.

Tab.1. Review of insecticides used in trial

Insecticide	*Active ingredient*	*Quantities of chem. (ml, g/plant)*
Confidor 200 SL	imidacloprid	0.05
Confidor 200 SL	imidacloprid	0.3
Mospilan 20 SP	acetamiprid	0.05
Mospilan 20 SP	acetamiprid	0.3
Actara WG 25	thiametoxam	0.04
Actara WG 25	thiametoxam	0.25
Calypso 480 SC	thialoprid	0.02
Calypso 480 SC	thialoprid	0.12

Results and discussion

Table 2 and 3 present the examination results of the two years investigation on efficiency of insecticides from the neonicotinoid group for prevention of the *Ph. citrella.*

Tabel 2. Efficiency of insecticides applied as drench treatment 18, 32, 52 and 67 day after the treatment (Bar, 09.08.2002., four month old nursery plants)

Insecticide	*Quantities of chem. (ml, g/plant)*	*Efficacy (%) 18 DAT*	*Efficacy (%) 32 DAT*	*Efficacy (%) 52 DAT*	*Efficacy (%) 67 DAT*
Confidor 200 SL	0.05	100	100	72.5	50.2
Confidor 200 SL	0.3	100	100	-	100
Mospilan 20 SP	0.05	100	73.5	45.6	10.05
Mospilan 20 SP	0.3	100	100	-	-
Actara WG 25	0.04	100	100	100	100
Actara WG 25	0.25	100	100	100	100
Calypso 480 SC	0.02	92.4	78.0	32.7	7.1
Calypso 480 SC	0.12	100	87.0	-	47.5

It can be noted that the longest protection period from the citrus leaf miner infestation was provided by insecticide Actara 25 WG. The efficiency was 100% during the 83-day-period in both concentration of application.

A high efficiency in the period of 83 days was also provided by insecticide Confidor 200 SL applied in quantity of 0.3 ml per plant. When applying the same insecticide in lower quantity (0.05ml per plant) somewhat lower persistency was noted. The efficiency was high and lasted for 33 days after the application, after which it decreased and came to 72.5% after 52 days and 50.2% after 67 days. Dates on the high efficiency for the prevention of the citrus leaf miner using insecticides based on imidacloprid were reported by Nucifora (1996), Leocata (1997) and Conti et al. (1998).

Tabel 3. Efficiency of insecticides applied as drench treatment 18, 32, 67 and 83 day after the treatment (Bar, 02.07.2004., three month old nursery plants)

Insecticide	*Quantities of chem. ml,g/plant)*	*Efficacy (%) 18 DAT*	*Efficacy (%) 32 DAT*	*Efficacy (%) 67 DAT*	*Efficacy (%) 83 DAT*
Confidor 200 SL	0.05	100	100	-	-
Confidor 200 SL	0.3	100	100	100	100
Mospilan 20 SP	0.05	100	-	56.7	12.5
Mospilan 20 SP	0.3	100	-	-	15.7
Actara WG 25	0.04	100	100	100	100
Actara WG 25	0.25	100	-	-	100
Calypso 480 SC	0.02	100	80.3	-	13.1
Calypso 480 SC	0.12	100	90.0	53.9	-

Mospilan 20 SP applied in quantity of 0.3g per plant shows high efficiency in control of the citrus leaf miner during 33 days. Further monitoring of the insecticides efficiency was not possible due to the lack of growth during treatment in the course of both research years. In 2004 during in this plot the presence of new growth was occur 83 days after the treatment and the efficiency was 15.7%. When the same insecticide was applied in lower quantity (0.05g per plant) high efficiency was noted 33 days after the treatment (table 2 and 3) after which it decreased to 12.5% after 83 days.

Insecticide based on thialoprid Calypso 480 SC showed satisfactory efficiency in prevention of the citrus leaf miner infestation during 18 days. When applying 0.12ml/plant after 33 days the efficiency was 87-90% and 47.5-53.9% after 67 days. In lower quantity of application (0.02ml/plant) somewhat lower efficiency was noted, 92.4 – 100% after 18 days and 78 – 80.3% after 33 days. The efficiency then considerably decreases and 52 days after the treatment it is 50%.

The soil drench application of insecticides based on imidacloprid, acetamiprid and thiametoxam is recommended in nurseries and young citrus plantation up to 5 years of age. In this way important citrus pest can be protected during a several-week period (Broeksma et al., 1993; Schonllau, 1995; Nucifora, 1996; Leocata, 1997; Iordanou and Charalambous, 1998; Conti et al., 1998; Mansanet et al., 1999; Perović et al., 1999). Nucifora (1996) noted extraordinary results in the protection of the citrus nursery plants by drenching with insecticide Confidor 200 SL. Quantities of 0.2, 0.4-0.6 cm^3 per plant (in pots with 25cm of diameter and height 40cm) provided complete protection of the nursery plants in the period of 80, respectively 105 days. When the same chemical was applied in the quantity of 0.1 ml/plant in the pots with 22cm of diameter, high efficiency was noted in period of 40 days after treatment.

The two-year-examinations of Conti et al. (1998) show that a successful protection of the nursery plants in the open, lasting 19 weeks, can be achieved with the soil drench application of Confidor 200 SL in quantity of 2 cm^3 per plant. In the trials were used plants 60 cm high, in the 25cm diameter pots. They also state high efficiency of the same chemical applied in quantity of 1.5 ml/plant in a greenhouse in 30 weeks period.

In the study conducted by Leocata (1997) the orange nursery plants were used in the pots 16x40 cm with 10 cm growth, soon after grafting. A high efficiency of Confidor 200 SL applied with 0.5, 1 and 2 ml/plant during 112 days was noted but also phitotoxity was noted. Therefore the author recommends only the lowest quantity of application.

Dates on the possibility for successful protection of citrus nursery plants using Confidor 200 SL applied though soil drench lasting up to 12 weeks were reported by Perović et al. (1999).

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Field evaluation of the influence of different citrus rootstocks on *Phyllocnictis citrella* Stainton, *Aphis spiraecola* Patch and *A. gossypii* Glover incidence on 'Clementina de Nules' trees

S. Trapero Muñoz, Á. Hervalejo García, M. Jiménez Pérez, J.R. Boyero, J.M. Vela, E. Martínez-Ferri
IFAPA. Centro de Churriana. Cortijo de la Cruz s/n. 29140 Churriana, Málaga, Spain

We have evaluated the influence of six different citrus rootstocks on the incidence of citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), and of the aphid species, *Aphis gossypii* Glover and *A. spiraecola* Patch (Homoptera: Aphididae), on trees of 'Clementina de Nules' (*Citrus clementina* Hort. *ex* Tan.). Sampling was made in a three-year-old grove in South Spain, where a completely randomised block was designed. The percentage of incidence was assessed fortnightly during 2005 and 2006. In parallel to incidence, a 'flushing index' was estimated as the percentage of shoots susceptible of being injured by phytophagous. Our results showed that contrasting factors affected the incidence of populations of *P. citrella*, *A. gossypii* and *A. spiraecola* on 'Clementina de Nules'. Incidence of *P. citrella* was significantly dependent on the flushing pattern observed throughout the study years whereas the reverse was true for the aphid species. Between these, *A. spiraecola* displayed similar levels of infestation in all study rootstocks whereas *A. gossypii* showed a preferential incidence ($P<0.05$) for leaves of 'Clementina de Nules' grafted on 'Cleopatra mandarin' (*Citrus reshni* Hort. *ex* Tan), affecting this rootstock almost exclusively. This result may have future implications on pest control.

Thrips, Whiteflies and Aphids

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IOBC/wprs Bulletin Vol. 38, 2008
pp. 200-203

Field evaluation on citrus fruit scars in Italy: the role of *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae)

Gaetano Siscaro[1], Giancarlo Perrotta[1,2], Filadelfo Conti[3] & Lucia Zappalà[1]
[1] *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi – Via S. Sofia, 100 – 95123 Catania, Italy;*
[2] *Assessorato Agricoltura e Foreste – Dipartimento Interventi Infrastrutturali – U.O.T. 50 – V.le Teracati, 39 – 96100 Siracusa, Italy;*
[3] *Assessorato Agricoltura e Foreste – Dipartimento Interventi Strutturali – O.M.P. – U.O. 54 – Via Sclafani, 32 – 95024 Acireale, Italy*

Abstract: The results of a field trial on the damage caused by *Pezothrips kellyanus* (Bagnall) on lemon fruits are reported. In order to carry out the trial, a rearing of *P. kellyanus* was started during spring 2006 and 2007. In the first year the trial was conducted in a twenty years organic lemon orchard. Six trees were selected and five fruits per tree, with a diameter of 1.5 cm, were chosen. All these fruits were caged with a fine polyester mesh bag; four of them were inoculated with five female thrips collected in the laboratory rearing, while the fifth was treated as control. The fruits were exposed to thrips activity for 1, 2, 3, and 4 weeks and then weekly checked in order to verify the surface conditions. In 2007, the trial was repeated in an integrated lemon orchard on older plants (30 years).

The trial conducted in 2006 inoculating *P. kellyanus* gave evidence of a presence of an epidermic alteration already after a week from fruit exposition to the thrips feeding activity. The damage became gradually more evident after 2, 3 and 4 weeks showing a presence of irregularly distributed scars that could interest large portions of the fruit surface. This kind of alteration was present on all fruits inoculated with *P. kellyanus* with high damage level. Differently, in 2007 the results were inconsistent, probably also due to extreme temperature conditions.

Key words: Kelly's thrips, rearing, lemon, damage

Introduction

In Italy, the records of alterations on citrus fruit surface have been increasing during the last years. Frequently, the commercial value of these fruits is heavily downgraded (Conti et al., 2001). This kind of damage affects mostly the upper layers of the epidermic tissue causing corky alterations on the rind; in the worst cases this alteration could induce deeper scars and deformations as well. Many causal agents are involved, such as insect and mite pests (Grafton-Cardwell et al., 2003) as well as fungi (Cutuli & Salerno, 1998), but some of them remain actually not clearly correlated to a specific alteration. Causal agent identification is essential for planning an efficient control strategy and thrips are one of the main pests generally considered able to cause scars on citrus fruits. In Italian citrus orchards the Kelly's citrus thrips *Pezothrips kellyanus* (Bagnall) is one of the most diffused; other species reported are the Western flower thrips, *Frankliniella occidentalis* (Pergande), the Onion thrips, *Thrips tabaci* Lindeman, the Yellow flower thrips, *Thrips flavus* Schrank, and the Greenhouse thrips, *Heliothrips haemorrhoidalis* Bouchè. Recorded in Italy in 1998 (Marullo, 1998), *P. kellyanus* is a polyphagous species, very common on white or yellowish flowers, infesting all citrus with a particular preference for lemon. The long blooming period of lemon induces to think that the thrips performs at least one generation on this host before reaching the highest levels of presence on the tree canopy during the initial growth of the young fruits (Marullo, 2003). The damage is related to their feeding activity on flowers and young fruits, generally causing scars at the stem end (*halo damage*) or between touching fruits. (Baker et al., 2000; Broughton & De Lima, 2002). This damage can be particularly severe on citrus varieties that retain their sepals, under which the thrips larvae shelter and feed (Webster et al., 2006). On the other hand, if the level of the infestation is very high, scars can extend on the other part of the fruit surface or on the entire fruit. Since thrips activity in citrus occurs early in the season, during blooming and fruit growth, it is very difficult to attribute certain kind of scars to these pests: when the peel alterations are evident, normally there are no more thrips specimens on the fruit due to their migration on other blooming hosts plants.

The aim of the survey is to assess the feasibility of this field experimental approach to verify the harmfulness of Kelly's thrips and to define the damage pattern caused by the species.

Material and methods

Thrips rearing

In order to obtain the specimens for the field trials a laboratory rearing of *P. kellyanus* was started during spring of 2006 and 2007. Plastic cylindrical boxes (diameter 20cm; height 22cm; volume 8.3l), with two opposite openings covered with polyester mesh to allow air circulation, were used as rearing cages ;the bottom of the boxes was filled with a 5-cm layer of vermiculite where 5 lemons were placed. Pollen of *Typha* spp. was then distributed on the fruits as additional protein supply. Sixty adult thrips specimens, collected from flowers picked up in Siracusa lemon orchards, were introduced in every cage. The cages were then placed in a climatic chamber with a temperature of 24$\pm$1°C, a relative humidity of 50–60% and a photoperiod of 14 h light and 10 h dark. Every week the cages were checked in order to add fresh fruits and new pollen. Under these climatic conditions *P. kellyanus* cycle lasts 21 days.

Field trials

In 2006 the trial was carried out in a 20 year-old organic lemon orchard. Six trees were selected and on each of them 5 young fruits, with an average diameter of 1.5 cm, were chosen. Each fruit was isolated with a fine polyester mesh bag (12x7 cm). Four fruits were inoculated with 5 females while the 5th was left as untreated control. The fruits were exposed to thrips activity for 1, 2, 3, and 4 weeks; after each exposition the specimens were removed and the fruits weekly checked in order to verify the surface conditions. Photo-recording of the damage evolution have been carried out for the following 3 months. In 2007, the trial was repeated with the same scheme but in this case an integrated lemon orchard was chosen.

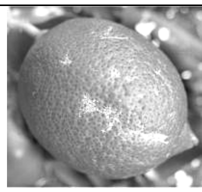
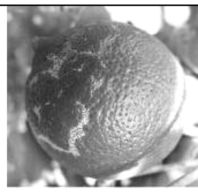
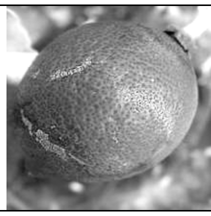
Results and discussion

During the field trials, it was verified that *P. kellyanus* females can feed on lemon fruits causing economic damage. The trial conducted in 2006 gave evidence of a presence of scars already after a week of exposition (Table 1). The rind alterations were noticed with a high intensity on all fruits inoculated with Kelly's thrips. The damages became gradually more evident after 2, 3 and 4 weeks of exposition with irregularly distributed scars that could affect, in some cases, large portions of the fruit surface. It could be worth mentioning that inside large scars, "islands" of healthy tissue have been also detected; the presence of these marks on fruits is considered by some authors (Broughton & De Lima, 2002) as distinctive of wind damage, therefore confirming the difficulties in assessing the precise causal agents of the various fruit scars.

No scars or alterations of the rind have been evidenced on the fruits not exposed to thrips (untreated control); besides, typical ring spots (*halo damage*) have been only rarely detected. Considering that thrips, as already mentioned, generally have a preference for protected parts of the trees, we assume that the experimental conditions could have influenced their normal behaviour, making them also feed on portions of fruits normally not attacked; this could happen in the field on fruits located in the internal part of the canopy and therefore someway protected.

The field trials conducted in 2007 gave inconsistent results, probably also due to extreme temperature conditions (over 45°C) occurred during the release of the thrips inside the small mesh bags.

Table 1. *Pezothrips kellyanus* (Bagnall): results of the field experiment on newly settled lemon fruits, after the exposition time and at the end of the trial.

Exposition time (days)	**Damage**	**Damage evolution**	
7	Slightly depressed scattered spots or patches	Small scars on a limited portion of the surface (+97 days)	
14	Elongate scars located mainly on the apical portion of the fruit	Marked scars in the same position (+90 days)	
21	Larger scars with irregular shape	Same shape but enlarged in function of the fruit development (+83 days)	
28	Similar scars but scattered irregularly on the whole surface	Scars with the same position and shape (+76 days)	

The data obtained during this field trial demonstrated the validity of this experimental approach which gave also positive results in a trial conducted on late summer production whose results are presently under analysis. Further trials will be conducted in order to verify the thrips damage also on orange and other citrus.

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IOBC/wprs Bulletin Vol. 38, 2008
pp. 204-209

A threshold hypothesis for an integrated control of thrips infestation on citrus in South-Eastern Sicily

Giancarlo Perrotta[1,3], Filadelfo Conti[2]

[1] *Regione Siciliana, Assessorato Agricoltura e Foreste, Dipartimento Interventi Infrastrutturali, Unità Operativa Territoriale 50 – Viale Teracati 39, 96100 Siracusa, Italy*

[2] *Regione Siciliana, Assessorato Agricoltura e Foreste, Dipartimento Interventi Strutturali, Servizio Fitosanitario - U.O. 54 – Via Sclafani 32, 95024 Acireale, Italy*

[3] *Di.S.Te.F., Università degli Studi – Via S. Sofia 100, 95123 Catania, Italy*

Abstract: Several species of Thysanoptera are able to cause characteristic scars on the peel around citrus fruit stem or on their contact surface. Since 1998 surveys on citrus thrips fauna composition have had a new impulse when the presence of *Pezothrips kellyanus* Bagnall was recorded on citrus first in Italy. This species has changed the interspecific relationship among citrus thrips fauna reducing the presence and the importance of *Heliothrips haemorrhoidalis* Bouché, once considered the most dangerous thrips species reported on citrus. At the moment the species reported on citrus flowers in south-eastern Sicily orchards largely belong to *P. kellyanus* and *Thrips flavus* Schrank, while *T. tabaci* Lindeman, *Frankliniella occidentalis* Pergande and *H. haemorrhoidalis* are less important.

The aim of the present work is to define a spraying threshold in order to support farmers with a good pest management technique. Monitoring activity has been carried out for four years (2003-2006) in two lemon orchards in Siracusa province. White cromo-attractive sticky traps were used to detect thrips population. Furthermore, a direct observation on flowers and fruitlets was started to record adults and larvae presence. They were collected around the canopy circumference of five lemon trees. Flowers or young fruits were picked up after an evaluation of their average presence in ten canopy portions assessed with a 25 x 25 cm quadrate (Q). At harvest, damage on fruit caused by thrips feeding activity was evaluated. White sticky traps have always recorded a peak of adult thrips captures within the first half of June whereas fruitlets control did not show any significant thrips presence.

During the four years period, flowers and young fruits monitoring gave evidence of a different thrips population extent. In 2004 the highest larvae and adult thrips presence was recorded, exceeding the peaks of 12 and 10 specimens/Q respectively; furthermore, during the whole survey period, a mean of 3.28 larvae per Q and 2.53 adults per Q was recorded. At that population level the highest fruit damage occurred at the harvest period (24%). Traps were unable to express any significant relationship between thrips infestation and damage at harvest. On the contrary flowers and young fruits monitoring showed a relationship between the number of specimens per canopy quadrate and damage at harvest. In conclusion, these observations allowed us to define a spraying threshold to manage citrus thrips infestation that we suggest should be fixed in 10 larvae/quadrate.

Key words: *Pezothrips kellyanus*, IPM, monitoring, threshold

Introduction

Citrus fruits often show several surface alterations that can be generically classified as scars. Many casual agents can determine alteration on different step of the fruit growth like that just reported in California (Grafton-Cardwell, 2003). Among them several species of Thysanoptera are able to cause characteristic scars on the peel around citrus fruit stem or on their contact surface removing the green pigmentation from the epidermal cells resulting in irregular white patches (Blank & Gill, 1997). Since 1998 surveys on citrus thrips fauna composition have had a new impulse when the presence of *Pezothrips kellyanus* Bagnall was recorded on citrus first in Italy (Marullo, 1998). Around the world this species is recorded and considered like one of the most harmful for citrus fruits together with *Scirtothrips aurantium* Faure and *S. citri* Moulton. In particular in Australia (Baker *et al*., 2000), New Zealand (Pyle & Stevens, 2004) and Italy (Conti *et al*., 2002) it is considered a serious citrus pest. *P. kellyanus* has changed in Italy the inter-specific relationship among citrus thrips fauna reducing the presence and the importance of *Heliothrips haemorrhoidalis* Bouché, once considered the most dangerous thrips species reported on citrus. At the moment the species recorded on citrus flowers in south-eastern Sicily orchards largely belong to *P. kellyanus* and *Thrips flavus* Schrank, while *T. tabaci* Lindeman, *Frankliniella occidentalis* Pergande and *H. haemorrhoidalis* are less important. *P. kellyanus* infests flowers just when they begin to open but this is a critical period for sprays; so that it is important to find out the best time for soft chemical sprays during the fruit growth according to an I.P.M. program.

Other works had already demonstrated a close correlation between severe commercial damage at harvest and young fruits infested by thrips larvae after petal fall (Grout *et al*., 1986; Perrotta *et al*., 2004); but since a simple evaluation of the percentage of thrips infested fruit early in the season would not give a true indication of the total population presence, it was useful to assess the thrips population density based on a unit of citrus canopy area (Baker *et al*., 2000). The aim of the present work is to correlate the thrips infestation density (adult and/or larvae) on tree canopy with the damage at harvest in order to assess a rational spraying threshold.

Material and methods

Monitoring activity has been carried out for four years (2003-2006) in two lemon orchards, [*Citrus limon* (L.) Burm. f.], Femminello Siracusano variety, in Siracusa province, South East area of Sicily in 1 hectare plots. Lemon trees were cultivated according to an Integrated Pest Management strategy with application of spray oils and few narrow spectrum pesticides when needed. Starting from March, every week five mature citrus trees were randomly chosen for the experimental purpose, located in plots where frequently thrips infestation have been recorded in the previous growing seasons. Two white cromo-attractive sticky traps (12 cm x 8 cm) were used in each plot to detect thrips population. Traps were hanged at eyes level in the southern external part of the canopy. Traps were collected in plastic cover and citrus thrips were later counted in laboratory under a stereomicroscope. The traps were used to monitor adult citrus thrips and provided a mean number of citrus thrips per week per card. After the first captures on sticky traps (March-April), a direct observation on flowers and fruitlets was started to record thrips presence. A monitoring was carried out for 10-12 weeks according to thrips activity. Six flowers and/or young fruits were randomly picked up weekly around the canopy circumference of five lemon trees per plot and stored individually in small plastic container. The presence of larvae and adult thrips was observed in laboratory under stereomicrosope. The relative proportion of flowers and fruits collected was based on the evaluation of their average presence in 10 canopy portions of each of five trees, delimited with a 25 x 25 cm quadrate (Q). The mean number of thrips specimens counted in laboratory was multiplied for the proportion of flowers or fruits per quadrate, in order to obtain the thrips density per quadrate in the canopy. All data are expressed as mean number of larvae and adults per quadrate per week with the aim of expressing the population density on a unit area.

Each year, at harvest time (December - January), damage on fruit caused by thrips feeding activity was evaluated on 20 fruits collected on 10 trees per experimental plot (400 fruits in total).

All data were submitted to ANOVA after appropriate transformation (ARCSIN and SQROOT) and means were separated with Duncan's multiple range test. The different four monitoring years were statistically analysed for the damage at harvest, larvae and adults density and traps captures. The statistical differences of these variables were compared and evaluated in order to observe any correspondence.

Results and discussion

The presence of flowers and fruits was similar in the 4 years of monitoring without any statistical significance (Fig. 1 and Table 1). The period of flowering was concentrated in April-May, with a peak of about 5-6 flowers per quadrate in the first part of May. During the flowering, the presence of larvae was consistent; they were found feeding inside the flowers. The peaks of larvae were observed in the first half of May, in particular in the 2004 monitoring year (Fig. 2).

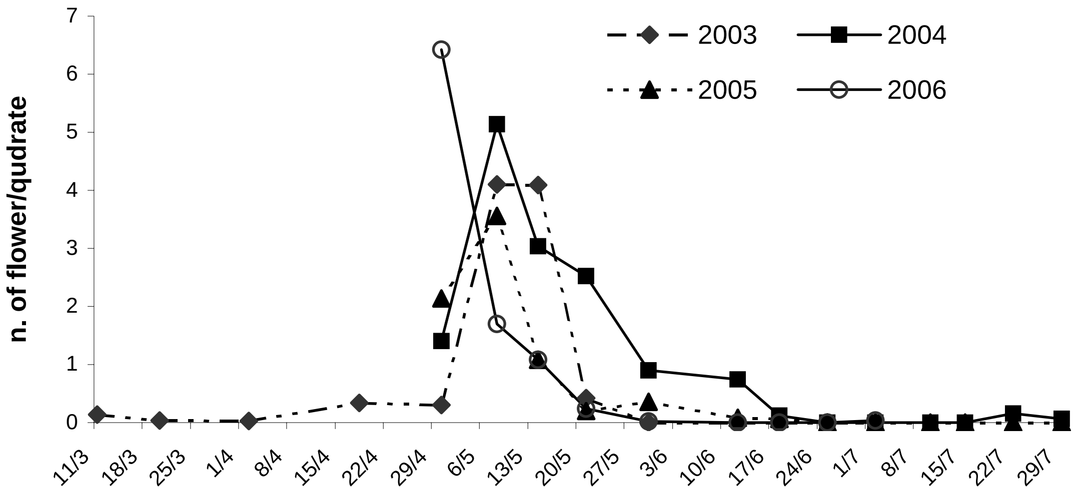

Fig. 1 Mean number of flower per quadrate (25 cm x 25 cm) in the monitoring period (2003-06) in Siracusa lemon orchard

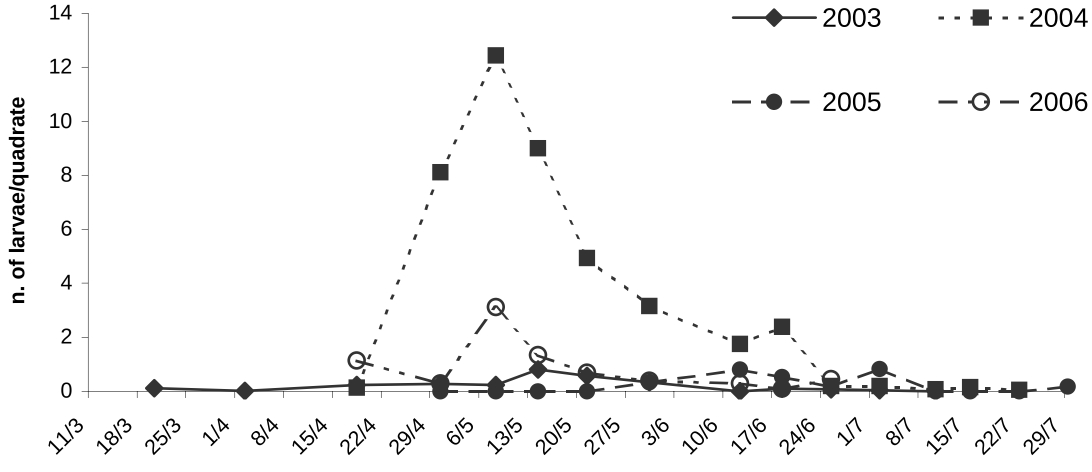

Fig. 2 Mean number of thrips larvae per quadrate in the monitoring period (2003-06) in Siracusa lemon orchard

The adults were found in coincidence of the flowering period with a peak in April, but a relevant presence was observed on the small fruits as well, with a peak in June (Fig. 3). After petal fall, some adults and larvae remained under the calyx of the fruit producing the damage due to feeding activity. When the fruits increased, it was very rare to detect feeding larvae or adults between touching fruits.

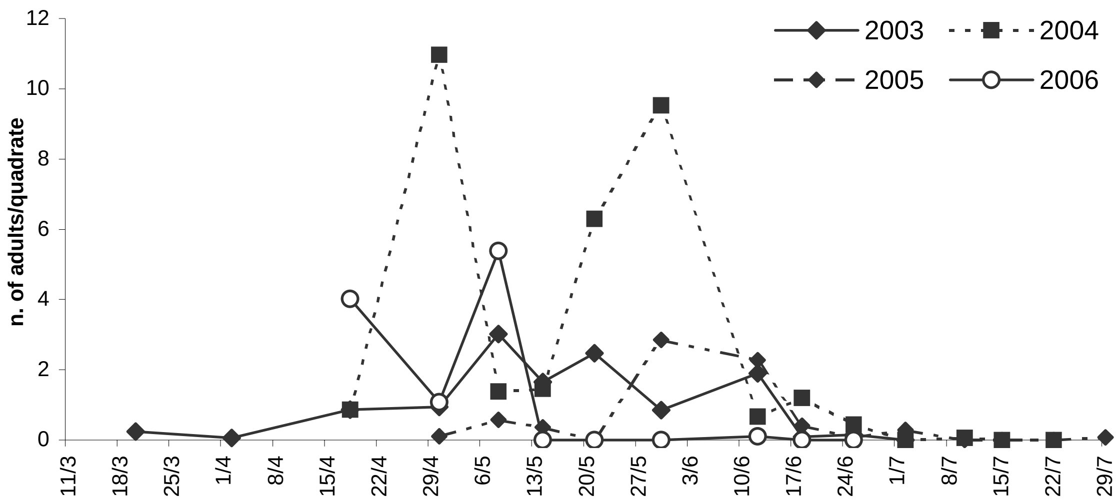

Fig. 3 Number of thrips adults per quadrate in the monitoring period (2003-06) in Siracusa lemon orchard

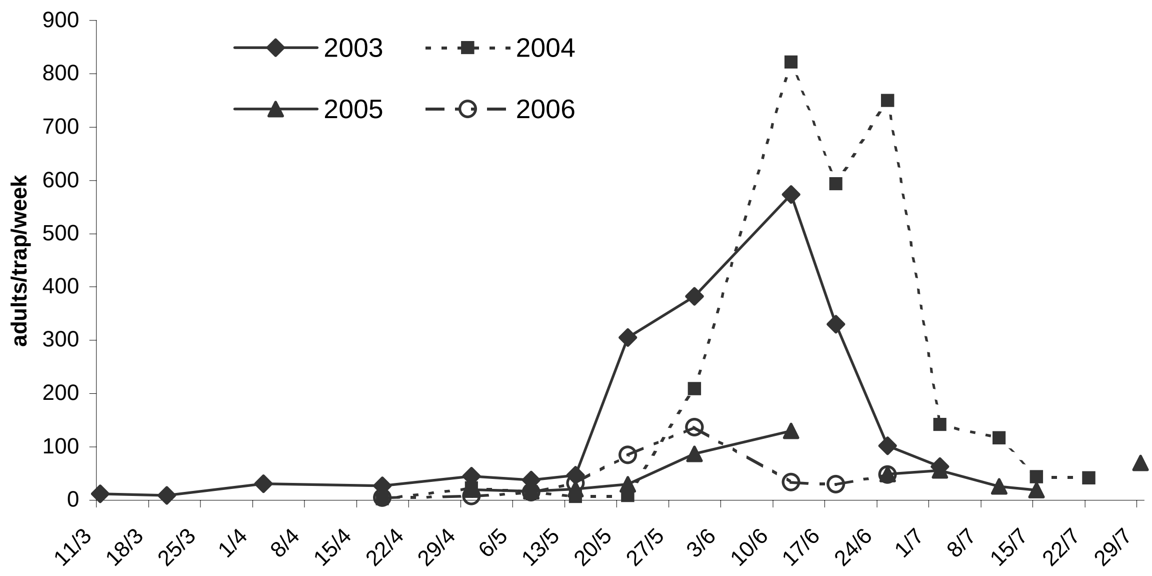

Fig. 4 Number of thrips adult per trap in the monitoring period (2003-06) in Siracusa lemon orchard

In 2004 the highest larvae and adult thrips presence was recorded at all, exceeding the peaks of 12 and 10 specimens/Q respectively (Fig. 2 and Fig. 3). The peak of adults captured on traps was the highest in 2003 and 2004 with a peak of 800 and 600 specimens per trap respectively (Fig. 4). The peaks on traps were recorded in mid–June, later than the maximum presence of larvae and adults detected on flowers and fruits. It is likely that adults captured on traps are the progeny of the population detected on the canopy.

During the 2004 survey period, a mean of 3.28 larvae per Q was recorded and it was statistically higher in comparison with the others survey years while a mean of 2.53 adults per Q was record, statistically different only with the 2005 monitoring year (Table 1). At that population level in 2004, the highest fruit damage (24%) occurred at the harvest period as reported in the Table 1. Flowers and young fruits monitoring showed a relationship between the number of thrips specimens per canopy quadrate and damage at harvest. In particular the differences in the number of larvae per Q were statistically comparable with the ranking of damage % during the 4 years of monitoring. The correspondence between the adult's presence and the damage at harvest was less clear, confirming that larvae are the major causal agent for fruit scarring. Traps were unable to express any significant relationship between thrips infestation and damage at harvest. The statistical differences among traps captures did not correspond to the ranking of damage at harvest and to the larvae or adult per Q (Table 1).

Table 1. Adult and larvae thrips density in relation to fruit and flower presence on canopy quadrate (25 x 25 cm) of lemon trees, trap captures and fruit damage at harvest in Sicily.

Year	fruitlet/Q (mean)	flower/Q (mean)	adult/Q (mean)	larvae/Q (mean)	adults/trap (mean)	fruit damage (mean %)
2003	2.34 a	0.73 a	1.02 ab	0.23 b	141.8 ab	13.0 b
2004	2.52 a	1.08 a	2.53 a	3.28 a	213.7 a	24.0 a
2005	2.74 a	0.58 a	0.54 b	0.22 b	47.7 ab	6.0 c
2006	3.07 a	1.06 a	1.18 ab	0.87 b	44.1 b	11.0 b

Statistical comparisons of data are made within the same columns and the different rankings are evaluated. Data were significantly different ($P< 0.05$) when followed by a different letter according to Duncan's test.

The dynamics of thrips presence on lemon trees canopy appears to be correlated with the trees physiology. A peak of larvae corresponds to the highest number of flowers and small fruits in spring. The thrips presence decreases drastically during the period of absence of flowers and fruitlets. We suppose that thrips population don't use bigger fruits for feeding activity. The evaluation of the data collected during four years of monitoring (2003-2006) showed a statistically close correspondence between fruits damage at harvest and density of thrips larvae on the canopy soon after the petal fall. When this presence exceeded the peak of 10 specimens per quadrate of canopy the damage was very high and not commercially suitable. Sticky traps don't seems to be a useful tools for assessing thrips density and predicting fruit damage at harvest.

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IOBC/wprs Bulletin Vol. 38, 2008
pp. 210-213

Citrus whiteflies in Israel

Dan Gerling[1] and Yael Argov[2]
[1] Department of Zoology, Tel Aviv University Ramat Aviv, Israel 69978 e-mail dangr@post.tau.ac.il [2] The Israel Cohen Institute for Biological Control, Plants Production and Marketing Board, P. O. Box 80, Bet Dagan 50250, Israel

Abstract: According to Evans (USDA: APHIS), there are about 105 species of whiteflies attacking citrus in the world. Their origin includes 59 neotropical species, 32 Asiatic species and a few which evolved in other parts of the world. Since the cultivated citrus plants have all been introduced into Israel, we are dealing with mostly an Asian flora whose whiteflies pests are introduced. Two of them *Aleurothrixus floccosus and Paraleyrodes minei* are from the Neotropics and two *Dialeurodes citri and Parabemisia myricae* are from East Asia. Less prominent pests are the Mediterranean *Acaud-aleyrodes rachipora* and *Aleurolobus marlatti* whose origin is Asiatic. Citrus trees are also occasional hosts to generalist whiteflies such as *Bemisia tabaci*. All citrus whiteflies in Israel (and most of those in the world) are polyphagous tree pests. The main relevant damage-related features are the life cycles of the different species and their relationship to the tree's phenology. Some life cycles are closely tied to that of the citrus tree, thus *D. citri* oviposits on young, but fully developed foliage, and produces three generations/year. *P. myricae* on the other hand which apparently evolved in the citrus centers of the world has eight to ten generations per year which oviposit exclusively on very young foliage. The South American species evolved under different conditions and on different hosts, therefore, *P. minei*, and *A. floccosus* are less dependent upon new foliation and have as many as six yearly cycles. The former is also unique in its frequent appearance on mature leaves.

The variability of the life cycles is also reflected in the different parasitoids that have been acclimated in Israel: *Encarsia lahorensis* for *D. citri*, *Eretmocerus debachi* for *P. myricae* and *Cales noacki* for *A. floccosus* by. No effective parasitoids for *P. mine are* known. Altogether, citrus whiteflies form a complex of extremely varied life histories and host adaptations that we should recognize if we want to manage them properly.

Key words: whiteflies, pest management, parasitoids, *Encarsia*, *Eretmocerus*, citrus, biological control

Introduction

Whiteflies (Homoptera: Aleyrodidae) are known pests of citrus and several species have been introduced into Israel during the last decades. These have caused outbreaks that usually were the subjects of numerous successful biological control campaigns (Argov et al 2003 and references therein). Examination of the makeup of the present whitefly fauna attacking citrus reveals that some are local, but the more pestiferous species are introductions either from the Far East (which is the presumable original home of citrus plants) or from Central and South America.

Examination of whiteflies biology shows that many of them are fine tuned to the trees on which they develop, especially to the foliating stage (eg. Walker 1985). Thus we should expect to find a variety of strategies in different species that had developed under different conditions. All of these should materialize together on the citrus in Israel. This difference in strategies should reflect on the life cycles of the pests as well as on their enemies. Its recognition could be helpful in planning biological control activities. Therefore, we examined the tritrophic plant-whitefly-parasitoid interrelationships and its relationship to the whitefly's origin. This paper summarizes the present situation as to the whiteflies and their parasitoids.

Material and methods

Most surveys, parasitoid introductions and disseminations, as well as appraisal of the results were made by The Israel Cohen Institute for Biological Control, Plants Production and Marketing Board, under the supervision of Dr. Y. Rössler and Y. Argov. Data were analyzed and summarized for presentation.

Results and discussion

Of the ca. 105 species of whiteflies listed by G. Evans (USDA:APHIS, personal communication) as attacking citrus, we found six to be worth noting. They include the Asian species *Dialeurodes citri* and *Parabemisia myricae*, the American species *Paraleyrodes minei* and *Aleurothrixus floccosus* and two locally occurring species *Acaudaleyrodes rachipora* and *Aleurolobus marlatti*. The latter is of special interest since its occurrence in Israel has only been recorded recently under the following circumstances. The first occurrence of *A. marlatti* was recoded in the sea port of Eilat in 1999. Since then it has been found widespread in all of the country as well as in Jordan occasionally reaching pest status. The name "marlatti" is a senior synonym (Martin 1999) of *A. niloticus* that has been described from Egypt already in 1934 and is widespread in the Eastern Mediterranean basin. Yet, until 1999, the insect was unknown as even a minor pest of citrus. Therefore, investigations as to the true nature of this species are underway.

As Table 1 shows, the whitefly species differ also in their bionomics which includes the number of generations per year and the capability to oviposit as related to leaf age.

Table 1. Origin and bionomic details of 6 whitefly species attacking citrus in Israel

whitefly	**origin**	**Generations Per year**	**Introduced bio control**	**Leaf age for oviposition**
Dialeurodes citri	Orient	3	+	Young mature
Parabemisia myricae	Orient	8 - 10	+	Very young
Aleurothrixus floccosus	Neotropical	5-6	+	Young mature
Paraleyrodes minei	Neotropical	5-6	–	Young + mature
Acaudaleyrodes rachipora	Mediterranean	3?	–	Young mature
Aleuorlobus marlatti	Orient - Mediterranean?	3	–	All ages

Whiteflies and their natural enemies

Most of the biological control work has been conducted with parasitoids, However, various predators, including phytoseiid mites, species of *Conwenzia* (Neuroptera: Coniopterigydae), and the Coccinellidae *Clitostethus arcuatus* and *Serangium montaserii* are often present and in some cases seem to be important controlling factors. No life table studies of their effects have been conducted.

Citrus whitefly *Dialeurodes citri*

Originating in the Far East, the citrus whitefly oviposits in young fully-developed, but occasionally in mature leaves. It has a life cycle peaking at 48-58 days (Vollka rootstock at 25^0C, L16: D8) but emergence continues for another ~ 40 days at low levels allowing for enough emergences during the next leaf flush to obtain oviposition on the new leaves. There are 3 generations/year: spring, Apr.-Jun. (65±18 days), summer, Jun.-Aug. (52 ±14 days) and Fall-winter, Aug.-Apr.~ 8 months. Five percent of the population has 4 generations per year and three percent has 2 generations per year (Argov et al. 1999).

The most effective parasitoid is *Encarsia lahorensis*, which oviposits in the fourth nymphal instar and, as most other *Encarsia* species, is an autoparasitoid with females being primary parasitoids and their males secondary ones.

Japanese bayberry whitefly *Parabemisia myricae*

The uniparental Japanese bayberry whitefly was discovered in the north of Israel in 1978 in citrus and avocado groves. It has 8 - 10 generations per year implying that it is not dependent upon the seasonal leaf flushes of the host plant. Instead, the whitefly adults may oviposit in the few young leaves that occur occasionally. The eggs, which are laid in circles, may occur on both sides of the leaf, but crawlers prefer to settle on the underside. Average life time oviposition is ca. 70 eggs/female. The eggs hatch after 6-8 days and the life cycle at 23^oC is 13-27 days from crawler to adult (Swirski et al 2002).

The whitefly is effectively controlled by the thelytokous parasitoid *Eretmocerus debachi* which was introduced from California in 1982 following its success there. It prefers to oviposit under the second host instar whereas host feeding is usually on the first instar.

Woolly whitefly *Aleurothrixus flocosus*

The woolly whitefly was first discovered in the Western Galilee in 1992, it spread rapidly and, like in other places, caused heavy infestations of citrus and other subtropical fruits. The whitefly oviposits circles of eggs on the underside of young fully-developed, leaves. It produces 5-6 generations/year, indicating again, that its probable host plant has not been citrus but other South American plants.

A number of South American parasitoids are known for this pest. We introduced *Cales noacki* which oviposits in instars 2-4 of the host and succeeded, like in most countries, to bring the pest under satisfactory management.

Nesting whitefly *Paraleyrodes minei*

This species was discovered in Israel in1993 and presently is mainly a pest of avocado although it abounds in many citrus plantations. It has not been reared or studied in detail in Israel, but data from Spain indicate that it has 5-6 generations/year whereas Californian data point to 4 generations. In Israel the pest is occasionally parasitized by *Encarsia hispida* whereas in the US it is known to be attacked by *E. variegata*.

Efforts to find more natural enemies for *P. minei* have, thus far not been successful. The 7-odd species of the genus, some of which have already established themselves as pests in Africa, are all considered relatively rare in the Americas and a special project for locating their specific parasitoids should be conducted.

Aleurolobus marlatti

As said, this might be a well established species in the Middle East, but not on citrus, where it has been recorded only since 1999. On citrus it reaches occasionally very high populations but usually does not become a pest. Like other *Aleurolobus* species, it oviposits and develops on both sides of the leaf; however, it prefers the leaf underside. The number of generations/year varies according to the host plant, with four on citrus but 2-3 on *Punica granatum* and *Zyziphus spina-christi*. It is known to be parasitized by *Encarsia elegans*, *Encarsia* spp. and by *Eretmocerus* (?) *aleurolobi*.

Babul whitefly *Acaudaleyrodes rachipora*
This is a widespread whitefly, which occurs on scores of wild hosts throughout the Mediterranean basin and reached the Far East. It is usually not considered a pest in Israel whereas in some Asian countries (notably India whence its common name comes from) it may reach damaging proportions. *A. rachipora* has not been reared in Israel but there are presumably 3 generations/year. The most prominent recorded parasitoids are *Eretmocerus roseni, Encarsia lutea* and *Encarsia davidi.*

In summary, some 4 species of very damaging whitefly species have been introduced into Israel during the last few decades. They originated in different parts of the globe and on different host trees. Thus they demonstrate a variety of life cycles. These species cause severe damage to citrus trees if not checked by natural enemies. On the other hand, the introduction and establishment of natural enemies has brought about excellent biological control and enabled the citrus industry to keep insecticidal treatments at a minimum. Exact knowledge and understanding of the tritrophic life cycles and relationships involved is essential to keep this system under proper management.

Acknowledgements

We wish to thank the staff and workers of the Israel Cohen Institute for Biological Control, Plants Production and Marketing Board and the Entomology group at Tel Aviv University for their encouragement.

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First observations on the influence of *Bacillus subtilis* on the populations of *Dialeurodes citri* (Ash.) (Hom. Aleurodidae) in various citrus fruit orchards of Mitidja (Blidean Atlas, Algeria): is there an insecticidal effect?

L. Allal-Benfekih[1], Z. Djazouli[1], F. Rezig[2], O. El Mokaïd[2], F. Hamaïdi[2]
[1] *Département d'Agronomie, Faculté agro vétérinaire, Université de Blida, DZ 09000, Algeria*
[2] *Département de Biologie, Faculté agro vétérinaire, Université de Blida, DZ 09000, Algeria*

The bacterium *Bacillus subtilis* was for the first time tested on populations of devastating insects of citrus fruits of Mitidja, in the Blidean atlas. We present in this work the preliminary results of bioessays using suspensions of spores of *B.subtilis* on the various stages of development of *Dialeurodes citri* (Ash.), from March to June 2004, periods of proliferation of this pest in the area. Significant death rates are observed after 7 days of treatment. An analysis of the variance enabled us to discuss the insecticidal effect of the bacterium with 3 various amounts (10^{-1}, 10^{-5} and 10^{-8} spores /ml) on the eggs, the young larvae (L_1-L_2) and the old larvae (L_3-L_4) of *D. citri*, on clementines, orange trees (Thomson Navel) and lemon trees.

Field evaluation of the entomopathogenic fungi, *Beauveria bassiana* and *Verticillium lecanii* against jasmine whitefly, *Aleuroclava jasmine* on citrus

H.F. Alrubeai, S.O. Klawi, J.B. Hammad, M.W. Khader
Ministry of Science & Technology, IPC Res. Ctr ., P.O.BOX 765, Baghdad, IRAQ

Pathogenicities of entomopathogenic fungi, *Beauveria bassiana* and *Verticillium lecanii* were evaluated against jasmine whitefly, *Aleuroclava jasmine*, infested citrus trees under field conditions of three different locations. Results indicated that parasitization percentages of *B. bassiana* on eggs and nymphs were relatively comparable to that of *V. lecanii* with some differences between locations. The parasitization percentages of eggs were, in general significantly lower than that of nymphs. It was found that parasitization of both fungal species tested, increased significantly with time. The results showed the probable negative effect of high temperatures and low humidity on both fungal parasitization potentials.

Control in Citrus Fruit Crops
IOBC/wprs Bulletin Vol. 38, 2008
pp. 216-224

Life cycle of *Aphis spiraecola* Patch (Homoptera: Aphididae) in East Mediterranean Region of Turkey and its development on some important host plants

Serdar Satar, Nedim Uygun
University of Çukurova, Faculty of Agriculture, Department of Plant protection, 01330, Balcalı-Adana, Turkey

Abstract- Aphids are important pests on citrus in East Mediterranean Region of Turkey. Five different aphid species were determined in citrus of Mediterranean region of Turkey. These are; *Aphis spiraecol*a Patch, *Aphis gossypii* Glover, *Aphis craccivora* Koch, *Myzus persicae* Sulzer and *Toxoptera aurantii* (Boyer de Fonscolombe) (Homoptera: Aphididae). *Aphis spiraecola* and *Aphis gossypii* are the most abundant and diverse species in the region.

In present study, host plants of *A. spiraecola* and its life cycles on these hosts were investigated in Eastern Mediterranean Region of Turkey. We also evaluated developmental periods, fecundity longevity and death ratios of the aphid on *Eriobotrya japonica, Pyracantha coccinea* and C*itrus sinensis* (Washington navel) at four constant temperatures (15, 17,5, 20 and 25 ± 1°C) under laboratory conditions.

According to the results, *A. spiraecola* has 11 host plants and overwinters on *E. japonica* as anholocyclic in Eastern Mediterranean Region of Turkey. *E. japonica, P. coccinea*, *Vibirnum tinus* and all varieties from genus citrus were found as important hosts of *A. spiraecola*. The developmental time of *A. spiraecola* was ranged from 14.4 days for 15°C to 6.5 days for 25°C on *E. japonica*, ranged from 13.3 days for 15°C to 6.7 days for 25°C on *P. coccinea* and ranged from 12.1 days for 15°C to 5.8 days for 25°C on Washington navel. While no death ratio was observed at lower temperatures (15, 17.5 and 20°C) and 6.8% death ratio at 25°C on Washington navel, the death ratio on *E. japonica* was ranged from 9.1% at 15°C and to 15.7% at 25°C. The intrinsic rates of increase (r_m) at 15°C were 0.1029, 0.1078 and 0.1551 ♀♀/♀/day^{-1} on *E. japonica P. coccinea* and Washington navel respectively. On the other hand, the highest r_m was calculated at 25°C (0.2891 ♀♀/♀/day^{-1}) on Washington navel. The lower developmental thresholds of *A. spiraecola* were 7.4, 5.8 and 7.2°C and it required, 113.6, 131.6 and 107.5 degree-day for a first instar to become adult, on *E. japonica, P. coccinea* and Washington navel respectively.

Key Words: *Aphis spiraecola*, Citrus, Life cycle, East Mediterranean Region of Turkey

Introduction

The spirea aphid, *Aphis spiraecola* Patch (Homoptera: Aphididae) is an important pest of citrus and some ornamental plants in Eastern Mediterranean Region of Turkey. The citrus plantations have five different aphid species which are *Aphis craccivora* Koch, *Aphis gossypii* Glover, *Aphis spiraecola*, *Myzus persicae* (Sulzer), *Toxoptera aurantii* (Boyer de Fonscolombe) (Homoptera: Aphididae) (Yumruktepe & Uygun, 1994). *Aphis spiraecola* is one of the most abundant aphids on citrus in Turkey (Uygun et al., 1992; Yumruktepe & Uygun, 1994). The primary economic impact of *Aphis spiraecola* not only arises from its foliage distortion but also promotes growth of sooty mold and attracts the ants which fend off natural enemies of aphids especially spring-time attacks are the most deleterious in young citrus orchards. During flowering, an attack causes the flowers to drop off.

Despite of the importance of the aphid, very little information is available about biology of *Aphis spiraecola* in the region (Uygun et al., 1992; Yumruktepe & Uygun, 1994). *Aphis spiraecola* was determined in 1970 in Israel (Zevahi & Rosen, 1987) and in 1980 in Turkey (Lodos, 1982). It is also an important pest of apple (Pfeiffer et al, 1989) but it rarely found on apple in Turkey.

Temperature and host plants affect insect population processes such as mortality, development and fecundity. Several papers have been published on effect of temperature (Wang & Tsai, 2000) and host plant (Tsai & Wang, 2001) on spirea aphid life histories. But none of this paper has provided information on how they interact to affect aphid development, reproduction and survival. And Also the importance of the result to Mediterranean region of Turkey is totally unknown. A thorough understanding of pest biology and population dynamics is necessary for establishment of management strategies (Campbell et al., 1974; Komazaki, 1982). Therefore the present study was designed to provide primarily data on developmental rate and fecundity of a local citrus population of *Aphis spiraecola* on different host plants and at different constant temperatures in the laboratory and also life cycle of *Aphis spiraecola*. This knowledge may provide useful information about understanding the population dynamics of *Aphis spiraecola* on citrus in the east Mediterranean region of Turkey.

Material and methods

Host plant and life cycle

To determine the host plants of *Aphis spiraecola* field survey were done periodically and/or unperiodically in East Mediterranean region of Turkey. Infected plant material brought the laboratory for identification of the aphid. Aphis identification was done by Serdar SATAR. After the identification of *Aphis spiraecola,* the plant was accepted as a host plant. Life cycle of *Aphis spiraecola* was investigated in the campus of the Çukurova University and its production field. Çukurova University has more than 2000 ha including parks, citrus orchards, stone fruits orchards, apple orchards, wheat, turf etc.

Aphid rearing

Aphis spiraecola were obtained from mandarin trees near Adana in the East Mediterranean region of Turkey in May 2002 and colonized on Mayer lemon and different satsuma mandarien varietes at 25 ± 1 °C, 65 ± 10 % Rh. and 16 h of artificial light of about 5,000 Lux in a insect rearing room. Aphids were reared in laboratory for two to four generations before individuals were used in the experiments.

Host plant testing

Two commercial host plants (*Eriobotrya japonica* Lindl. and *Citrus sinensis* Washington Navel) and one ornamental plant (*Pyracantha coccinea*) were tested to determine the host range and effect of temperature on *Aphis spiraecola.* To overcome the preconditioning effect of the prior host, aphids were reared for two generations on each variety before starting the experiment (Kindlmann & Dixon, 1989)

Development and survivorship of nymphs

Apterous adult aphids from the colony were transferred onto shoots which were maintained in small glass bottles, 1 by 4 cm (diameter by height), filled with a modified Hoagland solution containing the following nutrients per liter of distilled water: 0.69 g $Ca(NO_3)_2\ 4H_2O$; 0.29 g KNO_3; 0.08 g KH_2PO_4; 0.29 g $MgSO_4\ 7H_2O$; 0.31 ml $FeCl_3\ 6H_2O$; 0.04 g Na_2 EDTA $4H_2O$; 0.0072 g H_3BO_3; 0.0046 g $MnCl_2\ 4H_2O$; 0.0003 g $ZnCl_2$; 0.0001 g $CuCl_2\ 2H_2O$; 0.00008 g $Na_2MoO_4\ 2H_2O$; pH 6.2 (Hoagland & Arnon 1950). Aphids were then transferred to the shoot which was then covered with a transparent plastic cage, 15 by 5 cm (length by diameter), with a polyester organdy top. Nymphs born within 24 h were transferred individually by camels-hair brush and placed onto a single shoot and placed in environmental chambers at 15, 17.5, 20, and 25± 1 °C at a humidity and photoperiod as mentioned above. The aphids were checked daily for exuviae and survivorship at all temperature regimes. Aphids were transferred to new shoot every fourth day until the death of the test aphids. Shoots were cut directly from the tree to maintain a fresh supply of host material.

Adult longevity and reproduction

When the test aphid became reproductively mature, the number of offspring and mortality were determined daily. Nymphs were removed from the test arena after counting and these observations continued until the mature aphid died.

Data analysis and evaluation

The data were analyzed per temperature by analysis of variance (ANOVA) and differences determined by the Duncan test. Statistical tests were performed using PROC GLM (Anonymus, 1998). The relationship between temperature (T) and the rate of development (r_T) was described by a linear regression model where $r_T = a + bT$ following the method of Campbell et al. (1974). The thermal threshold (t) and the thermal constant (K) were estimated by the equation t=-a/b and $K = 1/b$, where a and b are estimated parameters and the data are expressed as degree-days (Campbell et al. 1974). Life table construction was done using age specific fecundity (m_x) and survival rates (l_x) for each age interval (x) per day (Andrewartha & Birch 1954) and the intrinsic rate of increase (r_m) was assessed by the equation:

$$1 = \Sigma\, e^{-r*x}\, l_x * m_x \qquad (1)$$

In which : x= age in days (including immature stages), r = intrinsic rate of increase, l_x = age-specific survival (including immature mortality), m_x = age-specific number of female offspring.

After r was computed for the original data (r_{all}), differences in r_m-values were tested for significance by estimating variances through the jack knife method (Meyer et al. 1986). The jack knife pseudo-value r_j was calculated for the n samples using the following equation:

$$r_j = n * r_{all} - (n-1) * r_i \qquad (2)$$

The mean values of (n - 1) jack knife pseudo-values for each treatment were subjected to ANOVA. The differences between the mean values of jack knife pseudo-values were analyzed by LSD test. Statistical tests was performed using PROC GLM (Anonymus, 1998).

Results and discussion

Field survey for determining the host plant and life cycle of *Aphis spiraecola* showed that *A. spiraecola* has 11 host plants in east Mediterranean Region of Turkey (Table 1). Out of these 11 host plants, six plants are economically important host plants while 5 of them are ornamental plants. All the host plants were determined along the East Mediterranean Region of Turkey except *Lavandula* sp., it could determined only in Hatay province.

All the determined host plants are shrubs and trees belonging to the families Rutaceae, Rosaceae, Caprifoliaceae and Rhamnaceae. According to Kranz et al. (1977) and Blackman & Eastop (1984) *Aphis spiraecola* is found on over 65 plant genera including economically important crops like citrus, cacao, papaya anona, *Malus* sp., *Pirus* sp., *Prunus* sp. etc. Except the *Lavandula* sp. and *Paliurus spina-christi*, all the host plants are evergreen and *Aphis spiraecola* is found on these two host plants during the flowering time and of the plant. Plant ingredients, particularly flowering time makes the plants as suitable host (Dixon, 1985).

Aphis spiraecola shows anholocyclic life cycle on its host plants in Eastern Mediterranean Region of Turkey as in Israel (Zehavi & Rosen, 1987). It overwinters on *E. japonica* as alatae and apterous viviparous females in the region. *E. japonica* is native to China and Japan. They are popularly grown as ornamentals in orchards in the southeast of the Mediterranean region of Turkey. *E. japonica* is grown in city parks or house gardens as an ornamental plant and serves as an overwintering host. Opposite of the rest of the host pants, *E. japonica* gives the flower and flushes during winter time, and also microclimate in city creates necessary condition to live on it.

Table 1. Life cycle (marked by dark colours) of *Aphis spiraecola* on determined host plant in Çukurova.

Host Plants	**Family**	**Months**											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Citrus limon	Rutacea												
Citrus paradisi	Rutacea												
Citrus reticulata	Rutacea												
Citrus sinensis	Rutacea												
Cotoneaster spp**.**	Rosacea												
Eriobotrya japonica	Rosacea												
Sipirae vonhautti	Rosacea												
Pyracantha coccinea	Rosaceae												
Viburnum tinus	Caprifoliaceae												
Palirus spina-christi	Rhamnaceae												

Table 2.Mortality (%) and development periods (Days ± SE) of immature stages of *Aphis spiraecola* on three host plants at four constant temperatures.

Temperature (°C)	n	Total nymphal mortality rate (%)	Developmental time (days)
E. japonica			
15	44	9.1	14.4 ± 0.23 d
17.5	44	4.6	12.1 ± 0.26 c
20	39	5.2	8.9 ± 0.23 b
25	52	15.7	6.5 ± 0.12 a
P. coccinea			
15	46	2.5	13.3 ± 0.20 d
17.5	40	2.2	11.6 ± 0.22 c
20	49	4.1	9.9 ± 0.18 b
25	41	19.0	6.7 ± 0.21 a
Washington			
15	43	0	12.1 ± 0.09 d
17.5	44	0	11.7 ± 0.13 c
20	44	0	9.1 ± 0.12 b
25	44	6.8	5.8 ± 0.08 a

* Means in columns (for each of plants) followed by the same letter are not significantly different by Duncan Multiple Range Test (α = 0.05). (α=0,05; FE. japonica Development.= 270.709, df = 3, 159; FP.coccinea Development.= 181.080, df = 3, 164; FWashington development.= 650.706, df = 3, 171)

Pyracantha coccinea is one of the important host plants of *Aphis spiraecola* and the aphid pass on it all year except winter months. *P. coccinea* was tried as a rearing plant for *Aphis spiraecola* which was collected from citrus plants. But it was shown that it is not a suitable host for rearing *Aphis spiraecola* because of not colonized on it. It may be that *Aphis spiraecola* on *P. coccinea* is different biotype than *Aphis spiraecola* on citrus. According to Komazaki (1988), *Aphis spiraecola* has different biotypes.

Developmental time of immature stages on the three plants at four temperatures is presented in Table 2. Temperature significantly affected the development of *Aphis spiraecola.* There were significant differences in development time of immature stages among the population on different host. Development time from birth to adult moult was 14.4 days at 15°C and 15.7 days at 25°C respectively on *E. japonica*, 13.3 days at 15 °C and 6.7 days at 25°C respectively on *P. coccinea* and 12.1 days at 15°C and 5.8 days at 25°C respectively on Washington navel orange.

Mortality during the birth to adult moult was lowest on Washington navel in all the constant temperatures compare to *E. japonica* and *P. coccinea*. Increased mortality of *Aphis spiraecola* was recorded at the (%9.1) lower and (%15.7) higher temperature on *E. japonica* whereas no mortality was recorded on Washington navel orange the temperature between 15 to 20°C. The highest mortality occurred mainly at highest temperature for all the plants (Table 2). Van Lenteren & Noldus (1990), stated that shorter developmental times and greater total reproduction on a host reflect the suitability of a host. Our results on development and mortality showed that the shortest development and the lowest mortality occurred on Washington navel orange than *E. japonica* and *P. coccinea*. Developmental time of *Aphis spiraecola* also on *E. japonica*, *P. coccinea* and Washington navel orange was shorter than those reported by Tsai & Wang (2000) for *Aphis spiraecola* on six different host plants at 25°C.

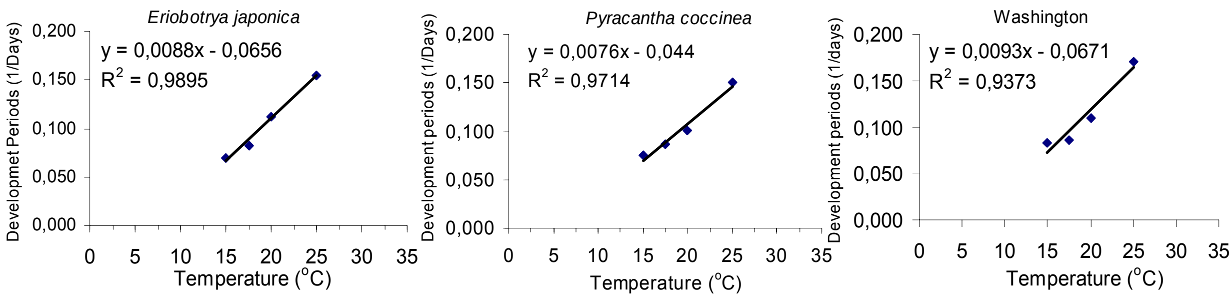

Figure 1. Developmental periods of *Aphis spiraecola* on three host plant at four constant temperatures. Line is the linear regression analysis of developmental period and temperature within the range of 15 – 25 °C.

A linear regression analysis was applied to the developmental points within the 15-25°C range for *Aphis spiraecola* on *E. japonica, P. coccinea* and Washington navel orange. Within the temperature range the developmental rates ($r_{[T]}$) of *Aphis spiraecola* increased linearly with increasing temperature (Figure 1). The theoretical development thresholds of *Aphis spiraecola* on *E. japonica, P. coccinea* and Washington navel orange were estimated as 7.4°C, 5.8 and 7.2, respectively. *Aphis spiraecola* on *E. japonica, P. coccinea* and Washington navel orange required 113.6 DD, 131.6 DD and 107.5 DD for a first instar to become adult based on the developmental threshold for overall immature stages (Figure 1). Komazaki (1982) calculated development thresholds and thermal constant of *Aphis spiraecola* on Satsuma mandarin as 7.9°C and 101 DD, respectively. On the other hand, Wang & Tsai

(2000), assessed development thresholds and thermal constant of *Aphis spiraecola* on *Polyscias crispate* (Bull) as 2.3°C and 197.8 DD.

Analysis of variance showed that temperature affect significantly on longevity, offspring per reproduction day and total number of offspring (Table 3). The longevity of individuals declined exponentially from 27.8 to 14.7 days for *E. japonica*; 23.8 to 10.9 for *P. coccinea* and 31.6 to 10.9 days for Washington. Offspring per reproduction day increased with increasing temperature (table 3). The highest number of offspring per female was recorded at 25°C for three host plants. Total number of offspring in all the working temperature is highest on Washington navel orange compare to *E. japonica* and *P. coccinea*.

The reproduction rate (R_o), intrinsic rate of increase (r_m) and Generation time (T_o) were calculated for the populations on 3 host plants at four temperatures (Table 4).The effects of temperature on intrinsic rate of increase and generation time were significant ($P<0.05$), however the effects of temperature on reproduction rate for *E. japonica* and *P. coccinea* were not significant ($P_{E.\ japonica} = 0.214$; $P_{P.\ coccinea}=0.709$). The populations kept in 25°C had the highest r_m and lowest T_o.

Table 3. Longevity, offspring per reproduction day, total number of offspring of *Aphis spiraecola* on three host plants at four temperatures (mean±SE)*.

Temperature (°C)	n	Longevity (Days)	Offspring per reproduction day (♀/♀/Day)	Total number of offspring (♀/♀)
E. japonica				
15	40	27.8 ± 1.85 b	0.5 ± 0.05 a	14.6 ± 1.79 ab
17.5	41	16.5 ± 1.45 a	0.6 ± 0.06 a	11.2 ± 1.37 a
20	36	16.1 ± 1.41 a	0.7 ± 0.09 a	12.9 ± 1.69 a
25	43	14.7 ± 0.94 a	1.2 ± 0.10 b	17.8 ± 1.38 b
P. coccinea				
15	45	23.8 ± 2.07 b	0.5 ± 0.04 a	12.4 ± 1.23 a
17.5	39	19.9 ± 1.53 b	0.7 ± 0.05 b	13.9 ± 1.28 ab
20	47	13.0 ± 0.99 a	1.0 ± 0.07 c	12.1 ± 1.03 a
25	33	10.9 ± 0.63 a	1.5 ± 0.10 d	16.7 ± 1.51 a
Washington				
15	43	31.6 ± 2.46 c	0.8 ± 0.05 a	24.0 ± 1.91 a
17.5	44	21.2 ± 1.58 b	1.0 ± 0.05 a	20.7 ± 1.65 a
20	44	23.1 ± 1.70 b	1.5 ± 0.07 b	33.9 ± 2.69 b
25	41	14.9 ± 1.10 a	1.6 ± 0.08 b	23.2 ± 1.62 a

*Means in columns (for each of plants) followed by the same letter are not significantly different by Duncan Multiple Range Test (α = 0.05). (α=0,05; df= 3, 159 $F_{E.\ japonica\ long.}=18.088$, $F_{E.\ japonica\ ♀/♀/day}=18.919$ $F_{E.\ japonica\ total\ offspring}=3.377$; df= 3, 163 $F_{P.coccinea\ long.}=15.981$, $F_{P.coccinea\ ♀/♀/day} = 42.004$, $F_{P.coccinea\ total\ offspring} =2.560$; df= 3, 171 $F_{Washington\ long.}=14.497$, $F_{Washington\ ♀/♀/day} =33.930$, $F_{Washington\ total\ offspring}=8.362$)

Intrinsic rate of increase (r_m) is the only statistics that adequately summarizes qualities of an animal relative to its capacity of increase (Andrewartha & Birch, 1954). Our result gave that populations raised at 25°C had highest r_m (0.2402 on *E. japonica*, 0.2443 on *P. coccinea* and 0.2891 on Washington) among the all the tested temperatures because it fastest development on this temperature (Table 4.). Also Wang & Tsai (2000) and Satar et al., (1998) had highest r_m on 25°C (0.308) for *A. spiraecola* on *P. crispate* and (0.3024) *Aphis gossypii* on Grapefruit, respectively.

Table 4. Reproduction rate (R_o), intrinsic rate of increase (r_m), and generation time (T_o), of *Aphis spiraecola* on three host plants at four constant temperatures (mean±SE)*

Temperature (°C)	n	Parameters		
		Reproduction rate (R_o) (aphids aphid^{-1})	Intrinsic rate of increase (r_m) (aphids aphid^{-1} day^{-1})	Generation time (To) (days)
E. japonica				
15	44	13.5 ± 1.74	0.1029 ± 0.0063 a	28.4 ± 1.01 d
17.5	44	10.5 ± 1.35	0.1246 ± 0.0079 a	20.9 ± 0.82 c
20	39	12.0 ± 1.66	0.1599 ± 0.0096 b	17.4 ± 0.64 b
25	52	14.8 ± 1.49	0.2402 ± 0.0122 c	12.6 ± 0.35 a
P. coccinea				
15	46	12.2 ± 1.23	0.1078 ± 0.0041 a	26.3 ± 0.92 d
17.5	40	13.5 ± 1.29	0.1364 ± 0.0054 b	21.3 ± 0.67 c
20	49	11.7 ± 1.05	0.1648 ± 0.0070 c	16.5 ± 0.41 b
25	41	13.3 ± 1.59	0.2443 ± 0.0131 d	11.8 ± 0.35 a
Washington				
15	43	25.2 ± 1.92 a	0.1551 ± 0.0030 a	26.3 ± 1.52 d
17.5	44	22.9 ± 1.74 a	0.1675 ± 0.0045 a	21.5 ± 0.82 c
20	44	34.1 ± 2.68 b	0.2245 ± 0.0044 b	18.3 ± 0.36 b
25	44	21.6 ± 1.75 a	0.2891 ± 0.0083 c	12.9 ± 0.31 a

*Means in columns (for each of plants) followed by the same letter are not significantly different by Duncan Multiple Range Test (α = 0.05) ((α=0,05; df= 3, 178 $F_{E.japonica\ Ro}$=1.508, F $F_{E.japonica\ rm}$=42.782 $F_{E.japonica\ To}$=86.907; df= 3, 175 F $_{P.\ coccinea\ Ro}$=0.462, F $_{P.\ coccinea\ rm}$= 52.940, F $_{P.\ coccinea\ To}$=95.613; df= 3, 171 $F_{Washington\ Ro}$=7.612, $F_{Washington\ rm}$= 125.819, $F_{Washington\ To}$= 40.226).

Survival rates (l_x) of *Aphis spiraecola* adults sharply decreased right after the peak of nymph production at 20 and 25°C temperatures, while a relatively long post-reproductive period was observed at 15 C and 17.5°C for three host plants (Figure 2). On the *E. japonica* the highest age-specific number of nymphs per female per day (m_x) ranged between 1.0 at 15°C and 2.08 at 25°C, while 1.29 at 15°C and 2.45 at 25°C on *P. coccinea* and 1.67 at 15°C and 2.39 the day 9 (2.61 the day 16) at 25°C on Washington. The reproduction periods lasted as long as adults longevity. Survival rates were 0.75, 0.80 and 0.93 on the peak point of m_x on *E. japonica*, *P. coccinea* and Washington navel orange, respectively.

Our result indicate that the differences associated not only temperature but also host plant cultivar on humidity, predation and parasitism can play important roles in the population growth of this insect.

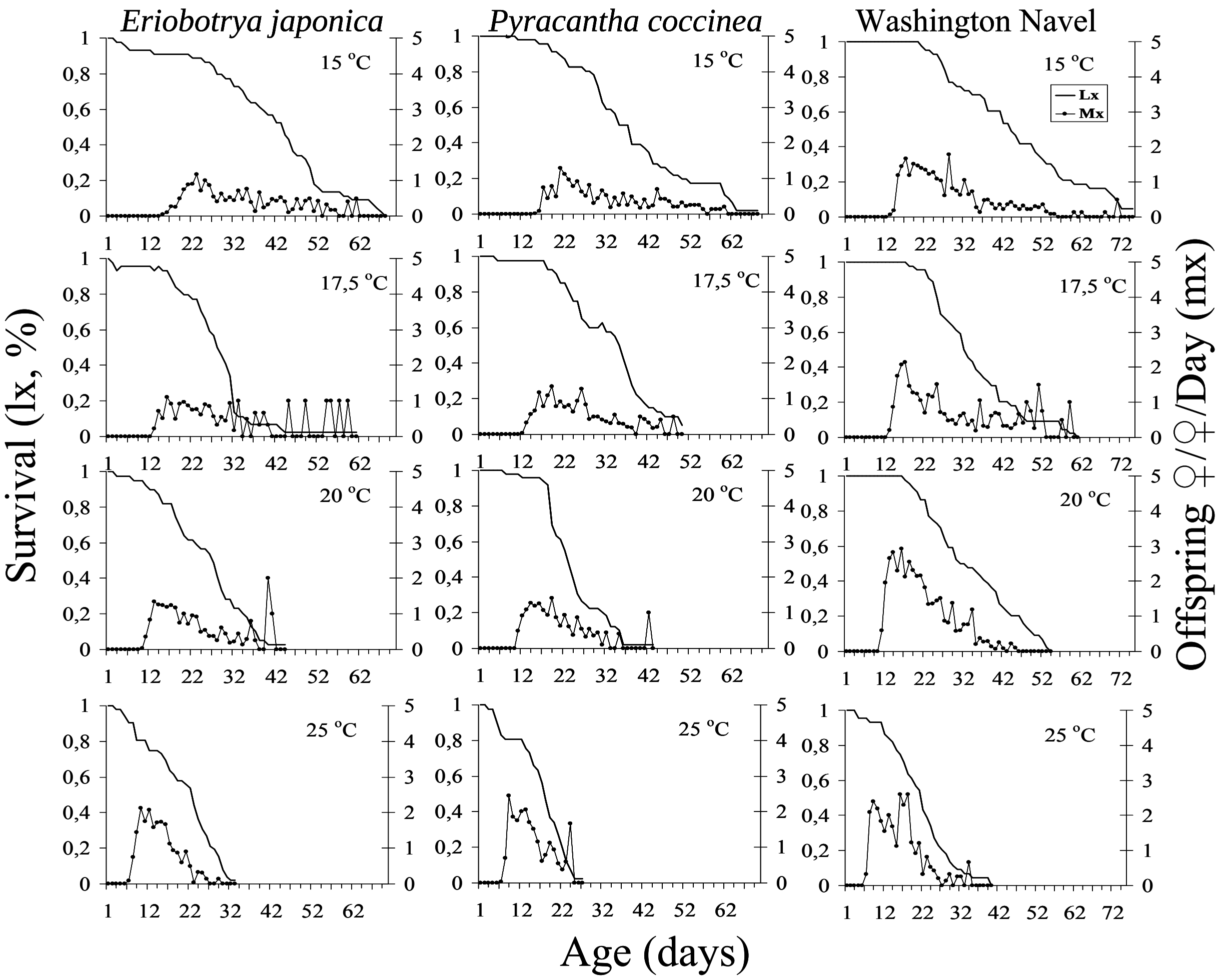

Figure 2. Age-specific survival rate (l_x) and age-specific fecundity (m_x) of *Aphis spiraecola* on three host pants at four constant temperatures.

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Control in Citrus Fruit Crops
IOBC/wprs Bulletin Vol. 38, 2008
pp. 225-232

Toxoptera citricida (Kirkaldy) (Hemiptera, Aphididae) and its natural enemies in Spain

Alfonso Hermoso de Mendoza[1], Almudena Álvarez[2], José Manuel Michelena[3], Pilar González[3], Mariano Cambra[1]
[1] Institut Valencià d'Investigacions Agràries, Apartat Oficial, 46113 Montcada, València, Spain. E-mail: ahermoso@ivia.es. [2] Laboratorio de Sanidad Vegetal, Consejería de Medio Rural y Pesca, c/ Lucas Rodríguez 4 bajo, 33011 Oviedo, Asturias, Spain. [3] Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, Apartat 2085, 46071 València, Spain.

Abstract: The aphid *Toxoptera citricida* (Kirkaldy) is the most efficient vector of *Citrus tristeza virus* (CTV) in the world, and it can transmit the more aggressive isolates of CTV. *T. citricida* is present in most of the zones growing citrus in the world, but it was absent from the Mediterranean Basin and North America until middle 1990's. Nevertheless, it was detected on citrus trees in 1994 in Madeira, in 1995 in Florida, in 2002 in Asturias, Spain (in yellow water traps), in 2003 in northern mainland Portugal, and in 2004 in southern Galicia, Spain, even though the three last detections were not published till 2005. As a consequence of its detection in Spain, several surveys and studies were undertaken from 2005. The main results are listed below.

Currently, *T. citricida* is present on citrus along the Atlantic coast in the northwest quadrant of the Iberian Peninsula. In Asturias, it presents a minimum in winter and other one in summer, but the last one is shorter than the minimum which Mediterranean citrus aphids have too. *Chaenomeles speciosa* (Rosaceae) has been found as an occasional alternative host for *T. citricida*. No winter eggs of *T. citricida* have been seen. CTV spread has not been detected in northern Spain. *T. citricida* is attacked in the Atlantic area by several natural enemy species, most of them present in the Mediterranean zone.

Key words: *Toxoptera citricida*, citrus, parasitoids, predators, *Citrus tristeza virus*

Introduction

Citrus tristeza virus (CTV) causes the most harmful and destructive disease affecting citrus (Bar-Joseph *et al*., 1989). In fact, the losses it causes are estimated at about 38 million trees in America, more than 55 million in the Mediterranean basin, especially in Spain, and about 5 million in other areas. Furthermore, to this we must add the low fruit quality and production loss of several million trees, grafted onto tristeza-tolerant stocks but that have been infected with severe CTV isolates, causing stem pitting in the rootstock and/or the variety (Bar-Joseph *et al*., 1989; Rocha-Peña *et al*., 1995; Cambra *et al*., 2000a).

CTV is transmitted by several aphid species (Hemiptera, Aphididae) in a semipersistent way. The most effective vector of CTV in the world is *Toxoptera citricida* (or *citricidus*) (Kirkaldy) (Meneghini, 1946; Bennet and Costa, 1949; Costa and Grant, 1951). However, *Aphis gossypii* Glover is the main vector in Spain, Israel, some citrus-growing areas of California (USA) and all those places where *T. citricida* is absent (Dickson *et al*., 1951; Bar-Joseph and Loebenstein, 1972; Raccah *et al*., 1976; Hermoso de Mendoza *et al*., 1984; Yokomi *et al*., 1989; Gottwald *et al*., 1996, 1997; Cambra *et al*., 2000a). In addition, other aphid species have been described, which are less effective CTV vectors: *A. spiraecola* Patch (Norman and Grant, 1954; Hermoso de Mendoza *et al*., 1984), *T. aurantii* (Boyer de

Fonscolombe) (Norman and Grant, 1956; Hermoso de Mendoza *et al*., 1984), *Myzus persicae* (Sulzer) (Varma *et al*., 1960), *A. craccivora* Koch and *Uroleucon jaceae* (Linnaeus) (Varma *et al*., 1965). On the other hand, *T. citricida* transmitted CTV 6-25 times more effectively than *A. gossypii* in parallel assays with the same virus isolates (Yokomi *et al*. 1994).

T. citricida is probably native to China, from where it must have spread to other countries in the East and South of Asia, Australia and sub-Saharian Africa. It seems quite probable that *T. citricida* went from South Africa to Brazil and Argentina with plant material, thus introducing tristeza into these countries (Moreno, 1995). After the epidemics in 1930-40 in Brazil and Argentina (in which 30 million citrus trees died), *T. citricida* advanced slowly toward the North of America until reaching Venezuela in 1976, where it caused the death of 6 million citrus trees over ten years (Rocha-Peña *et al*., 1995). In 1989, *T. citricida* was detected in Costa Rica (Lastra *et al*., 1991) and later in Belize (Pollard, 1997), in Guatemala (Palmieri, personal communication) and in Yucatan (Michaud and Álvarez, 2000), having also occupied the Caribbean islands, and in 1995 it arrived in the United States, to Florida to be precise (Moreno, 1995).

Concerning the Mediterranean basin, although citrus fruits had been cultivated for many centuries, the introduction of tristeza did not take place until the decade of 1920, and did so without the presence of *T. citricida*. Consequently, the tristeza problem here was relatively small and essentially centred in Spain and Israel. However, in 1994 *T. citricida* was detected on the island of Madeira, at the gateway to the Mediterranean (Fernandes and Cruz de Boelpaepe, 1994). Repeated surveys were made in continental Portugal without finding the aphid (Cruz de Boelpaepe and Ferreira, 1998) until 2003, when it was detected for the first time in the North of Portugal. In 2005 it was discovered that *T. citricida* had also been found in the North of Spain (in Asturias from 2002 and Galicia in 2004) (Ilharco *et al*., 2005) and this fact meant that *T. citricida* had been introduced into the Mediterranean basin. This posed a serious threat to the citrus industries in the area, both in terms of the pest itself and in its role as main vector of the tristeza, especially because the current strategy uses rootstocks that are tolerant to less aggressive strains of CTV, but cannot withstand such severe isolates of the virus.

The main Spanish citrus-producing regions are located in the East and South of the Iberian Peninsula, that is to say, quite far from the north-western areas where *T. citricida* had been detected. However, the danger of the aphid's propagation was evident, and therefore different plans of action were undertaken in Spain. On one hand, two projects were developed: "Preventive biological control to face the introduction of *Toxoptera citricida*" (INIA, 2005-08) and "Survey and studies of *Toxoptera citricida* on the Cantabrian cost" (IVIA, 2006-07).

Several surveys and different action-plans of another type (meetings, visits, etc.) were also carried out in the North of Spain from 2005. The aims of all these actions were, firstly, to study the situation of *T. citricida* in the North of Spain (geographical distribution, biological cycle and population dynamics, composition and dynamics of the fauna of natural enemies, search for alternative hosts to citrus, and survey of *Citrus tristeza virus*) and, secondly, to carry out comparative studies with other citrus aphids (population dynamics, and composition and dynamics of their natural enemies) in Valencia, the main Spanish citrus-growing area (located in the East of the Iberian Peninsula), where *T. citricida* has not appeared so far.

Material and methods

T. citricida has been surveyed using two different methods in the North of Spain. In the first place, square-based (60 x 60 cm) yellow water traps (Moericke, 1951) have been used, with captures being collected periodically to obtain data for the aphid flight graphs. One of these traps has been placed in each of the following locations: Asturias (Villaviciosa, 2002-07; Arbón, 2002; Tapia de Casariego, 2002; Pruvia, 2002; Niembro, 2002; Argüelles, 2002-03), Cantabria (Laredo, 2006-07; Novales, 2007) and Bizkaia (Bakio, 2006; Derio, 2007). In the second place, citrus were sampled to detect the presence of *T. citricida* in 2005 (Zaragoza, Gipuzkoa, Bizkaia, Cantabria and Asturias) and in 2006 (Pontevedra, A Coruña, Lugo and Asturias). Furthermore, intensive surveys of citrus have been carried out during 2006-07 in Asturias to determine the aphid’s diffusion, and eight lemon-tree plots have also been sampled periodically in order to study the pest’s development over time and that of its natural enemies. Meanwhile, samples of citrus have been taken throughout the North of Spain during 2005-07 to detect *Citrus tristeza virus* using the Tissue print-ELISA test (Cambra *et al*., 2000b).

During 2006-07, the citrus network *Plan de Vigilancia Fitosanitaria Citrícola* (Generalitat Valenciana, coordinated by F. García Marí and J.M. Llorens) has periodically provided samples of citrus aphid enemies for identification, collected throughout the Valencian territory. In addition, the development of the aphid colonies and their natural enemies on clementine trees has been followed up in two plots (L'Alcúdia in 2006 and Bétera in 2007).

Results and discussion

Figure 1 shows the results of the citrus aphids survey carried out in the North of Spain in 2005: *T. citricida* was detected on citrus in Asturias (where it had previously been found only in traps) and in Cantabria (where there were no references of its presence); however, it was not observed in any of the regions surveyed further East. Figure 2 shows a small change in the eastern boundary of *T. citricida* during 2006-07, since it was also detected in Bizkaia, although only one winged specimen was captured in a trap in 2006, while in 2007 none was trapped; furthermore, no live colonies of *T. citricida* have ever been seen in Bizkaia. Figure 2 gives the current distribution of *T. citricida* in Europe: it is present on citrus all along the north-western Atlantic coast of the Iberian Peninsula, from northern Portugal to Bizkaia, Spain [data of Portugal from 2006 (European Commission, 2006)].

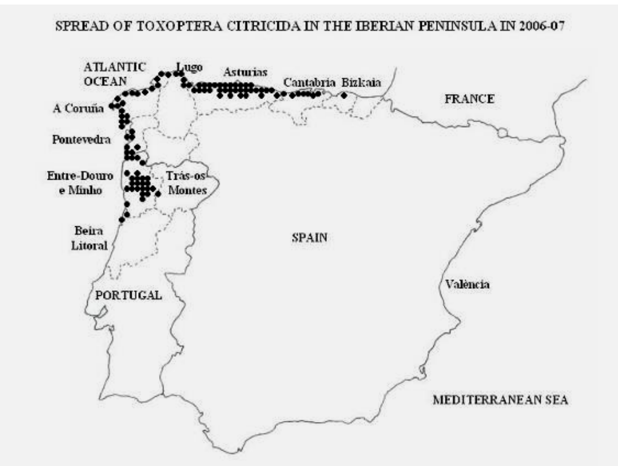

Figure 1

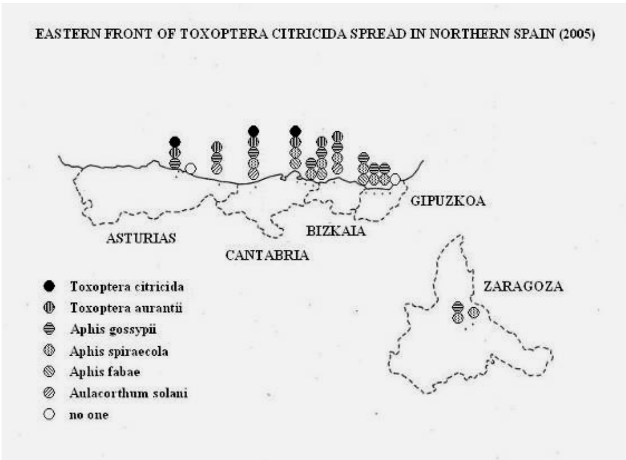

Figure 2

Figure 3 displays the time course of the winged *T. citricida* observed in Asturias, on the Atlantic, in the location where the trap has remained since 2002: In general there are two annual minimums (a very long one in winter and another very short one in summer) and two or three maximums (one or two in spring-summer and another in autumn). Figure 4 shows citrus aphid development during 2006-07 in the Valencian plots, on the Mediterranean: the two main species, *Aphis gossypii* and *Aphis spiraecola*, also usually display two annual minimums, in winter and summer (although the latter is longer than the Atlantic summer minimum), and two or three maximums (one or two in spring and another in autumn), which is common behaviour according to previous surveys (Hermoso de Mendoza *et al*., 1997).

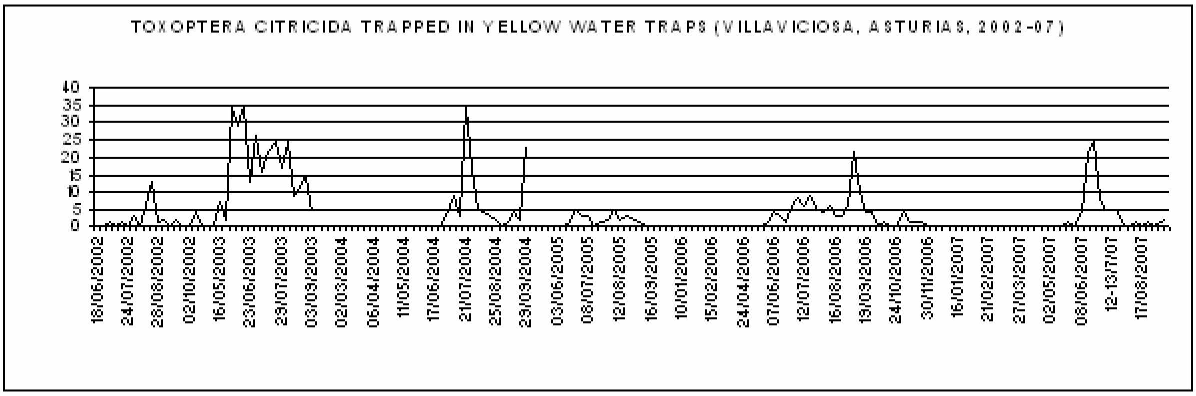

Figure 3

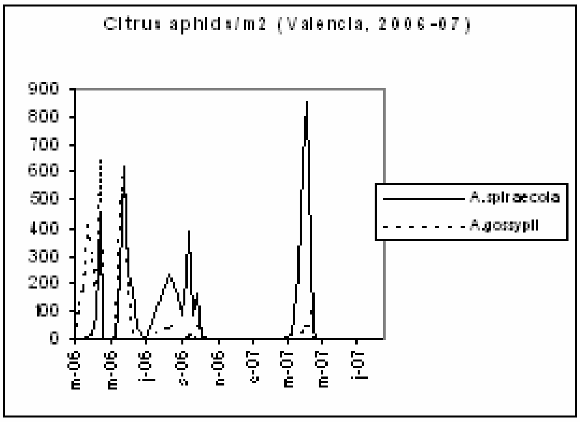

Figure 4

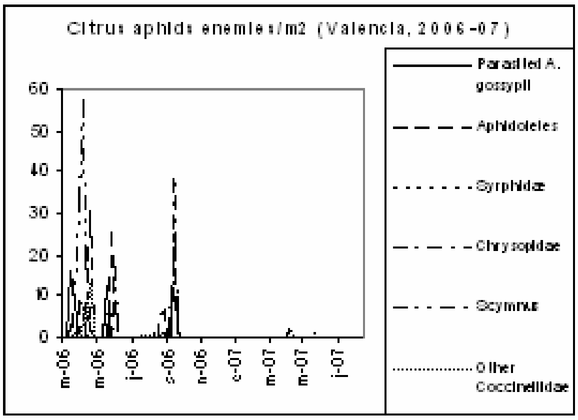

Figure 5

The natural enemies observed attacking *T. citricida* in Asturias during 2006-07 figure in Table 1. The enemies found in this period of time on the citrus aphids prospected in Valencia are also indicated there (both in the sampling plots and in the citrus network *Plan de Vigilancia Fitosanitaria Citrícola*), as well as the enemies observed in Valencia, not in these assays but in previous works (Quilis, 1930; Chalver, 1973; Michelena and González, 1987; Michelena and Oltra, 1987; González and Michelena, 1987, 1989; Llorens, 1990; Michelena *et al*., 1994; Michelena and Sanchis, 1997; Rojo, 1995; Urbaneja *et al*., 2005; Alvis Dávila and Garcia Marí, 2006). Looking at this Table, we can verify that *T. citricida* is attacked on the Spanish Atlantic coast by a large number of parasitoids and predators, most of which are also present on the Mediterranean coast.

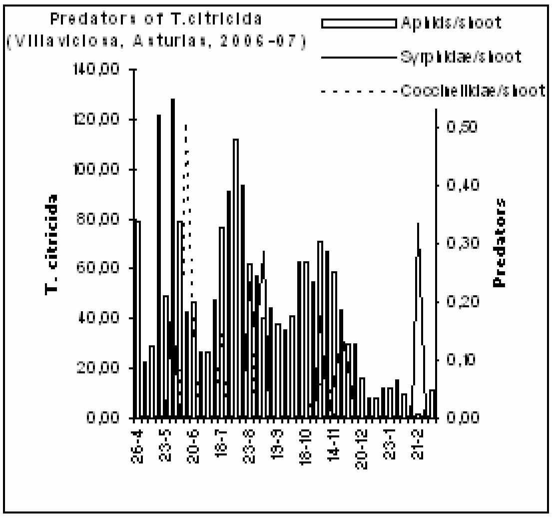

Figure 6

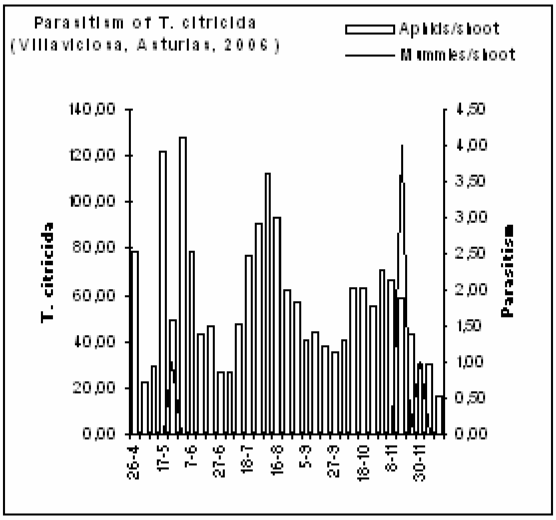

Figure 7

Table 1. Citrus-aphid enemies found in Spain on *T. citricida* (in Asturias) and on other citrus aphids (in Valencia) in 2006-07.

CITRUS APHIDS ENEMIES (2006-07)	on T. CITRICIDA (ASTURIAS)	on OTHER CITRUS APHIDS (VALENCIA) Plots	Citrus network	Previous
ACARI				X
Allotrombium pulvinum	X		X	
HYMENOPTERA APHIDIINAE				
Lysiphlebus fabarum	X			X
Lysiphlebus testaceipes	X		X	X
Trioxys angelicae	X			X
Trioxys acalephae			X	X
DIPTERA CECIDOMYIIDAE				
Aphidoletes aphidimyza	X	X	X	X
DIPTERA SYRPHIDAE				
Episyrphus balteatus	X			X
Syrphus ribesii	X			
Syrphus vitripennis	X			X
Epistrophe eligans	X			X
Meliscaeva auricollis	X			X
Eupeodes corollae		X		X
DIPTERA CHAMAEMYIIDAE				
Leucopis sp.	X			X
NEUROPTERA CHRYSOPIDAE				
Chrysoperla carnea	X	X	X	X
Chrysopa septempunctata	X			X
HEMIPTERA ANTHOCORIDAE				
Orius majusculus	X			
COLEOPTERA COCCINELLIDAE				
Adalia bipunctata	X			X
Coccinella septempunctata	X		X	X
Propylea quatuordecimpunctata	X	X	X	X
Scymnus subvillosus		X	X	X
Scymnus interruptus		X		X

Figures 6 and 7 show the time course of the main natural enemies (predators and parasitoids, respectively) of *T. citricida* in Asturias during 2006-07, with Syrphidae and Coccinellidae proving to be the most abundant predators. Figure 5 shows the same for the citrus aphid enemies in Valencia, but here the most numerous enemies are Cecidomyiidae (*Aphidoletes aphidimyza*) and Coccinellidae (particularly *Scymnus* spp.). The development of all the enemies is always synchronized, as is logical, with that of the aphids.

From the observations as to how *T. citricida* survives the winter in the North of Spain, it has been proven that it does so as nymphs or adults on shoots or buds of citrus in protected places. Neither eggs nor sexual forms have been found.

Once in Asturias, *T. citricida* was detected forming colonies on a different plant to citrus, the bush *Chaenomeles speciosa* (Rosaceae), never referred to previously as a host of this aphid.

Surveys of *Citrus tristeza virus* carried out on citrus in the North of Spain in 2005-07, have recorded only 3 CTV positive trees out of 1123 analyzed, that is to say, 0.26%. This represents a low incidence of the virus, which has not spread for the moment.

Conclusions

- *T. citricida* is present on citrus all along the NW Atlantic coast of the Iberian Peninsula, from northern Portugal to Bizkaia, Spain.
- In Spain, minimums are recorded for *T. citricida* on the Atlantic coast and citrus aphids on the Mediterranean coast both in winter and in summer, but the latter is shorter on the Atlantic.
- *T. citricida* is attacked on the Atlantic by several natural enemy species, most of which are present on the Mediterranean as well.
- One occasional alternative host plant species has been found for *T. citricida*: *Chaenomeles speciosa* (1st reference in the world).
- No sexual forms of *T. citricida* have been found.
- CTV spread has not been detected in northern Spain.

Acknowledgements

We would like to express our gratitude to: Miguel Cambra, Francisco Garín, Guillermo Urbieta, Alfonso González, Emilio Castro, Ana Feijoo, Severo Méndez, Pedro González, Máximo Braña, Rosa Pérez, M. Carmen Castaño, Raimundo Castaño, Lorenzo Molejón and Raquel Alzugaray for their help in the surveys of *T. citricida*; to Nicolás Pérez for identifying *T. citricida* the first time in Asturias; to M. Ángeles Marcos, Santos Rojo, Miguel Carles Tolrà and Arturo Goldarazena for identifying predators; to Vicente Borràs and Martín Llavador for lending us their plots; to Ferran Garcia Marí, José Manuel Llorens and people of Plan de Vigilancia Fitosanitaria Citrícola (Generalitat Valenciana); and to the INIA (project RTA2005-00095-00-00) and the IVIA (project 5608) for subsidizing this research.

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Ants, Coleoptera and others

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Survey of the ants (Hymenoptera Formicidae) in citrus orchards with different types of crop management in Sicily

Alessandra La Pergola[1], Antonio Alicata[2] & Santi Longo[1]
[1] *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università di Catania, Via S. Sofia 100, 95123 Catania, E-mail: a.lapergola@unict.it*
[2] *Dipartimento di Biologia Animale "M. La Greca", Università di Catania, Via Androne 81, 95124 Catania, E-mail: antonal@mail1.dba.unict.it*

Abstract: In the framework of a research on the interactions between honeydew-producing insects and ants, a survey on the species of Formicidae in citrus orchards was conducted by means of pitfall-traps distributed on the soil and manual captures on the canopy. In order to find analogies and differences about abundance and identity of ants in different types of crop management, captures were performed in three different citrus orchards in Catania plain, two under biological control and one under chemical control. During May-October 2006 and 2007 a total of 21,190 specimens of 25 species belonging to 17 different genera were collected and identified. *Linepithema humile* (Mayr, 1868), *Crematogaster scutellaris* (Olivier, 1791) and *Lasius alienus* (Förster, 1850), were found in a small number in the sprayed orchard. In the organic orchard a large number of *L. alienus* and *Formica cunicularia* Latreille 1798 was found; while *Tapinoma nigerrimum* (Nylander, 1856) and *Camponotus nylanderi* Emery 1921 are the most abundant species in the sprayed orchard. As regards the soil species *Pheidole pallidula* (Nylander, 1848) is not affected by chemical control while *Aphaenogaster semipolita* (Nylander, 1856) appears to be disturbed and was in fact found in lower number. A parallel trial on the symbiosis between ants and honeydew-producing insects disrupting natural biological control by predators and parasitoids is being carried out. *Aonidiella aurantii* (Maskell, 1879) and *Aphis gossypii* Glover, 1877 and the ant species connected with them are observed in this inquiry.

Key words: Citrus, Formicidae, sprayed orchard, organic orchard.

Introduction

A large number of pests, especially mealybugs and aphids, are key species of citrus orchards and are usually limited by natural predators and parasitoids (Longo *et al.*, 1994).

Ants, foraging in citrus trees, protect honeydew-producing insects from predators and parasitoids (Way, 1963); this mutual symbiosis can compromise biological balance increasing the populations of sap-sucking pests (Bartlett, 1961). The effects of these interactions are as serious as the phytophagous are under natural control by beneficial insects.

The ant fauna of Sicilian citrus groves is very rich and it is common to control them chemically; for this reason knowing the identity and the biology of the ant species inhabiting the orchards is necessary in order to understand the mechanism that rules the interaction with supsucking citrus insects.

This work aims at listing the species present in different Sicilian citrus groves and at laying the bases for subsequent investigation on the interaction between ants and other insects in order to try a new strategy of environmental friendly control.

Material and methods

Surveys were performed in three different citrus orchards in Catania plain: two organic (OCO1, monitored in 2006 and OCO2, monitored in 2007) using different types of crop management and one chemically managed (CCO).

On the three citrus orchards samples of leaves and twigs were collected to identify the sap-sucking species.

Sampling by means of pitfall-traps on the soil and manual captures on the canopy was conducted during May-October 2006 and 2007. The trap, placed on the soil to catch a large number of ant species, was a disposable plastic cup with saturated solution of salt and was changed every 20 days. A parallel trial was conducted with manual captures on the canopy and between the rows (Southwood, 1978).

Ants captured were removed from the traps and stored in 70% ethanol before being sorted and identified in the laboratory, based on keys of Agosti & Collingwood (1987).

The number of specimens captured was normalized according to the following mathematical formula: [Ns / (ndm x ntm)] x 100, where Ns = total number of specimens; ndm = number of days of exposure of traps and ntm = total number of traps in days of exposure.

Results and discussion

On the vegetal samples taken, the main sap-sucking species were *Aphis gossypii* Glover 1877, *Aonidiella aurantii* (Maskell, 1879), *Icerya purchasi* (Maskell, 1876) and *Planococcus citri* (Risso, 1813). A total of 21,190 ant specimens were collected and identified as 25 species belonging to 17 different genera (Table 1).

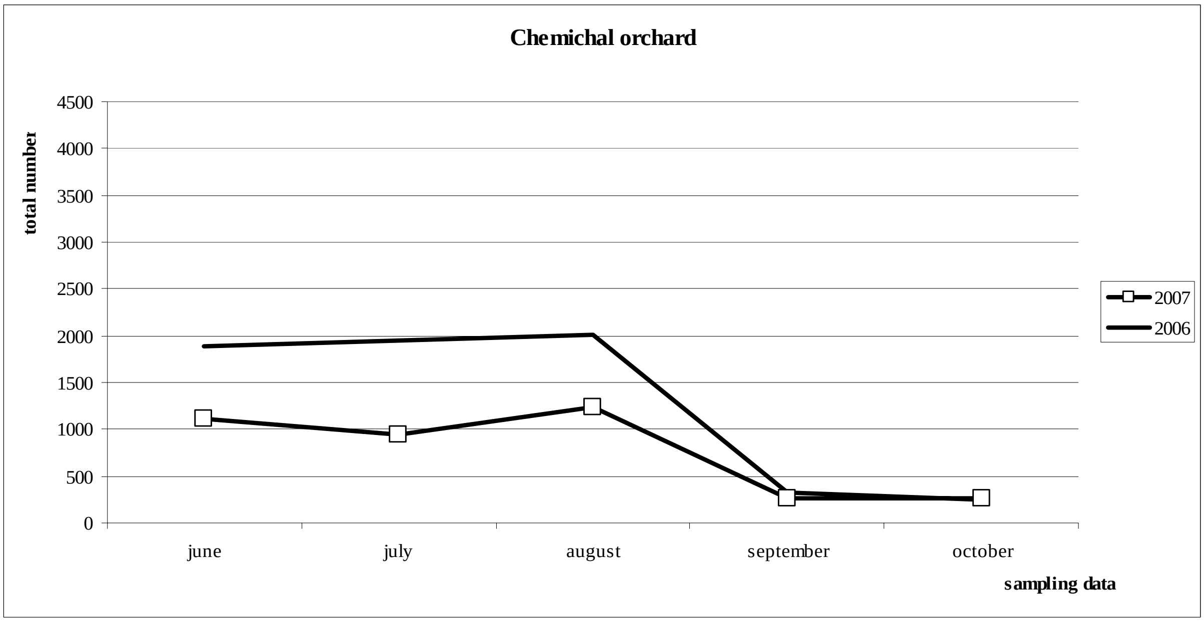

Figure 1. Phenological trend of ants in conventional citrus orchards

The data collected during the second year of study show a general decrease in the number of specimens, probably imputable to the high temperatures recorded in summer 2007, together with prolonged absence of rainfall. Ants' populations remained uniform during the two years of survey both for frequency and abundance in the sprayed citrus orchard (CCO, Figure 1); in the two different organic orchards, the activity trend of ant populations is different (Figure 2).

In the first orchard (OCO1), 20 species were collected in 2006 while in 2007, 13 species were captured in the second orchards (OCO2). Such diversity is certainly imputable to frequent soil tillage performed in the second orchard that has negative consequences on terricolous ants species, such as *Aphaenogaster semipolita* (Nylander, 1856) that was absent in OCO2, Figure 3); also *Camponotus nylanderi* Emery 1921 and *Formica cunicularia* Latreille 1798, species that forage on the canopy, appear to be disturbed by soil tillage and we can see a fall in number of specimens captured in both organic citrus orchards as to chemical citrus orchards. *Lasius alienus* (Förster, 1850), the most abundant specie in OCO1, is not influenced by soil tillage.

ECOLOGY	SPECIES	OCO1 (2006)	CCO (2006)	OCO2 (2007)	CCO (2007)
Arboreal	*Lasius alienus*	3785	0	2901	2
	Formica cunicularia	1273	296	163	170
	Camponotus aethiops	542	464	3	281
	Camponotus nylanderi	414	1309	81	903
	Tapinoma nigerrimum	228	1114	268	1751
	Linepithema humile	200	0	0	1
	Camponotus piceus	65	9	1	23
	Crematogaster scutellaris	37	0	0	0
	Plagiolepis pygmaea	20	38	0	40
	Tapinoma erraticum	5	241	0	0
	Plagiolepis schmitzii	3	0	0	0
	Camponotus lateralis	2	0	0	0
	Tapinoma simrothi	1	0	0	0
Terricolous	*Pheidole pallidula*	1069	998	497	443
	Aphaenogaster semipolita	1009	31	0	27
	Tetramorium semilaeve	60	11	144	30
	Hipoponera eduardi	20	1	10	0
	Solenopsis fugax	19	3	2	7
	Tetramorium caespitum	19	8	0	127
	Temnothorax recedens	1	0	0	0
	Aphaenogaster pallida	0	0	1	0
	Messor capitatus	0	2	2	0
	Messor structor	0	3	1	5
	Proceratium algiricum	0	1	0	0
	Pyramica membranifera	0	1	0	0
total number of specimens		8772	4530	4074	3810
total number of species	25	20	17	13	14

Table 1. Number of specimens (not normalized) identified in the three orchards in 2006 and 2007 (OCO= organic citrus orchard; CCO= conventional citrus orchard)

As regards the phenology of ants in CCO, among the species foraging on the canopy *Tapinoma nigerrimum* (Nylander, 1856) is the most abundant one and it showed a greater resistance and exceeded in number *L. alienus* (Table 1). The absence of *Linepithema humile* (Mayr, 1868) is not directly imputable to the kind of crop management, while that of *Crematogaster scutellaris* (Olivier, 1791), that prefers to nest in dead wood, is certainly attributable to nesting typology.

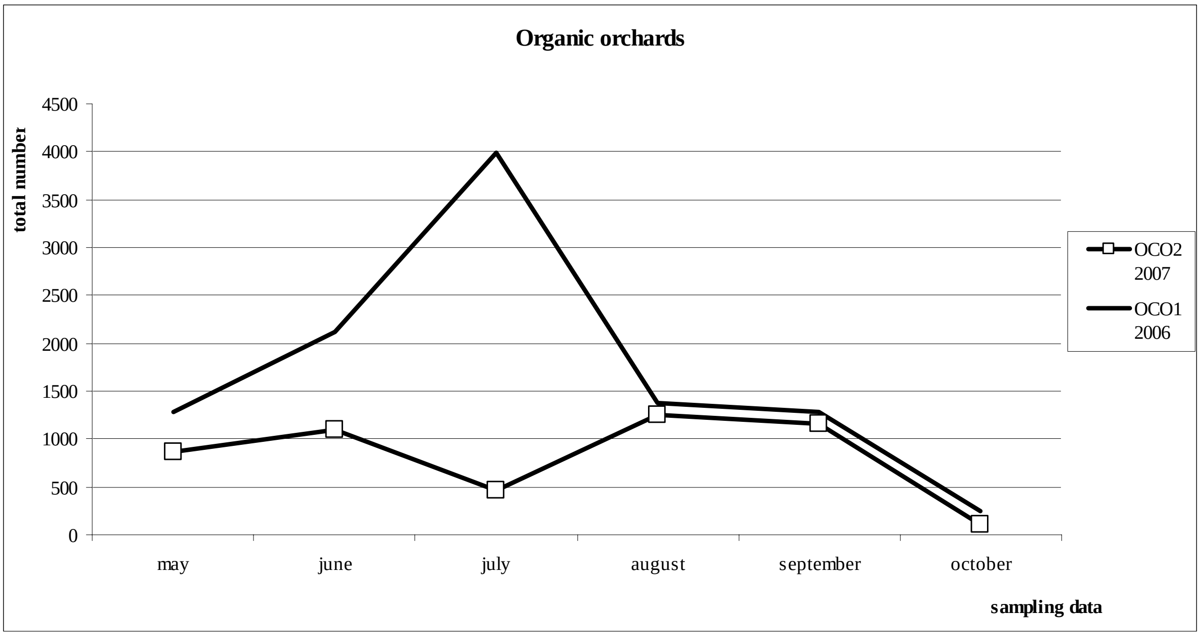

Figure 2. Phenological trend of ants in the organic citrus orchards

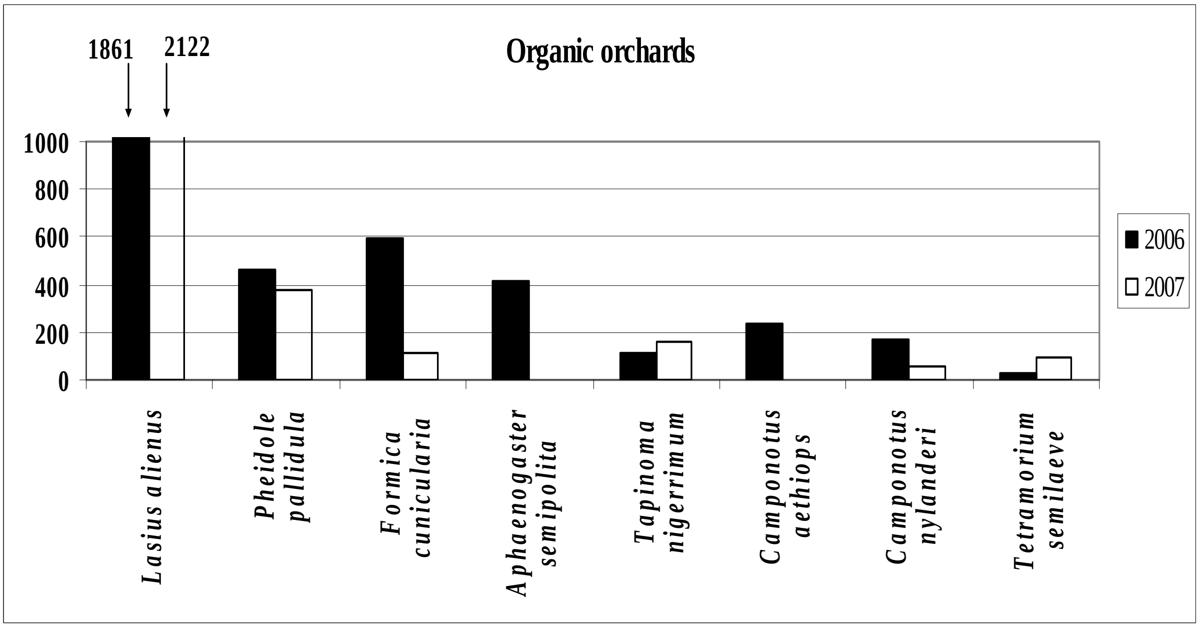

Figure 3. Number (normalized) of specimens of the main ant species captured in pitfall traps

The results obtained suggest that soil tillage is better than treatment on the canopy to control the activity of ants. Infact, in the CCO, where the canopies were sprayed, some species disappeared (*L. alienus*), while the more resistant species (*T. nigerrimum, Pheidole pallidula* Nylander 1848) continued their foraging activity without being disturbed.

Therefore, in order to control the ant fauna and to protect the environment, should be preferable an organic management typology, where soil tillage is performed (OCO2 2007) rather than organic management (OCO1 2006) with poor tilling or chemical management (CCO 2006/2007).

The ants have an important role in the citrus orchards because of their relationships with some phytophagous arthropods, thus it is indispensable to inquire the behaviour of the different species present in the citrus orchards in order to control the pest outbreaks.

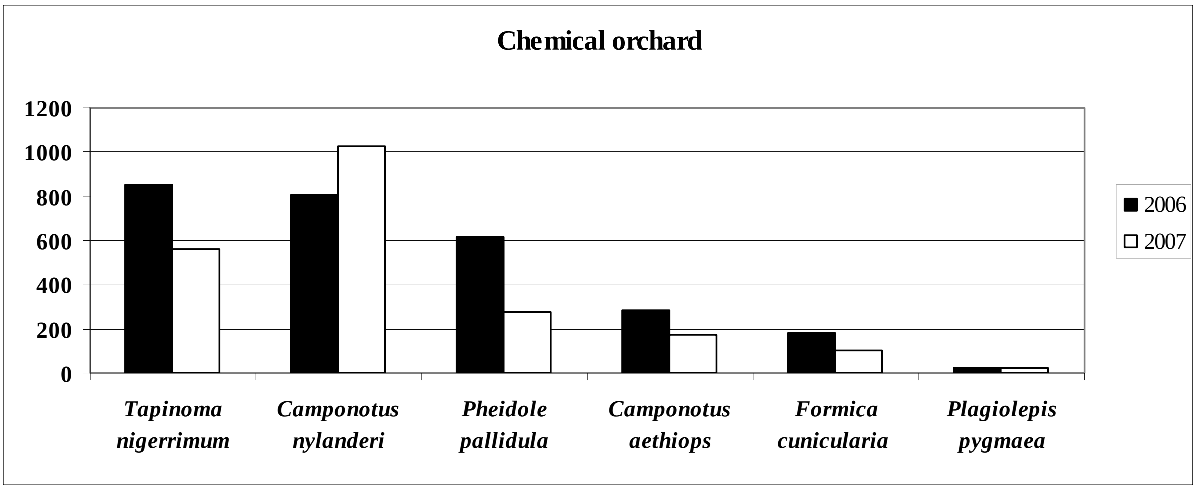

Figure 4. Number (normalized) of specimens of the main ant species captured in pitfall traps

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Potential natural enemies of the Citrus Longhorned Beetle, *Anoplophora chinensis* (Col.: Cerambycidae), an invasive Asian pest in Italy

F. Hérard[1], M. Ciampitti[2], M. Maspero[3], C. Cocquempot[4], G. Delvare[5], J. Lopez[1], N. Ramualde[1], C. Jucker[6], M. Colombo[6]
[1] *European Biological Control Laboratory, USDA-ARS, Montferrier-sur-Lez, France*
[2] *Regione Lombardia - Servizio Fitosanitario, Milano, Italy*
[3] *Fondazione Minoprio, Vertemate con Minoprio, Italy*
[4] *INRA, USC d'écologie animale et de zoologie agricole, Montpellier, France*
[5] *CIRAD, Campus International de Baillarguet-CSIRO Montferrier-sur-Lez, France*
[6] *Istituto di Entomologia Agraria, Università degli Studi di Milano, Italy*

The Citrus longhorned beetle (CLB) *Anoplophora chinensis* (Coleoptera, Cerambycidae) has been accidentally introduced in urban sites in Lombardy (Northern Italy) where it is considered as a serious threat to the urban and natural forests, and is subject to eradication. In its native area in the Far East, the pest is a major pest of *Citrus* spp. and it also causes serious damages to many deciduous trees, mainly in the genera *Populus*, *Acer* and *Salix*. In conjunction with the eradication programs, biological control studies were initiated in order to find, to identify, and to evaluate the parasitoids that could successfully control the pest. Exposure of early stages of the host, in sentinel plants placed in sites within, or outside, the area infested with CLB in Italy, showed that the egg parasitoid *Aprostocetus anoplophorae* Delvare (very likely originating from the Far East) is specific to CLB. Most of the early larval ectoparasitoids, *Spathius erythrocephalus* Wesmael (Hym.: Braconidae), *Eurytoma melanoneura* Walker (Hym.: Eurytomidae), *Calosota vernalis* Curtis (Hym.: Eupelmidae), *Cleonymus brevis* Boucek (Hym.: Pteromalidae, Cleonyminae), *Trigonoderus princeps* (Westwood) (Hym.: Pteromalidae, Pteromalinae), and *Sclerodermus* sp. (Hym.: Bethylidae) attacked *A. chinensis*. Life traits of some of the major parasitoids are currently studied to evaluate them as potential biological control agents. Data on the biology and behavior of *T. princeps* are presented.

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Present situation of *Anoplophora chinensis* (Forster) in Italy

C. Jucker[1], M. Maspero[2], M. Ciampitti[3], M. Colombo[1]
[1] *Istituto di Entomologia agraria, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy*
[2] *Fondazione Minoprio, V.le Raimondi 54, 20070, Vertemate con Minoprio (CO), Italy*
[3] *Servizio Fitosanitario Regione Lombardia, Via Pola 12/14, 20124 Milano, Italy*

Anoplophora chinensis (Forster), the white-spotted longicorn beetle, is one of the most dangerous exotic invasive species introduced in Italy. Known as 'Citrus Longhorn Beetle' (CLB) it is included in the A1 List of quarantine species of EPPO Region (European and Mediterranean Plant Protection Organization). The Cerambycid of the Lamiinae tribe is native from the Far East, where it is one of the most dangerous pests for fruit trees, in particular for *Citrus*, for poplars and other deciduous ornamental trees. Extremely polyphagous, damages are caused by the xylophagous larvae which bore tunnels into the trunk and roots. Hardly attacked plants can easily die.

A. chinensis was first reported in Lombardy (I) in 2001, when adults were captured in the municipality of Parabiago, north of Milan. The spread of the CLB is covering nowadays 21 Municipalities. Since the problem concerning this pest was strictly linked to Lombardy Region, a first Decree of Control and Eradication of *A. chinensis*, was issued during February 2004. Following this first Decree, thanks to the improving of the knowledge of this pest and the possible methods to control it, more useful techniques of control were issued through other updated Regional Phytosanitary Decrees. Symptomatic trees have to be destroyed and the wood burned too; the stump as well, has to be removed through the use of a stump erosion machine. Susceptible trees within the quarantine zone have to be checked and sprayed with chemicals during the flying period of the beetles. Furthermore, restrictions in planting new trees of the susceptible genera are compelled too.

As it is the first time in which CLB has been established outside its native area, a research to acquire data concerning the biology of the xylophagous in the new area was started, in order to verify if the information on this pest in its original country are suitable also for our country. For this reason, a biennial Project named BETOTAC "Biology, Ethology and Control of *Anoplophora chinensis* (Forster)" financed by D. G. Agriculture of Lombardy Region, started in 2005. The partners were Institute of Agricultural Entomology University of Milan, Minoprio Foundation and EBCL (European Biological Control Laboratory). The results of this work are reported.

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Anoplophora chinensis (Forster): a threat to *Citrus* and other ornamentals

M. Maspero[1], C. Jucker[2], M. Colombo[2]
[1] *Minoprio Foundation, V.le Raimondi 54, 22070 Vertemate con Minoprio, Como, Italy; biolomb@fondazioneminoprio.it*
[2] *Institute of Agricultural Entomology, University of Milan, Via Celoria 2, 20133 Milano, Italy; mario.colombo@unimi.it*

The Citrus Longhorned Beetle, *Anoplophora chinensis* (Forster) (Coleoptera: Cerambycidae – EPPO A1 list), originates from China, Japan and Korea where it is a serious pest of *Citrus* and many other deciduous ornamental trees. The first detection in Italy occurred in 2001, during a survey programme to check possible new introductions of exotic pests in nurseries and glasshouses near Milan, carried out by the Institute of Agricultural Entomology, University of Milan with the financial support from the Lombardy Plant Protection Service. The first adult of *A. chinensis* was collected by a technician in one of the nurseries.

A. chinensis is a regulated pest in Europe, according to EU Directive 2000/29/CE. In Lombardy many Decrees were issued aiming at eradicating the pest. Adults are present from late May to late August with a peak of abundance in mid-June. Males seem to emerge earlier than females. *A. chinensis* completes the life-cycle in one or two years in Northern Italy. Eggs are laid around the collar of plants or on accessible roots at ground level, and the xylophagous larvae bore tunnels into the trunks. *A. chinensis* is an extremely polyphagous species, attacking various species of *Acer, Platanus, Betula, Carpinus* and *Fagus, Aesculus, Corylus, Cotoneaster, Crataegus, Lagerstroemia, Malus, Populus, Prunus, Quercus, Rosa, Ulmus* and others.

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The fading of citrus fruits in the Mitidja (Algeria)

D. Toua, D. Fadil, S. Yahou, S. Lamine, T. Guettache, M. Benchabane
Université de Blida – Faculté Agro – Vétérinaire. Département des Sciences Agronomiques, Laboratoire de Phytopathologie, B.P. 270 Route de Soumaa – BLIDA 09000, Algérie; mssaoudh@yahoo.fr

A big part of the Algerian citrus orchards concentrate in the plain of the Mitidja (Algeria), known by its agricultural vocation. The cultivated species are variable (orange, lemon, mandarin, grapefruit, …) and the plantations date since the years 1950s. From the year 2001, the Algerian state threw an ambitious program (PNDA, national Program of agricultural development) to throw back the fruit trees in a general manner and the citrus in a particular way. We observed, these last years, the apparition of cases of fading and blight, in the beginning at sporadic state, but becoming increasingly frequent and troubling. Our work was aimed at establishing preliminary studies, while treating the etiological and epidemiological aspects of some cases of fading. We achieved a symptomatological diagnosis on six orchards of citrus fruits where this decline has been signaled and recorded. Various samples (plant and soil) have been collected according to the different cases of fading observed and compared with healthy topics. The analyzed results don't put in evidence the reasons of the etiological symptoms observed, but determined the susceptible situations of favorable conditions and possibly a fungal agent complex capable to provoke the fading or the blight. Some future studies are necessary to support our first hypotheses and to orient our work to identify more clearly the reasons of these diseases.

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Citrus phytosanitary survey project in the Comunitat Valenciana

J.M. Lloréns[1], F. Garcia-Marí[2]
[1] *Generalitat Valenciana. Conselleria de Agricultura, Pesca y Alimentación. Dirección General de Investigación e Innovación Agraria y Tecnología, València, Spain*
[2] *Institut Agroforestal Mediterrani,Universitat Politècnica de València, València, Spain*

A citrus phytosanitary survey project was initiated on October 2004 on the citrus crops of the Comunitat Valenciana (eastern Spain) with two main objectives, to determine and report on the citrus pest population levels along the year, and to detect new exotic pests that could arrive from abroad. A Survey Net was set with that purpose. The 250,000 Ha Citrus acreage from the Comunitat Valenciana was partitioned in 100 areas of 25 km^2 (5 x 5 km). One fixed and four mobile sampling points were established on each area. Each point is sampled fortnightly all along the year. Five (mobile point) to 10 (fixed point) trees are sampled per point, with four branches (including leaves, young shoots, flowers or fruits) observed per tree. All pests present are quantified following a numeric scale. Else, 10 different types of traps are placed on each fixed point. The information is placed in the web page of the Conselleria de Agricultura, Pesca y Alimentación. For each pest, a distribution or extension map and an abundance or intensity map are provided, together with presence and abundance indices.

Data for population levels of up to 30 species of arthropods, in some cases on several plan substrates, are routinely provided. Population trends along the year of the most common species in the last three years are also included. Overall, the most common species found are scales (*Aonidiella aurantii* (Maskell), *Parlatoria pergandei* Comstock, *Ceroplastes sinensis* Del Guercio, *Planococcus citri* (Risso), *Icerya purchasi* Maskell), aphids (*Aphis spiraecola* Patch, *A. gossypii* Glover), mites (*Panonychus citri* (McGregor), *Tetranychus urticae* Koch), ants, *Aleurothrixus floccosus* (Maskell) and *Phyllocnistis citrella* Stainton. Exotic or non-previously detected pests include *Coccus pseudomagnoliarum* (Kuwana), *Anatrachyntis badia* (Hodges) and *Pezothrips kellyanus* (Bagnall).

Mites

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Structure of *Tetranychus urticae* (Acari: Prostigmata) populations occurring in Spanish clementine orchards (*Citrus reticulata* Blanco) and its relevance for pest management

M. Hurtado[1], T. Ansaloni[1], J.A. Jacas[1], M. Navajas[2]
[1] *Unidad Asociada de Entomología UJI-Instituto Valenciano de Investigaciones Agrarias (IVIA), Universitat Jaume I; Campus del Riu Sec; E-12071-Castelló de la Plana, Spain*
[2] *INRA-CBGP; Campus International de Baillarguet; CS 30016; F-34988-Montferrier-sur-Lez Cedex, France*

Tetranychus urticae Koch is a cosmopolitan mite considered as the most polyphagous species among spider mites. This mite constitutes one of the key pests of clementine mandarins in the region of La Plana, where Spanish clementine production concentrates. Both the nature of the ground cover species and their management could affect the population dynamics of this mite and, consequently, its impact on the orchard. However, it is not clear whether there are host-specific races that might be associated to a particular plant species. Population genetic studies, using molecular markers as microsatellites, have been proved to be extremely informative to address questions about population structure, phylogeographic differences and feeding preferences. Our study included mite populations from commercial orchards located along the Mediterranean eastern coast of Spain, either feeding on trees or on associated weeds, where this mite is abundant. Our results show phylogeographic differences between populations, and one locus, Tu11, shows differences within localities, which makes it a proper marker for finding host-associated races. The final goal of our research has been to describe the genetic structure of these populations, which might help in the management of this pest.

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Economic thresholds for *Tetranychus urticae* (Acari: Prostigmata) in clementine: the effect of flushing on fruit damage

S. Pascual-Ruiz, M. Hurtado, E. Aguilar, T. Ansaloni, J.A. Jacas
Unidad Asociada de Entomología UJI-Instituto Valenciano de Investigaciones Agrarias (IVIA), Universitat Jaume I; Campus del Riu Sec; E-12071-Castelló de la Plana, Spain

One of the main problems for Spanish clementine growers is fruit scarring caused by *Tetranychus urticae* Koch, which can completely downgrade the commercial value of the fruit. *T. urticae* infestations can concentrate on fruit at the end of summer. This study assessed the influence of flush presence during summer and fall on the damage caused by *T. urticae* on clementine trees. Damage significantly increased in trees where summer and fall flushes were mechanically removed. This is indicative that the occurrence of summer and fall flushes is a key factor limiting the amount of mite damage on fruit. Both cultural and crop protection practices ensuring that abundant normal summer and fall flushes occurs seem to be critical to minimize the impact of *T. urticae* populations on fruit quality.

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The first record of *Tetranychus urticae* Koch. (Acarina, Tetranychidae) on citrus in Montenegro

Sanja Radonjić
University of Montenegro, Biotechnical Institute, Department of Plant Protection, Kralja Nikole bb, 81000 Podgorica, Montenegro

Abstract: The Presence of *Tetranychus urticae* Koch. on citrus was detected for the first time in November 2004, in one citrus nursery in the green house, in Djenovići (Boka Kotorska Bay). Out of 600 nursery plants (250 mandarins, 200 oranges and 150 lemons), leaves with symptoms of *T. urticae* attack were noticed on 2% of oranges (variety *Washington Navel*), with three to five infested leaves per plant. All development stages of the pest were found on those leaves. No symptoms were detected on the other citrus plants. In the following year, *T. urticae* was recorded in the same nursery again, in the last week of August, sporadically on a few lemon and orange nursery plants. Although less than 1% of both together were attacked, more than two infested leaves per plant were found. As in the previous years, *T. urticae* was found in the same nursery in 2006, as well. During the first week of September infested leaves were detected on 2%, out of 150 nursery lemon plants (variety *Meyer*). Infestations of several orange plants, and for the first time on mandarin (variety *Owari*), were detected in the next 3 weeks, the attacked lemons. At least three leaves per plant were infested. During all those years the symptoms of the *T. urticae* presence were not detected in any other citrus nurseries inspected along the Montenegrin seacoast, as well as in citrus producing orchards. In the first week of June 2007 infestations were detected for the first time in mandarin producing orchard, in Zoganje (Ulcinj citrus producing area). Out of approximately 500 mandarin trees in the orchard, symptoms of the attack were detected, on 5% of 100 examined plants.

Key words: the two spotted mite, *Tetranychus urticae*, symptoms, intensity of attack, citrus nursery, citrus producing orchard

Introduction

The two spotted mite *Tetranychus urticae* Koch. (Acarina, Tetranychidae) is a very polyphagous pest, attacking over 200 different species of plants (vegetables- indoor and outdoor, fruit, ornamental, trees, weeds). It is considered to be one of the most economically important spider mites (Fasulo and Denmark, 2000). The two spotted mite is particulary damaging to vines, beans, cucumbers, hops, cotton, clover, sunflower, on fruit trees (Fasulo and Denmark, 2000). This cosmopolitain species is considered to be a temperate zone species, although it is also found in the subtropical regions. It is marked as a very important pest in the southern regions of Europe and Asia, as well as in many other places (Dobrivojević & Petanović, 1982). Out of a wide range of host plants, *T. urticae* is considered as an important citrus pest (Laffi and Ponti, 1997; Fasulo and Denmark, 2000; Garcia-Marí, 2002; Abad et al. 2006; Ruiz et al. 2006).

On the underside of the leaves the mite developes its colonies and secretes silklike webbing underneath development stages live. During feeding, it damages leaf cells by sucking out its contents with its piercing sucking mouthparts. It causes cells to collapse and die. As a result of chlorophyll and assimilation disorderes as well as increasing of transpiration, leaves become wilted and later dry (Dobrivojević & Petanović, 1982).

In September 2004, in one citrus nursery, some unusual symptoms in the form of yellowish convexities were noticed on the upper sides of the leaves and corresponding concavity on the lower surface with tiny, silky webbing. Pale green leaves with many withish spots were noticed as the other type of symptoms. After those symptomatic leaves were checked under a stereomicroscope, different development stages of *Tetranychus urticae* were found. It was the first recording of this species on citrus plants in Montenegro. The aim of the study was to follow further appeareance and distribution in other nurseries and producing orchards as well.

Material and methods

The study was conducted in one citrus nursery in Djenovici (Boka Kotorska Bay), where mandarin (var. *Owari*), orange (var. *Washington Navel*) and lemon (var. *Lisbon* and *Meyer*) are grown. The trial lasted three years, from 2004 until 2006, although an inspection was carried out in 2007. During the period from June to November nursery plants were inspected twice. All citrus plants which showed visible symptoms of *T. urticae* attack were marked. Ten leaves were cut off and the number of those attacked were counted. In addition, ten mandarin, orange and lemon nurseries without visible symptoms of attack were chosen. From those, five leaves were randomly taken per plant (50 per species) and inspected under a stereomicroscope in the laboratory. In this nursery chemical treatments against *T. urticae* were applied. The presence of *T. urticae* was noticed in two other citrus nurseries in the area of the city of Bar. Inspections were also carried out in three producing orchards (mostly mandarin). Five leaves per 100 plants were inspected.

Results and discussion

In the period from 2004 until 2006, the presence of *T. urticae* was detected in all citrus species in a nursery in Djenovici (orange, mandarin and lemon). In June 2007 its presence was detected on the mandarin (var. *Chahara* and var. *Owari*) in the producing orchard in Zoganje (area of the city of Ulcinj), and in September in the lemon nursery plants which are grown in a mixed (fruits and ornamentals) nursery in Šušanj (area of the city of Bar).

On the orange and mandarin, initial symptoms of attack were visible on the upper surface as a slight, elongated convexity. It turned yellowish over time. On the lower surface, in the corresponding concavity, slight webbing were formed and beneath those the mite had fed on. All development stages were found on the underside of the attacked leaves as well. As result of feeding, a number of chlorotic, diffused, spots were noticable, although not on the upper side of the leaves. Sphaerical, yellowish eggs were mostly deposited along the midrib.

In the lemon (var. *Lisbon* and var. *Meyer*) symptoms of attack were somehow different. As a result of feeding, huge number of chlorotic spots were detected as an initial symptom. Those spots were visible from the upper, as well as lower surface. Appearence of slight convexity which turned yellowish (on the upper side) and bronze (corresponding concavity on the lower side) was detected as an advanced symptom. The two spotted mite is considered an important citrus pest in Spain, as well as in other Mediterranean regions, especially for lemons and mandarins, mostly clementine (Abad et al; 2006). On the lemon the mite developes on the lower surface causing decoloration as an initial symptom (Laffi And Ponti, 1997). It is followed by bronzing of the area where the colony developes. Yellow convexity is formed on the upper side. On attacked mandarins, clementines and oranges diffused discoloration is a noticable symptom. The mite spreades out on lower as well as the upper surface with no presence of convexity on the upper side.

In 2004, symptoms of attack were detected on oranges, during the second inspection in November. Out of 200 orange nurseries, symptomatic leaves were noticed on 4 plants or 2% of the total number. After 40 leaves were inspected it was found that 17 were attacked, or 42%.

The number of infested leaves ranked from three to five per plant, whilst two plants had five attacked leaves.

In addition, after 150 leaves were randomly taken from oranges, lemons and mandarins which showed no symptoms of attack although sporadic presence of mobile forms were detected.Out of 150 inspected leaves only 12 leaves (8%) showed a slight presence of *T. urticae*. Among those, five (41%) were lemons and seven were oranges (58%). The number of leaves of which the presence of mobile forms were detected are ranked one to two. No presence was detected on the mandarin.

After the first inspection was done in June 2005, almost no symptoms of visible attack were detected. Out of 150 inspected leaves only two symptomatic leaves were found on the lemon. Two months later, during the last week of August, symptoms of a slight attack were detected on lemons and oranges. Only three nursery plants (two lemons and an orange), or less than 1% of total number of both species, showed visible symptoms of attack. After 20 lemon leaves were inspected nine were found to have been attacked, or 45%. On the orange three symptomatic leaves, 30% of those inspected, were detected.

As a result of 150 randomly leaves taken with no visible symptoms of attack, mobile forms of the mite were found on every citrus species. Out of 150 inspected leaves, the presence of *T. urticae* was detected on 16 (11%). Among those attacked, nine (56%) were lemons, five (31%) oranges and two (12%) manadarins. The number of leaves on which the presence of aligible forms were detected is ranked from one to three on the lemon, up to two on the orange, and two on the mandarin.

During the first inspection in September in 2006 symptomatic leaves were noticed only on the lemon. Out of 150 nursery plants the symptoms of attack were detected on 3 plants or 2% of the total number. After 30 leaves were inspected it was found 14 were attacked, or 46%. Number of infested leaves per plant are ranked from four to six, whilst two plants had four attacked leaves.

As result of 150 randomly taken leaves with no visible symptoms of attack, mobile forms of the mite were found on every citrus species. Out of 150 inspected leaves, the presence of *T. urticae* was detected on 14 (9.3%). Among those attacked, seven (50.0%) were lemon, three (21.4%) were orange, and four (28.5%) were mandarin. The number of leaves on which the presence of mobile forms were detected are ranked from one to two on the lemon, one to three on the orange, and two on the mandarin.

Three weeks later, during the second inspection, symptomatic leaves were detected on oranges and for the first time on mandarins, in addition to attacked lemons. Out of 40, 30 and 40 leaves of lemon, orange and mandarin which were inspected, it were found 19 (47%), 12 (40%) and 17 (42%) were attacked. It was also found that at least three leaves per plant were infested. The number of leaves attacked were ranked from three to six on the lemon, three to five on the orange, and three to six on the mandarin. The maximum number of leaves attacked were six on the lemon and the orange.

As a result of 150 randomly taken leaves with no visible symptoms the presence of mobile forms of the mite were found on every citrus species. Out of 150 inspected leaves, the presence of *T. urticae* were detected on 19 (12%). Among those attacked 10 (52%) were lemon, three (16%) were orange and six (31%) were manadarin. The number of leaves on which the presence of the pest were detected, as well as visible symptoms of attack are ranked from one to two on the lemon and the orange, and one to three on the mandarin. During the three years of this study 800 leaves (symptomatic and those without visible symptoms as well) from the nursery in Djenovici were inspected. It was found 149 were attacked, or 18% of those inspected. Among those attacked 73 (49%) were lemon leaves, 47 (32%), orange and 29 (19%) mandarin. Our results also showed that among all citrus species the lemon was the most preferable for the *T. urticae* attack. This conclusion is also supported by the latest data (September 2007) obtained from the nursery in Šušanj where the presence of all development stages of the *T. urticae* was detected only on the lemon (var. *Lisbon* and *Meyer*), although some other nursery plants are grown (the mandarin, the orange, the kiwifruit, ornamentals). According to Laffi and Ponti (1997) *T. urticae* attacks all citrus species, although particulary is damage to the lemon (varieries *Interdonato* and *Monachello*).

The presence of *T. urticae* was detected for the first time in the mandarin producing orchards in Zoganje (Ulcinj citrus producing area) during the first week of June in 2007. Although sporadically visible, symptomatic leaves were detected. After 500 leaves were randomly taken from 100 trees, mobile mite forms were found on 5% of the plants. Out of 500 inspected leaves 11 (2.2%) were infested, with one to five attacked leaves per tree.

Acknowledgements

I sincerely thank Bojan Stojnić (University of Belgrade, Faculty of Agriculture-Zemun, Department of Plant and Food Protection, Entomology Division) on usefull suggestions at the beginning of the trial.

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Phytoseiid mites on Citrus in Souss valley, Morocco

M. Bounfour
Direction de la Protection des végétaux, des Contrôles Techniques et de la Repression des Fraudes, B.P. 1308, Rabat, Morocco

Spider mites are among key pests of Citrus crops in Souss valley along with medfly and California red scale. Many predators are associated with these pests in the Moroccan citrus orchard. Most of them are still unidentified to species level. These include chrysopids, *Stethorus* sp. as well as anystides, tigmaeides,.thrombidiides and Phytoseiid mites. The later make up the most important and common predators of spider mites.

Phytoseiid mites that can be found on Citrus in Morocco are *Proprioseiopsis messor* (Wainstein, 1960), *Phytoseiulus persimilis* Athias-Henriot (occasional), *Euseius scutalis* Athias-Henriot and *Euseius stipulatus* Athias-Henriot. *E. scutalis* is the dominant species in inland dry arid areas, while *E. stipulatus* is more common in the cooler coastal and northern areas of the country. Thus, *E. scutalis* is the dominant phytoseiid predator in Souss valley Citrus orchards.

The predator could be found all year round but mostly from May through October, with population decline in August. Adult predator had an overall aggregated distribution but females were more consistently aggregated at the upper part of the canopy than males.
Data for within trees distribution indicate that predators tend to aggregate in the spring and then randomly disperse within the tree in summer then aggregate again in fall. Female to male ratio vary from 1.5 to 3.6. The ratio becomes female biased as distribution becomes random. The predatory role of *E. scutalis* on *citrus* in Morocco and conservation tactics to enhance its effectiveness will be discussed, with special reference to its habitat.

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Prospecting of the phytoseiid species on citrus in Malaga (Spain)

M.E. Wong[1], A.L. Márquez[2], E.J. García[3], J. Olivero[2]
[1] *Centro de Investigación y Formación Agraria "Campanillas", IFAPA, Málaga (Spain);*
[2] *Dept. Biología Animal, Universidad de Málaga, Spain*
[3] *Dept. Sanidad Vegetal de Málaga, Consejería de Agricultura y Pesca, Junta de Andalucía, Spain*

We prospected the beneficial arthropods for the control of the oriental red mite, *Eutetranychus orientalis*, in the three citrus areas of Malaga province (Western coast, Axarquia and Guadalhorce), Spain. We determined their relative abundance, as well as if they were found in the same samples of the oriental red mite. We collected 152 samples, each one with 50 lemon, orange or mandarin citrus leaves, between March and October 2005. The samples were kept in Berlese funnels for 48 hours. The arthropods collected were analysed with a stereomicroscope to distinguish phytoseiids from other mites. All species were determined following the protocol described by usual protocols for the identification of mites.

We obtained 392 phytoseiid individuals, 88% of which were found on lemon leaves, 7% on orange leaves, and 5% on mandarin leaves. In the three citrus areas of Malaga, the highest phytoseiid abundance was observed in spring. The abundance sharply decreased in summer, reaching values next to 0, and recovered in autumn. The most abundant species was *Euseius stipulatus*, followed by *Euseius scutalis.* The oriental red mite was present in some of the samples where we found phytoseiid mites, which might be indicative of a biological control on this pest.

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Conservation of natural enemies of *Tetranychus urticae* (Acari: Prostigmata) in clementines: the effect of ground cover management

E. Aguilar, S. Pascual-Ruíz, J.A. Jacas
Unidad Asociada de Entomología UJI-Instituto Valenciano de Investigaciones Agrarias (IVIA), Universitat Jaume I; Campus del Riu Sec; E-12071-Castelló de la Plana, Spain

Tetranychus urticae Koch is a polyphagous pest which can become a serious problem in citrus. Clementines are especially sensitive to this mite which downgrades the commercial value of fruit. This mite can also feed on many weeds appearing in Citrus orchards. These mites, as well as their natural enemies, can move up to the trees and back to weeds. Therefore, any perturbation of the green ground cover (either by mowing, plowing, or herbicide applications) can dramatically affect the dynamics of this pest and their predators and as a consequence citrus damage. Since spring 2006, the influence of three different ground cover management techniques (bare soil, natural ground cover and *Festuca arundinacea*-sown cover) on the dynamics of this predator-prey system in four different commercial Clementine orchards has been studied. The acarofauna associated both to the green cover and to the tree has been collected every 15 days and identified.

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Intraguild predation between *Euseius stipulatus* and the phytoseiid predators of *Tetranychus urticae* in clementines, *Neoseiulus californicus* and *Phytoseiulus persimilis*

R. Abad[1], A. Urbaneja[1], P. Schausberger[2]
[1] *Unidad de Entomología. Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias (IVIA). Carretera Moncada-Náquera, Km 4.5, 46113 Moncada (Valencia), Spain; rabad@ivia.es*
[2] *Department of Applied Plant Sciences and Plant Biotechnology, Institute of Plant Protection, University of Natural Resources and Applied Life Sciences. Peter Jordanstrasse 82, A-1190 Vienna, Austria*

The current management of the two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) in clementines is based primarily on applications of acaricides. In recent years, emphasis has been placed on implementing more environmentally safe measures to control *T. urticae* in Spain. To this end, inoculative releases of the predator phytoseiids *Neoseiulus californicus* (McGregor) and *Phytoseiulus persimilis* Athias-Henriot are currently being implemented. Indeed, phytoseiid releases have demonstrated to be successful in controlling *T. urticae* on clementine under laboratory and semi-field conditions.

Both phytoseiids are naturally present in the citrus agro-ecosystem although at low levels. However, *Euseius stipulatus* Athias-Henriot is the most abundant phytoseiid in citrus and its conservation is a key component in the citrus IPM, due to its positive action on different pests. Therefore, before using *N. californicus* and *P. persimilis* to control *T. urticae* in inoculative releases in commercial orchards, we need to clarify the relationship between these phytoseiid species. With this aim, we conducted two experiments of intraguild predation between *E. stipulatus* and *N. californicus,* and between *E. stipulatus* and *P. persimilis*. First, we tested the aggressiveness (propensity of an individual to attack and kill another individual) of adult females on hetero-specific larvae. Second, we assessed survival and development of immature individuals in presence and absence of intraguild adult predators and alternative food.

Efficacy of some acaricides on *Tetranychus urticae* (Acari: Prostigmata) and their side-effects on selected natural enemies occurring in citrus orchards

A. Urbaneja[1], S. Pascual-Ruiz[2], T. Pina[1], R. Abad[1], P. Vanaclocha[1], H. Montón[1], P. Castañera[3], J.A. Jacas[2]
Unidad Asociada de Entomología IVIA-UJI-CIB CSIC
[1] *Instituto Valenciano de Investigaciones Agrarias (IVIA); Ctra. Moncada-Náquera Km 4.5; E-46120-Moncada (Valencia), Spain*
[2] *Universitat Jaume I (UJI); Campus del Riu Sec; E-12071-Castelló de la Plana, Spain*
[3] *Departamento Biología de Plantas, CIB, CSIC, C/ Ramiro de Maetzu, 9. E-28040, Madrid, Spain*

Three groups of natural enemies are fundamental in citrus Integrated Pest Management strategies in the Mediterranean. These are the coccinellids, the hymenopterans and the phytoseiids. The spider mite, *Tetranychus urticae*, is an important pest of citrus whose biological control has not yet been fully achieved. Therefore, acaricides are usually applied against this pest when problems appear. In this study, the efficacy of six different acaricides on this mite and their side-effects on three selected natural enemies (the coccinellid *Cryptolaemus montrouzieri*, the phytoseiid *Neoseiulus californicus* and the braconid *Aphidius colemani*) have been measured. Our results indicate that highly effective products harmless to *A. colemani* exist (mineral oil, tebufenpyrad, clofentezin and fenazaquin). However, almost all tested products resulted slightly harmful for both *C. montrouzieri* and *N. californicus*, and fenazaquin was even moderately harmful for this phytoseiid.

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Evaluation of a mixture of caraway oil and fatty acid potassium salts on *Tetranychus urticae* Koch (Acariformes, Tetranychidae) in laboratory trials

H. Tsolakis, S. Ragusa
Dipartimento S.EN.FI.MI.ZO. sez. Entomologia, Acarologia, Zoologia, Università di Palermo, Viale delle Scienze 13, 90128 Palermo, Italy; tsolakis@unipa.it

Laboratory trials were carried out to evaluate the toxicity and the influence of a commercial mixture of caraway essential oil and potassium salts of fatty acids (ACARIDOIL 13SL®) on the population growth rate (r_i - instantaneous rate of increase) of the phytophagous mite *Tetranychus urticae* Koch. A moderate mortality on treated eggs and on larvae hatched from treated eggs (53.8%) was caused by 1.3 mg/cm^2 of pesticide solution. Moreover a delay in the postembryonic development of the tetranychid was noted. Furthermore, the pesticide influenced negatively the treated females (83.4% mortality) and the population growth of *T. urticae*, which showed a very low rate of increase (r_i = 0.07), compared to that obtained in the control (r_i = 0.68). Results obtained indicate a considerable acaricidal activity of potassium salts of fatty acids and caraway oil on the phytophagous mite.

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Effects of *Melia azedarach* L. extracts on *Panonychus citri* (McGregor) (Acariformes, Tetranychidae) in laboratory trials

H. Tsolakis, R. Jordà Palomero
Dipartimento S.EN.FI.MI.ZO. sez. Entomologia, Acarologia, Zoologia, Università di Palermo, Viale delle Scienze 13, 90128 Palermo, Italy; tsolakis@unipa.it

Acetone extracts of *Melia azedarach* L. leaves were tested in laboratory trials on the citrus red mite *Panonychus citri* (McGregor). A dose of 5,000 ppm at 1.9 mg/cm^2 of pesticide solution showed a very high ovicidal effect (100% mortality), while with a concentration of 2,500 ppm the mortality was lower (21%). The latter dose caused a residual toxic effect on larvae (26.6% mortality). The juvenile stages were susceptible at the different concentrations of *Melia* extracts while adult females were vulnerable only at high doses of the different extracts.

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Experimental evaluation of spirodiclofen efficacy in the control of spider mites and armored scales in Sicilian citrus orchards

Giovanna Tropea Garzia, Gaetana Mazzeo, Pompeo Suma
Università degli Studi di Catania, Dipartimento di Scienze e Tecnologie Fitosanitarie, Via Santa Sofia 100, 95123 Catania, Italy

Abstract: The study aimed at evaluating the efficacy of spirodiclofen, a molecule belonging to the new class of tetronic acids recently produced by Bayer CropScience. Concerning spider mites, in compliance with EPPO methods, two-year experimental evaluations were performed to determine the action of spirodiclofen against *Tetranychus urticae* Koch, attacking citrus crops in Sicily, in comparison with older acaricidal standards (fenazaquin and etoxazole). All tested products provided high or satisfactory efficacy, but spirodiclofen showed 100% egg mortality and a longer residual activity on mobile stages. The molecule seemed also to be safe on other mites with different feeding habits.

In a preliminary trial the new acaricide was employed also against *Aonidiella aurantii* (Maskell) in order to verify its efficacy, according to the common practice, on the second generation crawlers in comparison with chlorpyriphos-methyl, pyriproxifen and sprirodiclofen in combination with mineral oil. On twigs, spirodiclofen applied alone or in combination with mineral oil showed a higher efficacy than the other products; it moreover determined the lowest rate of infested fruit at harvest. In relation to the activity of *Aphytis melinus* DeBach, parasitoid of the California red scale, no significant differences were recorded between spirodiclofen and untreated plots.

Key words: field test, effectiveness, tetronic acids, *Tetranychus urticae*, *Aonidiella aurantii*

Introduction

Spirodiclofen is an acaricide from the new class of tetronic acids and it was registered by Bayer CropScience in January 2007 on different crops. It is active on phytophagous mites, like spider mites, *Tetranychus* and *Panonychus* spp. and eriophyoids such as *Phyllocoptruta*, *Calepitrimerus*, *Aculus* and *Aculops* spp. (Guerra et al., 2002). Its activity was also evaluated against other noxious organisms, like psyllids and scale insects (De Maeyer et al., 2002).

Spider mites and armored scales are among the most important and highly destructive arthropods infesting citrus groves and causing significant yield losses worldwide. Pest control failure is usually due to resistance development, facilitated by many factors such as the high reproductive potential of the pests, their short life cycle and also the frequent application of pesticides. For this last reason, the availability of compounds that act on a new target site is extremely important in resistance management programs and the occurrence of a favorable evironmental profile is requested in IPM practices.

In this context, the aim of the work was to evaluate the efficacy of spirodiclofen against spider mites and armored scales.

Material and methods

The molecule was investigated under field conditions in Sicilian citrus groves in a two-year experimental trial on *Tetranychus urticae* Koch. A preliminary test against *Aonidiella aurantii*

(Maskell) was also carried out. Data were subjected to one-way ANOVA and the means were separated by applying the Least Significant Difference (LSD) test. Mortality was corrected with Abbott's formula.

Tetranychus urticae

According to EPPO methods, experimental evaluations were performed to determine the action of spirodiclofen against the two-spotted spider mite both to test ovicidal and adulticide efficacy.

Spirodiclofen has been applied in 2005 and 2006 in comparison with two older acaricidal standards (fenazaquin and etoxazole). Water application volume was 1500 l/ha. Commercial compounds, active ingredients and application rates are shown in Table 1. Samplings were performed before the treatment, 7 to 21 (in 2005) and 7 to 15 days after (in 2006), collecting 40-50 leaves/treatment where eggs and active stages were counted.

Table 1. Commercial compounds, active ingredients and application rates in the experimental trials on *Tetranychus urticae*.

Thesis	Commercial pesticide (c.p.)	Active ingredient (a.i.)	g/l a.i. & formulation	Application rate (cc or g/hl c.p.)
2005				
1	Control (treated with water)			
2	Envidor	Spirodiclofen	240 g/l SC	40
3	Magister	Fenazaquin	200 g/l SC	75
2006				
1	Control (treated with water)			
2	Envidor	Spirodiclofen	240 g/l SC	40
3	Magister	Fenazaquin	200g/l SC	75
4	Borneo	Etoxazole	110 g/l SC	50

Aonidiella aurantii

The preliminary test, following EPPO Standards [PP1/152 (2) and 135 (2)] was executed in 2005 in order to evaluate the efficacy of the compound on the second generation crawlers according to the common practices, in comparison with chlorpyriphos-methyl, pyriproxifen and spirodiclofen in combination with mineral oil (see Table 2 for details on compounds). The efficacy was evaluated by sampling 12 twigs/treatment 15 after treatment and 200 fruits/treatment just before harvest.

Results and discussion

Efficacy on* T. urticae *eggs

The effectiveness on *T. urticae* eggs was evaluated 7 days after the treatment. In 2005 the initial presence of spider mites was low and the action of spirodiclofen was complete (100% mortality); it was the only tested ovicidal product. The following year, when a higher density of eggs was found, mortality levels of 98.35% for spirodiclofen and of 96.63% for etoxazole were obtained (Figure 1).

Table 2. Commercial compounds, active ingredients and application rates in the experimental trial on *Aonidiella aurantii*

Thesis	Commercial pesticide (c.p.)	Active ingredient (a.i.)	g/l a.i. & formulation	Application rate (cc or g/hl c.p.)
1	Control (treated with water)			
2	Envidor	Spirodiclofen	240 g/l SC	40
3	Etifos M	Chlorpyriphos methyl	225 g/l LE	250
4	Juvinal 10 EC	Pyriproxyfen	100 g/l EC	75
5	Envidor + Oliocin EC	Spirodiclofen + Mineral oil	240 g/l SC 820,8 g/l	40 500

Figure 1 - Percentage of egg mortality registered in 2005 and 2006.

Efficacy on* T. urticae *active stages

During 2005 the degree of efficacy of spirodiclofen, evaluated 7 and 21 days after the treatment on mobile forms, showed an increase while fenazaquin firstly was more efficient but a strong collapse of its activity was registered at the end of the trial. In 2006, 7 days after the treatment all the products showed more than 90% efficacy (Figure 2).

Effects on other mites

During the samplings the presence of different mite species has been observed. In July 2006, dense and widespread populations of Tydeid and Oribatid mites were noted on foliage. After the treatment in the two first theses (control and spirodiclofen) all the mites were alive, while in the plot treated with fenazaquin 100% mortality was registered and in etoxazole caused 30% mortality (Figure 3).

***Insecticidal efficacy on* Aonidiella aurantii**

The mortality of crawlers on twigs recorded 15 days after the treatment with spirodiclofen alone or in combination with mineral oil, was significantly different from the control as from the other compounds (Figure 4).

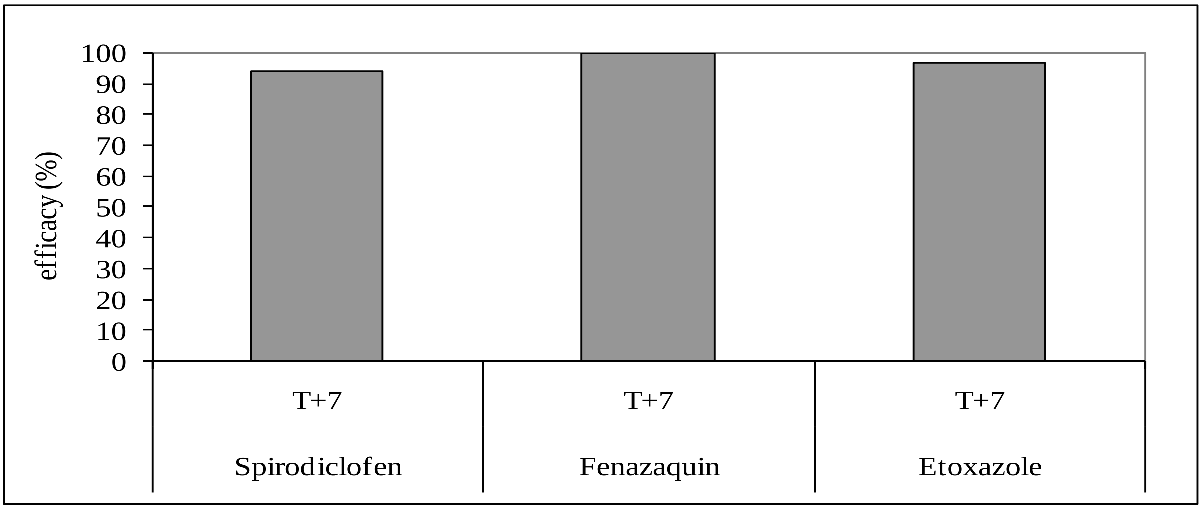

Figure 2 - Degree of efficacy on mobile forms seven days after the treatment in 2006.

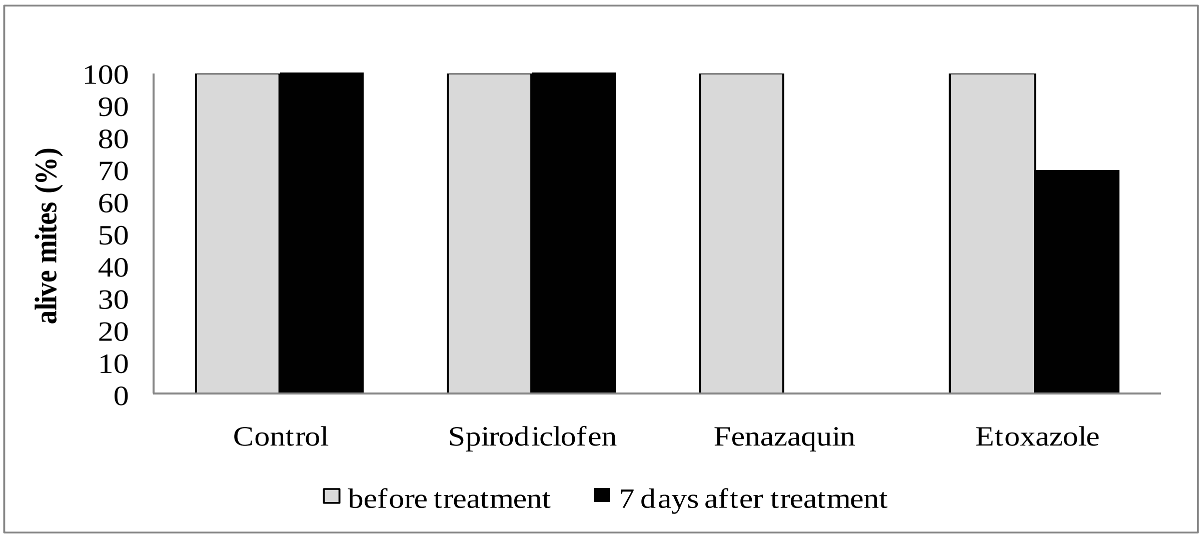

Figure 3 - Percentage of alive Tydeid and Oribatid mites registered in 2006 before treatment and seven days after.

At harvest the percentage of infested fruits was lower in thesis 5 (Spirodiclofen+Mineral oil). The parasitization rate of *Aphytis melinus* DeBach, the most important natural enemy of California Red Scale, in thesis 2 (Spirodiclofen) was close to that of the untreated control (Figure 5).

This experimental field evaluation showed the high ovicidal action of spirodiclofen seven days after the treatment; interesting was also the efficacy on active stages and the residual activity of the molecule. Moreover spirodiclofen allowed all mites with different feeding habits to survive acting as an indicator of a favorable evironmental profile, feature even more requested in IPM practices.

Spirodiclofen demonstrated a fairly good efficacy on *A. aurantii* crawlers on twigs that increased when the compound was in combination with mineral oil. It showed to be comparable with the other compounds and to have a low toxicity on natural enemies such as *A. melinus*. This preliminary test needs to be replicated in relation to the peculiar biological and morphological characteristics of the armored scale that interfere with the activity of the insecticides used for its control.

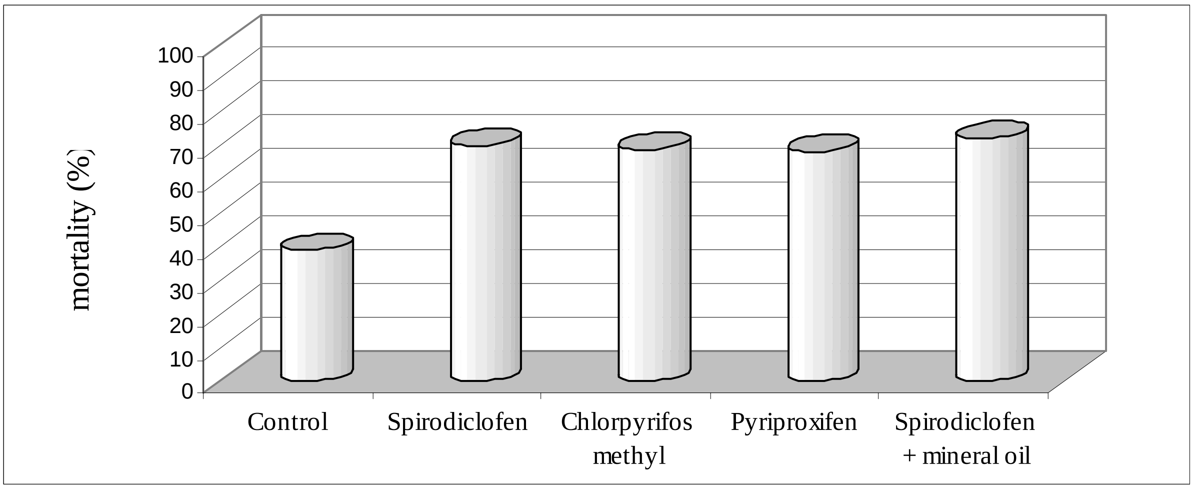

Figure 4 - Percentage of mortality of crawlers on twigs 15 days after treatment.

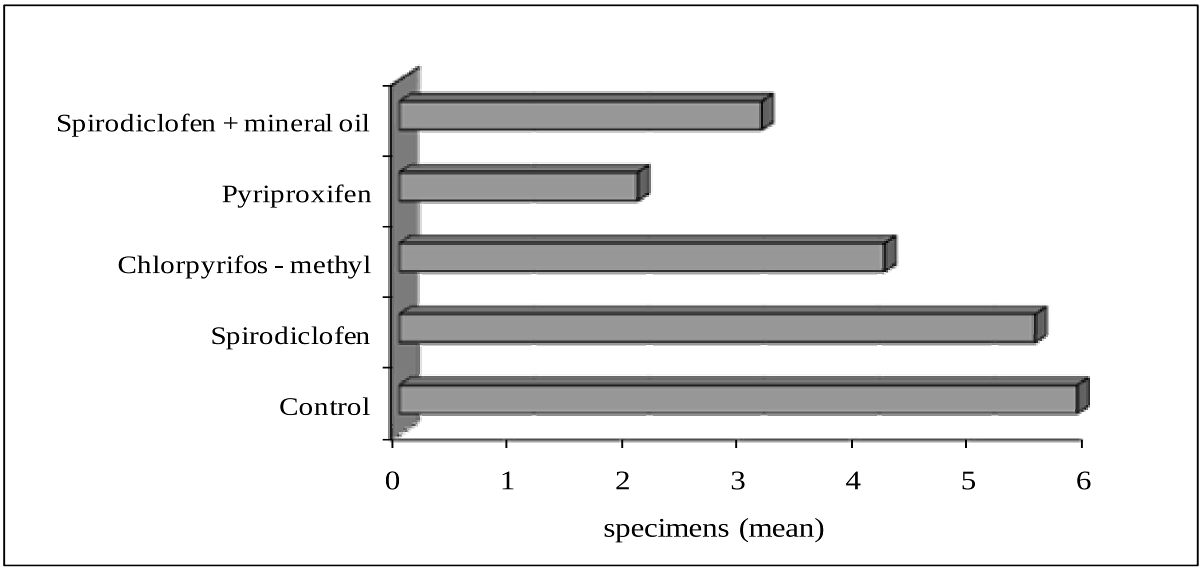

Figure 5 - Mean number of *Aonidiella aurantii* specimens parasitized by *Aphytis melinus* on fruits at harvest.

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Beneficials and Biological Control

Control in Citrus Fruit Crops
IOBC/wprs Bulletin Vol. 38, 2008
pp. 261-266

Seasonal and spatial population trend of predatory insects in eastern-Spain citrus orchards

Pablo Bru, Ferran Garcia-Marí
Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, España.

Abstract: The seasonal trend and spatial distribution of predatory species of arthropods found on Citrus trees in the main Spanish citrus area (País Valencià, east of Spain) was determined in 2005 and 2006. Seven different types of sticky traps were placed and periodically collected on 100 citrus orchards distributed throughout the 200,000 Ha of theValencia citrus belt. In all, 39,016 specimens included in 31 taxons were identified. The most common families were Neuroptera Coniopterygidae (66% of all insects found) and Chrysopidae (15%), followed by Coleoptera Coccinellidae (13%), Diptera Syrphidae (4%), and Hemiptera Anthocoridae (1%) and Miridae (1%). The most common species among the Coniopterygidae were *Conwentzia psociformis* (Curtis) (51% of all specimens in the family), which increased in winter, and *Semidalis aleyrodiformis* (Stephens) (39%), more abundant in summer and at the southern areas. Most Chrysopidae were *Chrysoperla carnea* (Stephens), which showed its highest population in early summer and at the North. Coccinellidae included as most abundant species *Rodolia cardinalis* (Mulsant) (42%), *Scymnus* spp. (26%), *Propylea quatuordecim-punctata* (L.) (13%) and *Stethorus punctillum* (Weise) (13%). All species of coccinellids showed a similar population trend, with maximum population levels in the spring and early summer.

Keywords: Predators, Citrus, Coniopterygidae, Chrysopidae, Coccinellidae, biological control

Introduction

Predatory insects are a group of natural enemies that can play a decisive role in the biological control of potential pests. Some families and species are very common in the citrus orchards. Their feeding habits are usually polyphagous, helping to prevent the build up of populations of phytophagous arthropods. The identity and abundance of predatory insects has been studied in several citrus producing areas around the world. Insects belonging to the orders Neuroptera, Coleoptera and Hemiptera are usually considered the most common groups of predators. Studies on Neuroptera have been carried out in Florida (Muma, 1971), South Africa (Smith Meyer and Schwartz, 1998), Russia (Agekyan, 1978), Portugal (Pantaleaô *et al*, 1994; Carvalho and Franco, 1994), Turkey (Davarci, 1996) and Greece (Katsoyannos, 1996). Papers on Coleoptera have been published in Italy (Longo and Benfatto, 1987) and Portugal (Franco *et al*., 1992). Hemiptera have been studied in California (IPM for citrus 1991), South Africa (Gilbert and Bedford, 1998) and the Middle East (Luck *et al.*, 1996).

Previous references on insect predators in Citrus in Spain are usually of species found preying on particular citrus pests (Limón et al, 1976; Carrero et al, 1977; Panis et al, 1977; Ripollés y Meliá, 1980; Llorens, 1990; Llorens and Garrido, 1992; Garrido and Ventura, 1993; Garijo et al, 1995; Lucas, 1995; Soto, 1999; Soler et al, 2002; Alonso, 2003; Urbaneja et al, 2001). Systematic studies on particular groups of predatory species found on spanish citrus orchards have been published by Rojo (1995) (Syrphidae), Alvis et al (2002; 2003) (Neuroptera, Coleoptera) and Ribes et al (2004) (Hemiptera). The objectives of this paper are to complete the identification and abundance of species of predatory insects in Spanish citrus orchards, to determine their seasonal trend of abundance along the year and to study their geographic distribution along the eastern-Spain citrus belt.

Material and methods

The study was carried out between January and December, 2005, on 100 commercial citrus orchards distributed through the País Valencià (eastern Spain) citrus belt. This region is the most important citrus producing area in Spain, with near 200,000 Has. These orchards were included in the Citrus Phytosanitary Survey Project established by the local government (Generalitat Valenciana) to study the population trend of existing pests and detect the introduction of new pests. Predatory insects were determined on three types of sticky traps on each orchard, a white cardboard delta trap, with its sticky surface (15x15 cm) horizontal, a white cardboard tent trap, with its sticky surface (10x15 cm) almost vertical, and a yellow plastic vertical trap (10x15 cm). Traps were sampled at regular intervals ranging from two weeks in summer to six weeks in winter. In all, 6,184 traps were observed.

Results and discussion

The highest number of insects belonging to predatory families found on the sticky traps were Neuroptera Coniopterygidae (24,519 specimens identified) and Chrysopidae (5,458), followed by Coleoptera Coccinellidae (4,578), Diptera Cecidomyidae (2,279) and Syrphidae (1,660), and Hemiptera Anthocoridae (351) and Miridae (160).

Table 1. Total number of the most common species of predatory insects identified on sticky traps from 100 citrus orchards sampled along the year 2005 on Valencia (eastern-Spain).

Family and species	nº	Family and species	nº
Coniopterygidae		**Syrphidae**	
Conwentzia psociformis (Curtis)	12,634	*Eupeodes corollae* (F.)	755
*Semidalis aleyrodiformis (*Stephens)	9,524	*Episyrphus balteatus* (De Geer)	442
Coniopteryx spp.	2,357	*Sphaerophoria* spp.	203
Chrysopidae		*Syrphus* spp.	85
Chrysoperla carnea (Stephens)	5,334	**Anthocoridae**	
Coccinellidae		*Cardiastethus* spp.	336
Rodolia cardinalis (Mulsant)	1,924	*Orius* spp.	15
Scymnus spp.	1,193	**Miridae**	
Propylea quatuordecimpunctata (L.)	608	*Campyloneura virgula* (Herrich-Schaeffer)	97
Stethorus punctillum (Weisel)	595	*Deraeocoris ruber* (L.)	17
Rhyzobius lophanthae (Blaisdell)	136		

The Neuroptera Coniopterygidae was by far the most common family found, with 66% of all predatory insects identified. These insects are very common on the citrus canopy but their relative importance can be overestimated due to the sampling method. It is known that coniopterygids are specially attracted to yellow sticky traps. Two species stand out as the most abundant, *Conwentzia psociformis* (Curtis) and *Semidalis aleyrodiformis* (Stephens). In spite of their abundance, the seasonal trend of their populations along the year was rather irregular (Fig. 1). Apparently *C. psociformis* prefers colder periods as it shows a minimum in summer, whereas *S. aleyrodiformis* develops better in the warmer period of the year as its populations show a minimum in winter. Neuroptera Chrysopidae were also very abundant, with 15% of all the insects identified. Almost all the chrysopids belonged to the species, or complex of species, *Chrysoperla carnea* (Stephens). Adults proliferated between May and August (Fig. 1).

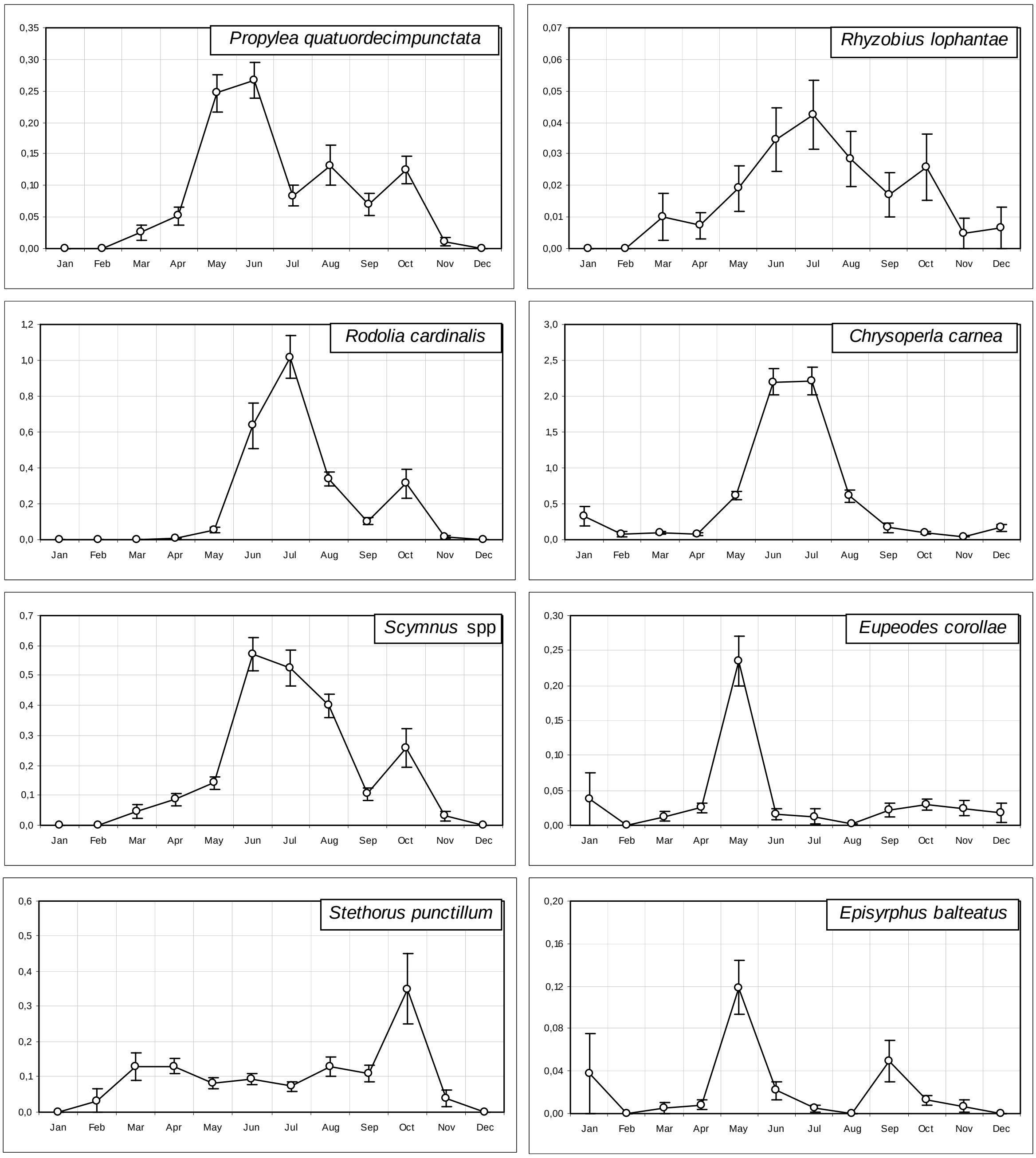

Figure 1. Seasonal trend in abundance along the year showed by different species of predatory insects found on citrus orchards in the País Valencià (eastern Spain) citrus producing area. Values represent average number of insects captured per trap and per three weeks in 100 citrus orchards sampled along the year 2005. Vertical bars indicate standard error of the means.

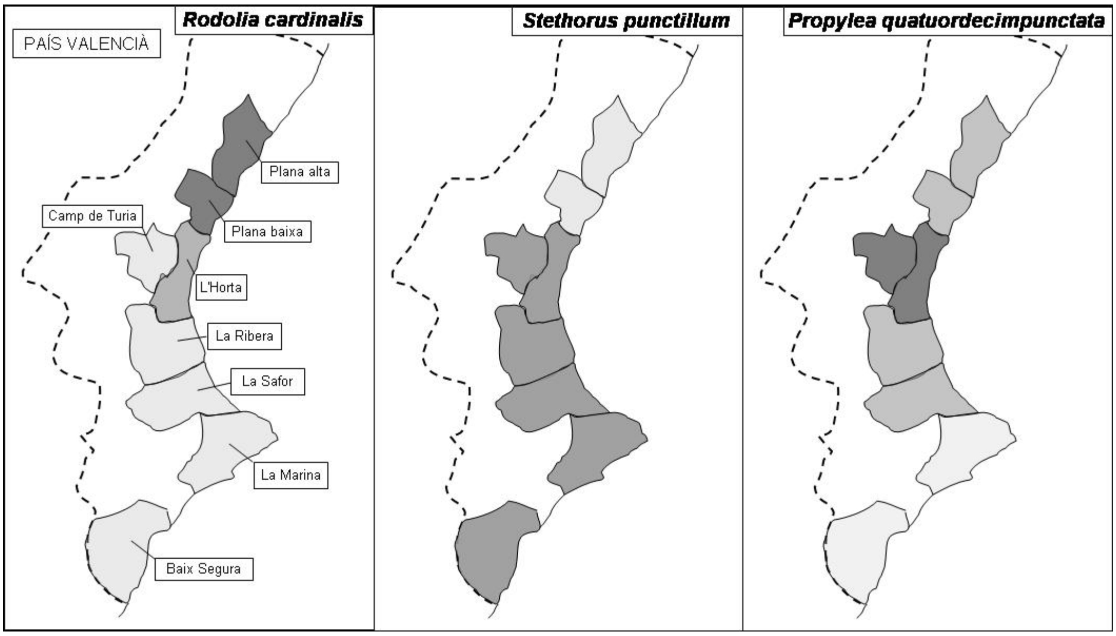

Figure 2. Relative abundance of three species of predatory insects in the eight most important regions of the País Valencià citrus producing area. Between 10 and 15 orchards were sampled on each region. Darker areas represent higher yearly average population densities.

Rodolia cardinalis and *Scymnus* spp were the most abundant species among the Coleoptera Coccinellidae, followed by *Propylea quatuordecimpunctata, Stethorus punctillum* and *Rhyzobius lophanthae* (Table 1). The seasonal trend along the year was similar *in R. cardinalis*, *Scymnus* spp and *R. lophantae*, with maximum population levels between June and August. *P. quatuordecimpunctata* peaked earlier, in May-June, and *S. punctullum* later, in October. These trends reflect possibly the abundance of their preferred prey, aphids for *P. quatuordecimpunctata* and mites for *S. punctillum*. *R. cardinalis* was more abundant at the two regions located at the north, Plana alta and Plana baixa (Fig. 2), whereas *S. punctillum* was less abundant just in these two regions. *P. quatuordecimpunctata* showed higher population densities at the north and interior regions. Differences in incidence of their preferred prey could explain the uneven geographical abundance along the Valencia citrus belt showed by coccinellids, though a direct preference of the species for warmer or colder climates cannot be excluded.

Eupeodes corollae and *Episyrphus balteatus* were by far the most common Diptera Syrphidae species identified. Almost three out of four adult syrphids found on traps belonged to one of these two species (Table 1). Whereas vertical yellow traps were the preferred for all other predatory insects, adult syrphids were captured almost exclusively on white horizontal delta traps. Captures were clearly concentrated during May (Fig. 1) and were less abundant in the colder (north and interior) regions of the area sampled. Hemiptera Anthocoridae and Miridae were captured in low numbers in citrus orchards, compared with other crops. Among the anthocorids, *Cardiastethus fasciiventris* is the most common species, whereas *Campyloneura virgula* predominates among the mirids (Table 1). Maximum population levels were observed between May and July in hemipters and they were more abundant at the southern regions.

Acknowledgements

We thank the Conselleria de Agricultura of the Generalitat Valenciana for financial support of the Citrus Phytosanitary Survey Project, and the technicians of Tragsatec, S.A. (A. Castaño, B. Escrig, M. Guillén,, O. López, M. Llopis, A.B. Martínez, L. Peris, J.J. Pérez, J. Sepúlveda, A. Vázquez and M. Vicente) for the field samplings.

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Ground-dwelling spiders (Araneae) in citrus orchards in Spain

C. Monzó[1], O. Mollà[1], H. Montón[1], A. Melic[3], P. Castañera[2], A. Urbaneja[1]
[1] *Unidad Asociada de Entomología IVIA - CIB CSIC. Centro de Protección Vegetal y Biotecnología (IVIA), Ctra. de Moncada a Náquera km. 4.5; 46113 - Moncada, Valencia, Spain; cmonzo@ivia.es*
[2] *Unidad Asociada de Entomología IVIA - CIB CSIC. Departamento Biología de Plantas, Centro de Investigaciones Biológicas (CIB). Consejo Superior de Investigaciones Científicas (CSIC). C/ Ramiro de Maeztu, 9, 28040 Madrid, Spain*
[3] *Sociedad Entomológica Aragonesa. Avda. Radio Juventud, 37; 50012 Zaragoza, Spain*

A survey of the ground-dwelling spiders (Araneae) was conducted in four citrus orchards in the province of Valencia, Spain. The sampling period was extended from August 2003 to August 2007. A total of 12 pitfall traps were diagonally distributed per orchard being regularly changed every 14 days. More than 3,000 individuals belonging to more than 25 species of 12 different families were collected. The family Lycosidae was the prevalent group, being *Pardosa cribata* Simon the main species of this family. Otherwise, *Zodarion pusio* Simon was the most abundant species of all the spiders captured. Ground-dwelling spiders were active throughout the year with peak populations in late spring and early summer.

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IOBC/wprs Bulletin Vol. 38, 2008
pp. 268-274

Studies on pest and beneficial insects of citrus in İzmir province (Turkey)

Ali Güncan[1], Zeynep Yoldaş[1], Türkan Koçlu[2]
[1] *Ege University, Faculty of Agriculture, Department of Plant Protection, 35100 Bornova, İzmir, Turkey*
[2] *Bornova Plant Protection Research Institute, Gençlik Cad. No:6, 35040 Bornova, İzmir, Turkey*

Abstract: This study was carried out in the town of Gümüldür and the Seferihisar district, which are the most important citrus, especially tangerine production and exportation areas of Izmir province (Turkey) between the years 2006-2007. Purpose of this study was to determine population fluctuations of some important pests on tangerine orchards and natural enemies of them, particularly aphids. Because aphid population increases remarkably year by year when compared to other pesticide-free tangerine orchards due to using broad-spectrum insecticides (4-5 times per year) to reduce the population of aphids without considering natural balance in tangerine agroecosystem by the producers. *Aphis gossypii* Glover, *A. spiraecola* Pagenstecher, *A. craccivora* Koch, *Toxoptera aurantii* (Boyer de Fons.), *Myzus persicae* (Sulzer), *Aleurothrixus floccosus* (Maskell), *Icerya purchasi* Maskell, *Ceroplastes rusci* L., *Saissetia oleae* (Oliv.), *Aonidiella* spp., *Coccus* spp., *Phyllocnistis citrella* Stainton, *Archips rosanus* L. and *Ceratitis capitata* (Wiedemann) were determined as pests. Species of Coccinellidae (Coleoptera), Chrysopidae (Neuroptera), Cecidomyiidae and Syrphidae (Diptera) families as predators and species of Braconidae (Hymenoptera) family as parasitoids were found as natural enemies of aphids.

Key words: citrus, pests, natural enemies, biological control, Turkey

Introduction

Citrus fruits are one of the most important crops of the Turkey. Species like orange, lemon, grapefruit and tangerine are widely produced. Tangerine production and exportation is remarkable in the Aegean Region of Turkey. According to the Food and Agriculture Organization (FAO), Turkey takes fifth place, with around 3% of the world's total tangerine production in 2005 (FAO, 2007).

Citrus is host to a large number of pests. Nearly 87 species of insects are considered to be a major pests of citrus in worldwide (Ebeling, 1959), more than 20 in Turkey (Anonymous, 1997; Uygun, 2001). Numerous studies were carried on beneficial fauna and biological control of citrus pests. Serious pests of citrus except The Mediterranean fruit fly *Ceratitis capitata* Wied. (Dip.: Tephritidae) can be suppressed by natural enemies (Öncüer, 1991). This paper presents some important citrus pests and beneficial insects and population fluctations of some of them in İzmir province of Turkey.

Material and methods

Four experimental tangerine orchards, two in the town of Gümüldür (38^{0}04'N, 27^{0}01'E; 38^{0}04'N, 27^{0}00'E) and two in the Seferihisar district (38^{0}11'N, 26^{0}48'E; 38^{0}15'N, 26^{0}49'E) were choosen in cooperation with local agricultural departments in İzmir province.

Aphids and their natural enemies were counted on 100 leaves particularly on new shoots. Aphids were counted as nymphs and adults. Individuals of predatory families of aphids, Coccinellidae and Chrysopidae were counted as egg, larvae, pupae and adult stages, totally whereas individuals of Syriphidae and Aphidoletes aphidimyza (Rondani) (Dip.: Cecidomyiidae) were counted as larvae only. Whiteflies and citrus leafminers were counted also on 100 leaves.

Studies were carried out weekly in spring and summer, monthly in autumn and winter periods between January 2006 and October 2007. The other pests and natural enemies were recorded when they have seen on leaves, shoots, branches, fruits or trunks in all experimental orchards. Samples were taken and brought to the laboratory, prepared considering their families and sent to the experts for identification.

Results and discussion

Pests of tangerine and their natural enemies in Izmir

Pests and their natural enemies that found in experimental orchards in were given at Table 1. Aphid population increases remarkably year by year when compared to other pesticide-free tangerine orchards due to using broad-spectrum insecticides (4-5 times per year) to reduce the population of aphids without considering natural balance in tangerine agroecosystem by the producers. Five species of aphids were found in experimental orchards. Worldwide, 16 species of aphids are reported to feed regularly on citrus (Halbert & Brown, 1996), 9 in southeastern Europe (Kavallieratos et al., 2005) and 5 in Eastern Mediterranean Region of Turkey (Yumruktepe & Uygun, 1994). Parasitoid (Starý,1976; Öncüer, 1991, Yumruktepe & Uygun, 1994) and predator (Öncüer,1991; Yumruktepe & Uygun, 1994, Hodek & Honek, 1996) species were considered a typical complex on aphid species.

The Mediterranean fruit fly *C. capitata* is important and a common polyphagous pest in citrus growing areas in Aegean Region of Turkey. Adults are monitored by traps by local agricultural departments and malathion based sprays are commonly used to control this pest otherwise it is capable of causing fruit loss of tangerine. This control method is very successful and relatively non-disruptive to natural enemies. All of other pests except aphids and the Mediterranean fruit fly is not caused important damages on tangerine. Their population densities fluctuate generally below the economic injury levels by the native and introduced natural enemies.

Population fluctuations of some tangerine pests and their natural enemies in Izmir

Population fluctuations of aphids and their natural enemies, citrus woolly whitefly *Aleurothrixus floccosus* (Maskell) (Hom.: Aleyrodidae) and citrus leafminer *Phyllocnistis citrella* Stainton (Lep.: Gracillaridae) in four orchards were given at Figure 1 and 2.

Aphids population were firstly seen at beginning of April in four experimental orchards in both years. Then activities of predators and parasitoids were started at the begining of May. Parasitization rates were increased gradually and reached 100% at the end of June except second orchard in Seferihisar because aphid population was not higher when compared the other orchards. Population of aphids was decreased to zero at the beginning of July. Predators that belong to Coccinellidae family were abundant at first orchard of Gümüldür. Both populations of parasitoids and predators were not differed too much by orchards and enough to reduce aphid population in a satisfactory time period.

Table 1. List of pests of tangerine and their natural enemies found in the experimental orchards.

Pests	**Natural Enemies**
Aphis gossypii Glover, *A. spiraecola* Pagenstecher, *A. craccivora* Koch, *Toxoptera aurantii* (Boyer de Fons.), *Myzus persicae* (Sulzer) (Hom.: Aphididae)	*Aphidius colemani* Vier. (Hym.: Braconidae) *Binodoxys angelicae* (Haliday) (Hym.: Braconidae) *Ephedrus persicae* Frog. (Hym.: Braconidae) *Lysiphlebus testaceipes* (Creson) (Hym.: Braconidae) *Adalia bipunctata* (L.) (Col.: Coccinellidae) *Coccinella septempunctata* L. (Col.: Coccinellidae) *Coccinula quatuordecimpustulata* (L.) (Col.: Coccinellidae) *Hippodamia variegata* (Goeze) (Col.: Coccinellidae) *Oenopia conglobata* (L.) (Col.: Coccinellidae) *Propylea quatuordecimpunctata* (L.) (Col.: Coccinellidae) *Scymnus* sp. (Col.: Coccinellidae) *Aphidoletes aphidimyza* (Rondani) (Dip.: Cecidomyiidae) (Dip.: Syrphidae) *Chrysoperla carnea* Stephen (Neu. Chrysopidae)
Aleurothrixus floccosus (Maskell) (Hom.: Aleyrodidae)	*Cales noacki* Howard (Hym.: Aphelinidae) *Clitostethus arcuatus* (Rossi) (Col.: Coccinellidae)
Dialeurodes citri (Ashmead) (Hom.: Aleyrodidae)	*Encarsia lahorensis* (Howard) (Hym.: Aphelinidae) *Serangium parcesetosum* Sicard. (Col.: Coccinellidae) *Clitostethus arcuatus* (Rossi) (Col.: Coccinellidae)
Empoasca decipiens Paoli *Asmetrasca decedens* Paoli (Hom.: Cicadellidae)	
Icerya purchasi Maskell (Hom.: Margororidae)	*Rodolia cardinalis* (Muls.) (Col.:Coccinellidae)
Ceroplastes rusci L. (Hom.: Coccidae)	*Chilocorus bipustulatus* (L.) (Col.:Coccinellidae) *Oenopia conglobata* (L.) (Col.: Coccinellidae) *Scymnus* sp. (Col.: Coccinellidae)
Coccus spp. (Hom.: Coccidae)	*Chilocorus bipustulatus* (L.) (Col.:Coccinellidae) *Oenopia conglobata* (L.) (Col.: Coccinellidae) *Rhyzobius lophantae* (Blaisd) (Col.:Coccinellidae) *Scymnus* sp. (Col.: Coccinellidae)
Saissetia oleae (Oliv.) (Hom.: Coccidae)	*Adalia bipunctata* (L.) (Col.: Coccinellidae) *Chilocorus bipustulatus* (L.) (Col.:Coccinellidae) *Oenopia conglobata* (L.) (Col.: Coccinellidae) *Propylea quatuordecimpunctata* (L.) (Col.: Coccinellidae) *Scymnus* sp. (Col.: Coccinellidae)
Aonidiella spp. (Hom.: Diaspididae)	*Chilocorus bipustulatus* (L.) (Col.:Coccinellidae) *Rhyzobius lophantae* (Blaisd) (Col.:Coccinellidae) *Scymnus* sp. (Col.: Coccinellidae)
Phyllocnistis citrella Stainton (Lep.: Gracillaridae)	*Ratzeburgiola incompleta* (Boucek) (Hym.:Eulophidae) *Cirrospilus* sp. near *lyncus* (Walker) (Hym.:Eulophidae) *Pnigalio* sp. (Hym.:Eulophidae)
Archips rosanus L. (Lep.: Tortricidae)	
Ceratitis capitata (Wied.) (Dip.: Tephritidae)	

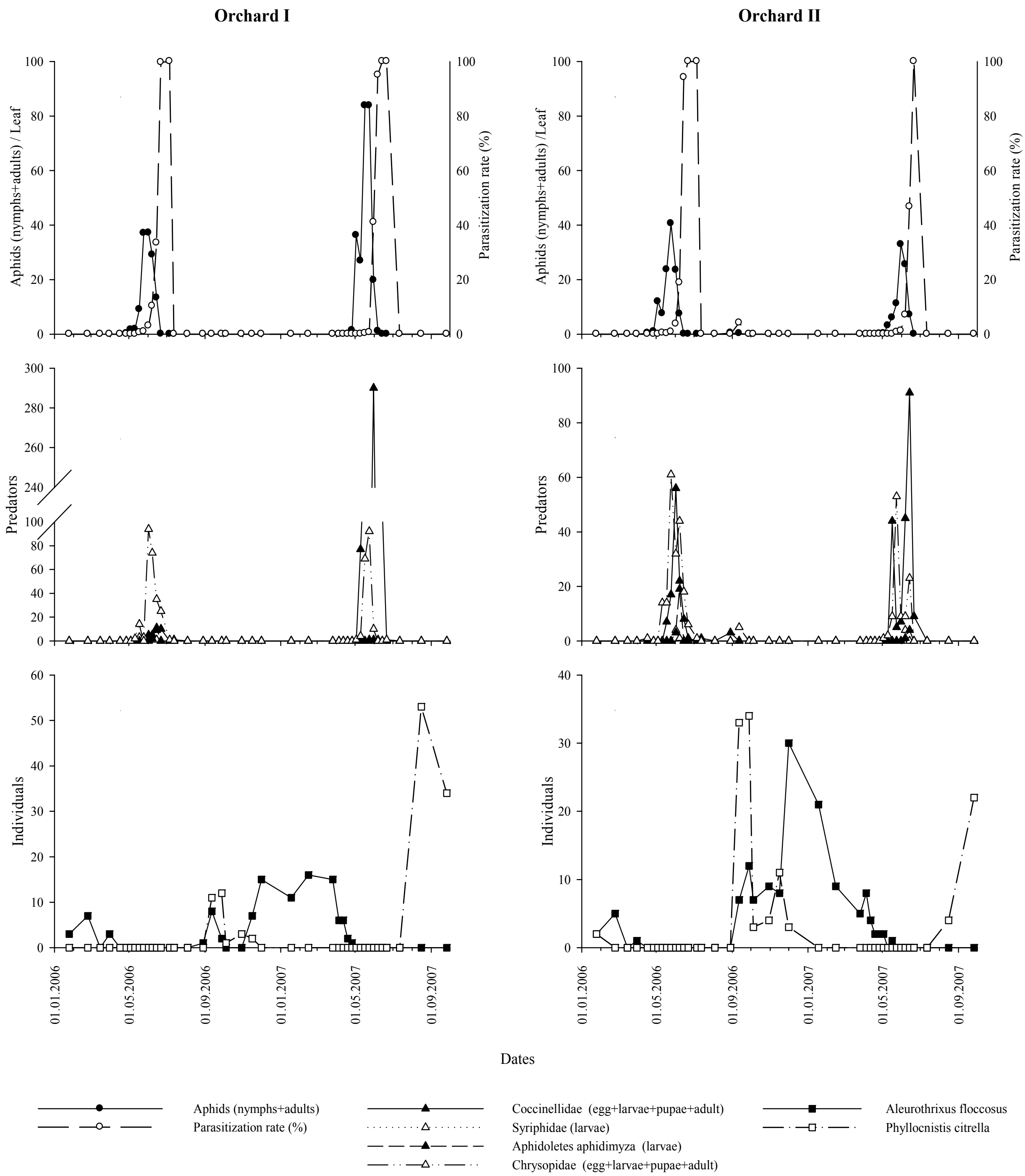

Figure 1. Population fluctuations of aphids and their parasitization rates, their predators, *Aleurothrixus floccosus* (Maskell) and *Phyllocnistis citrella* Stainton on tangerine orchards in Gümüldür between January 2006 and October 2007

Both populations of *A. floccosus* and *P. citrella* were not fluctuated above the economic thresholds. In second year population of *P. citrella* was higher in all orchards. Chemical control is not recommended against this pest in older trees. Cultural control practices consisted of reduced irrigation and fertilization to suppress the summer and autumn flush, early fertilization to stimulate the spring flush, and pruning of infested shoots to destroy egg and larva populations. *C. capitata* was not met in four orchards because of chemical control.

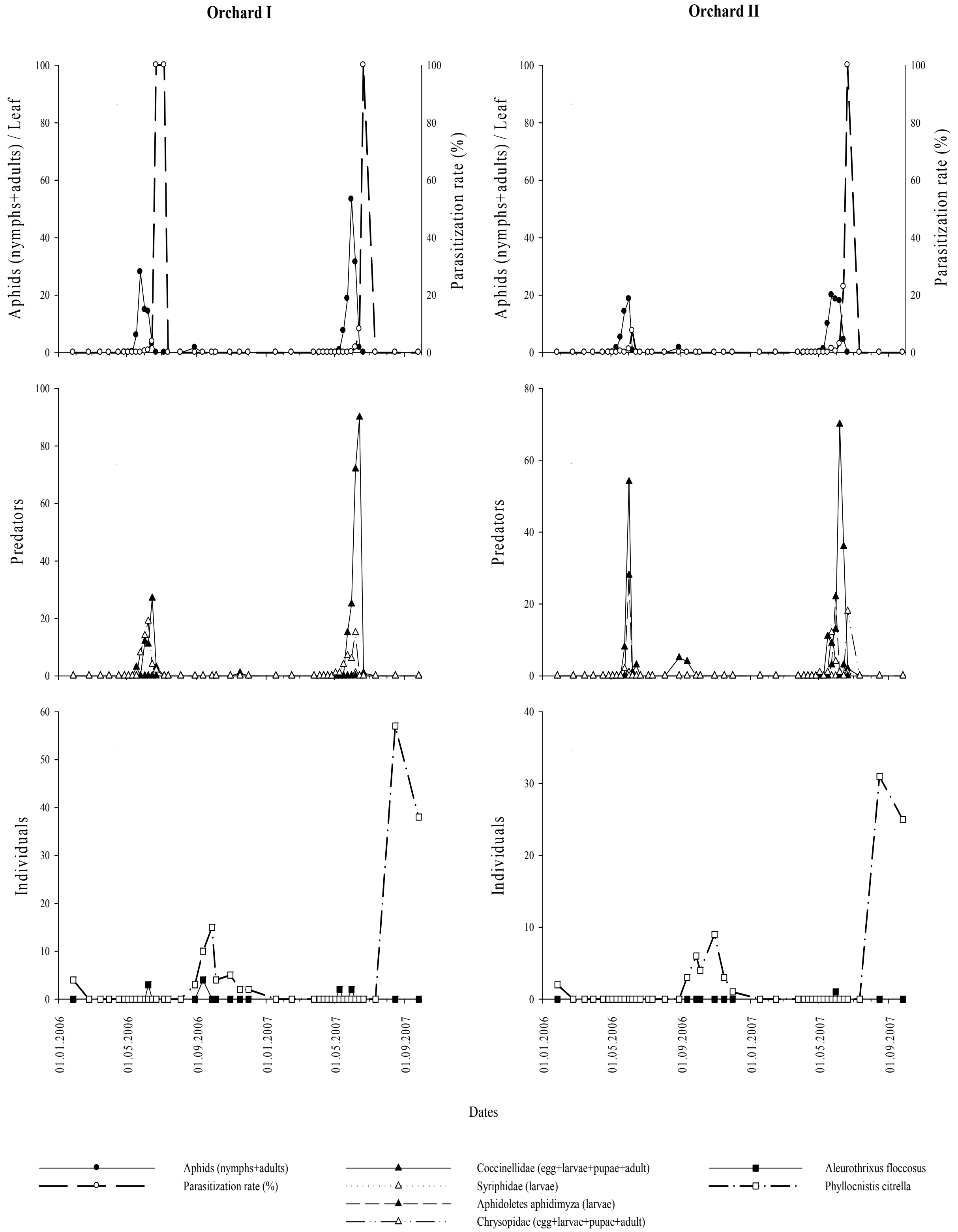

Figure 2. Population fluctuations of aphids and their parasitization rates, their predators, *Aleurothrixus floccosus* (Maskell) and *Phyllocnistis citrella* Stainton on tangerine orchards in Seferihisar between January 2006 and October 2007

Rosen (1993) stated that many biological control projects in citrus agroecosystem have been spectacularly successful than any other crops. Much of the methodology and theory of biological control has been derived from work on citrus. Within the past twenty years several new pests accidentally introduced to citrus grown areas of the Aegean Region of Turkey. All of the pests of citrus except *C. capitata* can be suppressed by natural enemies as a result of several successful biological control studies, especilally citrus whiteflies (Öncüer, 1991; Yoldaş et al., 2006).

Some pests may become important due to using broad spectrum pesticides to reduce the population of these pests without considering natural equilibrium in citrus orchards. Therefore it is essential that researchers related with plant protection should attract the attention of producers to the success of biological control in citrus agroecosystem and demonstrate it with more studies. Also the most important step for using biological control lies on producers' willingness to accept and thus on training for increasing sensitivity to human and environment health.

Acknowledgements

We thank Dr. Serdar Satar (Çukurova University, Turkey), Dr. Işıl Özdemir (Ankara Plant Protection Research Institute, Turkey) for identifying aphid species and RNDr. Petr Starý DrSc. (Institute of Entomology, Czech Republic) for identifying parasitoids of aphids. This study was funded in part by The Scientific and Technological Research Council of Turkey (TÜBİTAK), Project Number: 104O102.

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Control in Citrus Fruit Crops
IOBC/wprs Bulletin Vol. 38, 2008
pp. 275-279

Biodiversity and distribution of beneficial arthropods within hedgerows of organic Citrus orchards in Valencia (Spain)

González, S.[1], Vercher, R.[1], Domínguez Gento, A.[2] and Mañó, P.[1]
[1] Institut Agroforestal del Mediterrani (UPV). Camino de Vera 14, 46022 Valencia, Spain.
[2] Estació Experimental Agraria de Carcaixent (IVIA). Spain.

Abstract: A study of natural enemies within hedgerows and on ground covers was carried out in two organic citrus orchards in two areas of Valencia (Spain) using two sampling methods, yellow sticky traps and a vacuum machine. Hedgerows had significantly higher levels of natural enemies, followed by citrus and ground covers. The species of natural enemies in hedgerows were similar to those found in citrus orchards, but different from those identified on ground cover. In hedgerows and citrus the predominant predators were Coniopterygidae (Neuroptera) and Cecidomyiidae (Diptera), and the most abundant parasitoids were Aphelinidae (Hymenoptera).

Key words: predators, parasitoids, hedgerows, citrus, biological control, population dynamics.

Introduction

The intensification of agricultural production systems has produced a rapid decline in biodiversity in agroecosystems (Barr et al., 1993; Chamberlain et al., 2000; Robinson and Sutherland, 2002; Benton et al., 2003), reductions in landscape heterogeneity (Weibull et al., 2000) and the loss of non-cropped habitats (Marshall and Moonen, 2002; Petit and Usher, 1998). Non-crop habitats such field margins, fallows (setaside land), hedgerows and wood lots are relatively undisturbed and temporally permanent areas that hold a substantial proportion of the biodiversity in agricultural landscapes (Bianchi et al., 2006). In recent years, the strategies of conservation biological control have had great importance, and though it is said the more diverse natural vegetation species are, the more diversity of natural enemies is found there, the role of certain hedgerow species and ground covers associated to citrus orchards has not been studied in depth (Boriani et al., 1998; Burgio et al., 2004; Franco et al., 2006; Maudsley, 2002; Pollard and Holland, 2006).

This study is the first step toward establishing the role of certain Mediterranean hedgerows and ground covers in the natural enemies populations associated to organic citrus orchards in Valencia (Spain). The main objectives of the present study are: (1) to determine natural enemies of several hedgerows, in order to catalogue predatory species and study their population dynamics; (2) to compare the diversity of predators in the three substrates, hedgerows, orchard and ground covers.

Material and methods

Samples were taken in citrus and hedgerows of two organic citrus orchards located in Alcudia and Alzira, in the region of Valencia (Spain). A total number of 23 field samplings were carried out in Alcudia from May 2006 to May 2007 and 12 in Alzira from October 2006 to May 2007. Samples were made fortnightly, except in winter when samples were collected monthly.

The hedgerows sampled included three monospecific species: *Cupressus sempervirens* L., *Ailanthus altissima* Mill. and *Punica granatum* L., and four mixed species typical on the Mediterranean forest: *Pistacia lentiscus* L., *Crataegus monogyna* Jacq., *Rhamnus alaternus* L. and *Pistacia terebinthus* L. Two kinds of ground covers were also sampled, a spontaneous one and another with sown alfalfa. Plant species found in the spontaneous cover varied depending on the time of the year. In winter, more than 60% were *Bromus* spp., *Echinocloa* spp. and *Hordeum murinum* L., and in summer, more than 70% was *Cynodon dactylon* (L.) Pers.

Arthropods were collected with two methods, a portable vacuum device and yellow sticky traps, with four repetitions per plant species sampled. Samples with the portable vacuum consisted of 2 minutes of suction per plant species. During all the sampling period, 949 suction samples and 973 sticky traps were collected.

Results and discussion

A total of 118,176 arthropods were collected, belonging to 13 different orders. The distribution and abundance of natural enemies differed depending on the plant strata: hedgerows, citrus or ground cover. There were significantly higher numbers of Diptera in the herbaceous strata compared with hedgerows and citrus orchards (Figure 1). Only two families were studied in this order: Cecidomyiidae and Syrphidae. The family Cecidomyiidae was the most abundant. Captures in the Syrphidae were very low, maybe due to the fact that the sampling methods used were not suitable for this family.

Neuroptera were quite common in all sampled trees, been slightly more abundant in *Citrus* sp. and being absent on weeds (Figure 1). Previous studies had already emphasized the importance of this predators on citrus orchards (García-Marí et al., 1991; Llorens 1990; Llorens and Garrido, 1992; Ripollés et al., 1995), but it was not known that they were very abundant in Mediterranean hedgerows.

Very low numbers of Coccinellidae were found in ground covers in comparison with hedgerows and citrus orchards. The species of coccinellids identified were similar to those reported in the studies carried out in citrus orchards by Alvis Dávila (2003) and Bru (2006), although their relative importance varied.

The highest number of families was found in the order Heteroptera. They appeared more frequently in ground covers than in hedgerows, whereas in citrus orchards their presence was very low (Figure 1). Four predatory families were identified: Anthocoridae, Miridae, Reduviidae and Nabidae. Mirids were the most common family in all strata. Nabidae tended to inhabit in great numbers in ground covers, whereas Reduviidae were rare in all plant species. Our results differ from those obtained by Ribes et al. (2004) in organic citrus orchards in Tarragona (Spain). In their study, the proportion of anthocorids in citrus plants was higher than other predatory heteropterans. In our study, the most abundant family was the Miridae, confirming previous studies carried out in woody strata (Pollard and Holland, 2006; Fauvel, 1999).

This study also compared natural enemies found on different hedgerows species and citrus orchard. We found that there were significant differences between plant species and between groups of predators, indicating that plant species influence the diversity and abundance of natural enemies (Figure 2). Neuroptera were common in all plant species, but significantly more abundant in *Rhamnus alaternus*. Heteroptera were more frequent in *R. alaternus*. Coccinellidae were more frequent in *Citrus* sp., Diptera Cecidomyiidae in *Crataegus monogyna* and Formicidae and Araneae in *Cupressus sempervirens*. Aphelinidae were the most abundant parasitoids, being less common in *C. sempervirens* and more predominant in *Citrus* (data not shown).

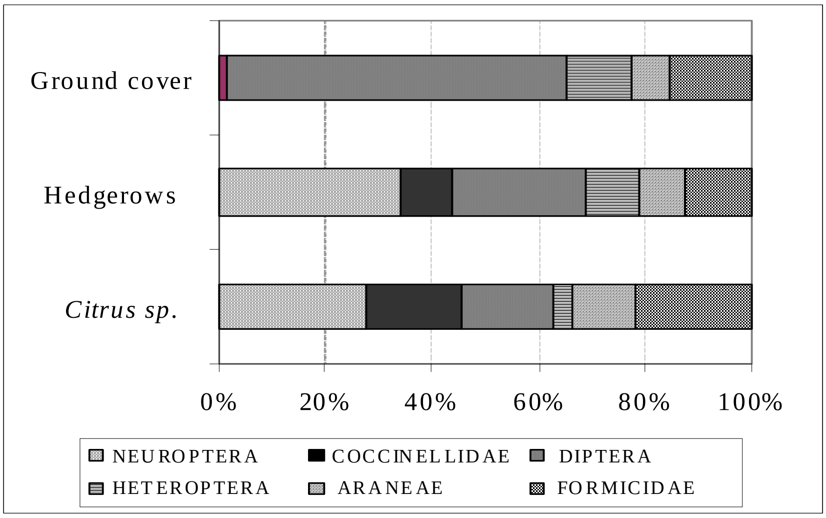

Figure 1. Abundance and diversity of predatory insects and Aranaea captured in two organic citrus orchards located in Valencia (Spain) from May 2006 to May 2007.

The population dynamics throughout the year indicated that certain natural enemies, like the coccinellid *Stethorus punctillum*, are distributed during the year among all plant species studied. Other natural enemies were more frequent at a particular time of the year in one plant species, and later on they moved on to another plant species. This is the case of *Conwentzia psociformis*, which inhabited *R. alaternus* in winter but was found in other plant species, like *Citrus* sp. and *P. terebinthus*, during the summer months (Figure 3).

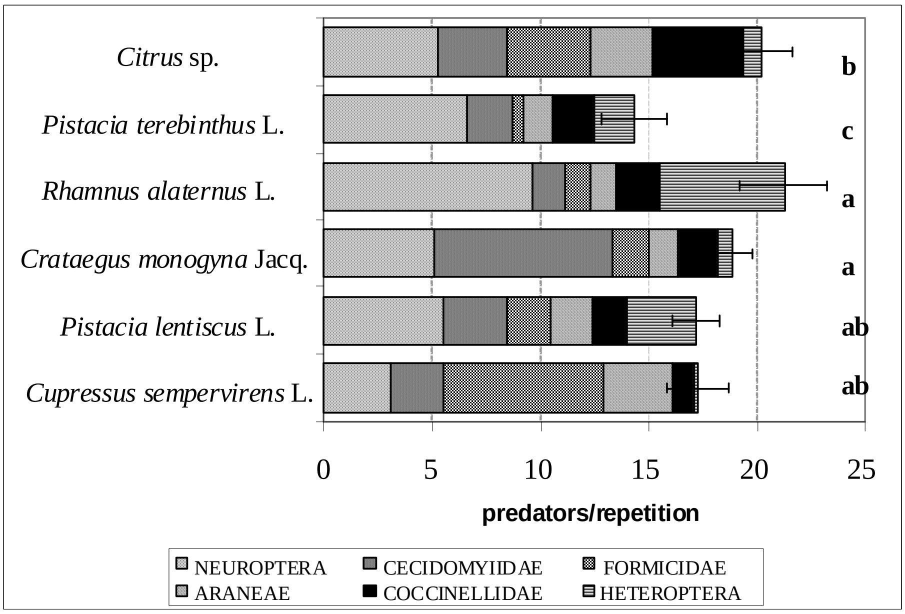

Figure 2. Relative abundance of groups of predators on hedgerows and citrus.

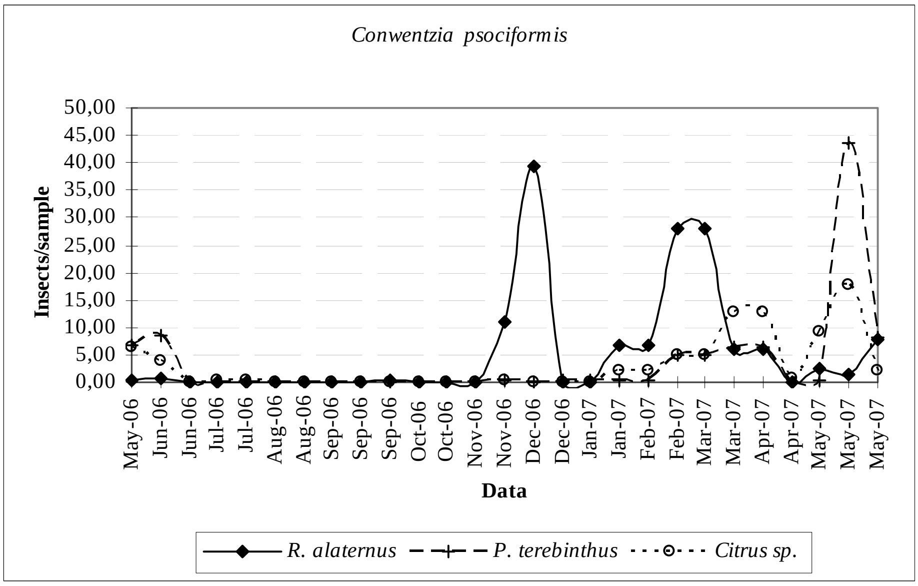

Figure 3. Population dynamics of the neuropteran predator Conwentzia psociformis.

Conclusions

The present study revealed a very high diversity and abundance of natural enemies within hedgerows. It also showed that citrus and hedgerows had similar species of predators, but differ to those species found on ground covers. In hedgerows the abundance of predators changed with the plant species considered. In conclusion, hedgerows present a wide variety of natural enemies that could help to control pests in citrus orchards. This could be linked to an increase in the diversity of vegetation.

Acknowledgements

We appreciate the Cooperative La Vall de la Casella (Alzira) and the owners of the field in L´Alcudia for allowing the sampling in their fields. Also thanks to Ferran García-Marí and Pablo Bru for helping to classify the arthropods.

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Control in Citrus Fruit Crops
IOBC/wprs Bulletin Vol. 38, 2008
pp. 280-283

Establishment of *Neodryinus typhlocybae* (Ashmead) (Hymenoptera: Dryinidae) in Sicilian lemon orchards

Lucia Zappalà, Gaetano Siscaro & Santi Longo
Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, via S. Sofia 100, 95123 Catania, Italy. E-mail: lzappala@unict.it; gsiscaro@unict.it; longosan@unict.it

Abstract: The results of a classical biocontrol program against *Metcalfa pruinosa* (Say) (Homoptera: Flatidae) introducing the specific parasitoid *Neodryinus typhlocybae* (Ashmead) (Hymenoptera: Dryinidae) are reported. The antagonist was released in a 15-ha lemon orchard in Eastern Sicily where the infestation levels and the population dynamics of *M. pruinosa* have been monitored, on a fortnightly basis, since March 2004. One hundred adults of *N. typhlocybae* were released in an area of the orchard with a high density of the pest and where a *Pittosporum* hedge was planted. The periodical sampling revealed the presence of the first pupae of the parasitoid only after January 2006 on trees close to the release point. The parasitoid was then found at a density of 1-10 pupae per tree, up to 100m far from the release point. The diffuse presence of *N. typhlocybae* on *Pittosporum* is of particular interest because of the potential use of these hedge plants as reproductive refuge of the antagonist. In the 3 years of the survey the infestation levels on lemon trees, expressed as mean percentage of new shoots bearing more than one *M. pruinosa* instar, were respectively 13.71%, 27.56% and 25.09%. Further observations are still being conducted in Sicily in order to find other areas of presence of the dryinid as well as to evaluate its activity and efficacy in controlling the citrus flatid.

Key words: *Metcalfa pruinosa*, biocontrol, parasitoid, citrus

Introduction

Metcalfa pruinosa (Say) (Homoptera: Flatidae), the citrus flatid planthopper, is a nearctic species accidentally introduced in Italy at the end of the 1970s (Zangheri & Donadini, 1980) and recorded in Sicily since 1997 (Lo Pinto et al., 1997). It is a highly polyphagous species, recorded on more than 200 woody and herbaceous host plants, cultivated as well as spontaneous, including those belonging to the genus *Citrus* and particularly lemon (Bagnoli & Lucchi, 2000).

Although it is normally considered a secondary pest on citrus, easily contained by the common cultural and control methods, due to the increasing organic citriculture, a classical biological control program was started. It consisted in the introduction of *Neodryinus typhlocybae* (Ashmead) (Hymenoptera: Dryinidae), specific parasitoid of *M. pruinosa*, already successfully released on other crops in Italy (Girolami & Mazzon, 1999) as well as in other European countries (Girolami & Mazzon, 2001; Jermini et al., 2000; Malausa et al., 2000). This species attacks *M. pruinosa* young instars (from 3rd instar nymph), and when its ectoparasitic larva has totally emptied the host, it builds a cocoon, normally on the leaf lower surface, where it overwinters. The dryinid adult females also directly feed on *M. pruinosa* nymphs, thus contributing to the control of the planthopper (Girolami et al., 1996).

Material and methods

The antagonist was introduced (with strains from the Universities of Padova and Pisa) in a 15-ha lemon orchard in Eastern Sicily (Giarre, Catania) where the infestation levels and the population dynamics of *M. pruinosa* have been monitored, on a fortnightly basis, during 2004-2006. The observations were carried out in the field on 10 shoots/20 trees (10 pruned and 10 not pruned). The presence of the pest was evaluated dividing the shoots into 5 infestation classes based on the number of young instars observed: class 0 (no specimens), class I (1-5 specimens), class II (6-10 specimens), class III (11-20 specimens), class IV (>20 specimens). The number of adults was also recorded. Besides, 100 flatid specimens were collected in order to conduct laboratory observations and define the population composition.

In June 2004, 100 adults of *N. typhlocybae* were released in an area of the orchard with a high density of the pest and where a *Pittosporum* hedge was planted. The presence of the parasitoid was evaluated during the survey.

Results and discussion

In the three-year survey the infestation started in the second half of May with the emergence of the 1st instar nymphs from the overwintering eggs (Figure 1). The flatid adults started to be recovered in July and they began laying overwintering eggs in October (Figure 1).

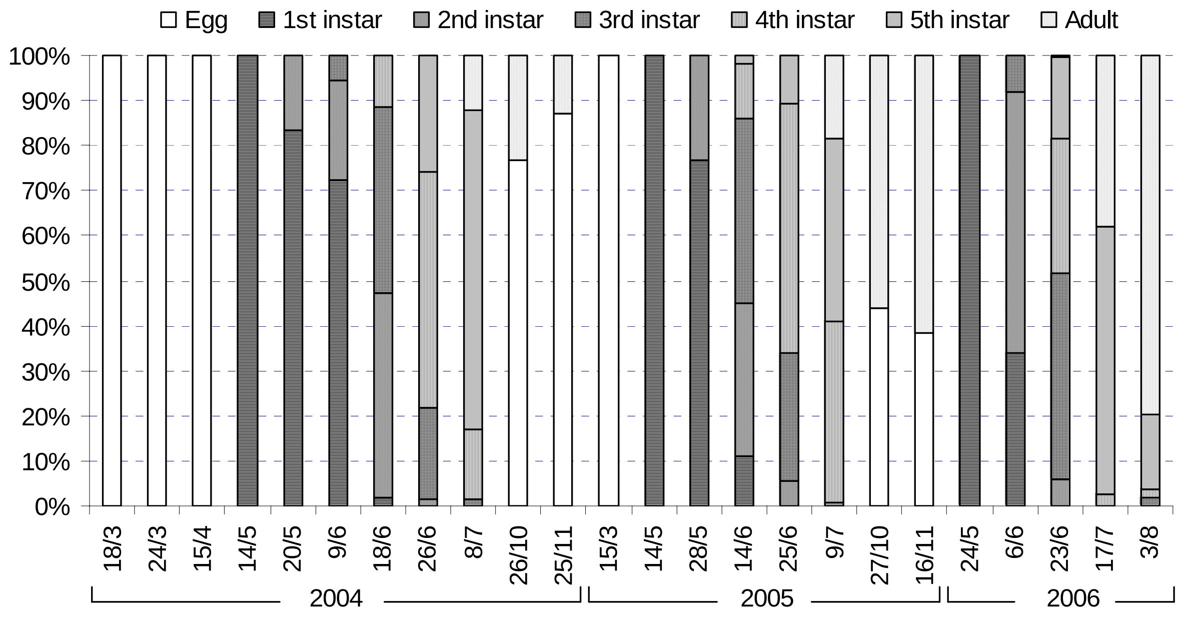

Figure 1. Composition of *M. pruinosa* population in 2004-2006.

The periodical sampling revealed the presence of the first pupae of the parasitoid only after January 2006 on trees close to the release point. The area monitored was progressively expanded and the establishment of the dryinid, as well as its presence 100m far from the release point, was confirmed. The parasitoid was found at a density of 1-10 pupae per tree, sampling on 10 infested plants in various areas of the orchard.

During the survey a diffuse presence of *N. typhlocybae* on *Pittosporum* was also observed. This result could be of particular interest because of the potential use of these hedge plants as reproductive refuge of the antagonist. Besides, as regards *M. pruinosa* infestation on lemon trees in the 3 years of the survey, the levels recorded, expressed as mean percentage of new shoots bearing more than one planthopper instar (Classes I-IV), were respectively 13.71%, 27.56% and 25.09% (Figure 2). These data show that after an initial increase in the percentage of infested shoots, probably related also to the absence of parasitic activity by *N. typhlocybae*, in 2006, when the first pupae of the parasitoid were found, the levels of infestation started to slightly decrease.

Further observations are still being conducted in Sicily in order to find other areas of presence of the dryinid as well as to evaluate its activity and efficacy in controlling the flatid on lemon trees and on other spontaneously growing or cultivated host plants.

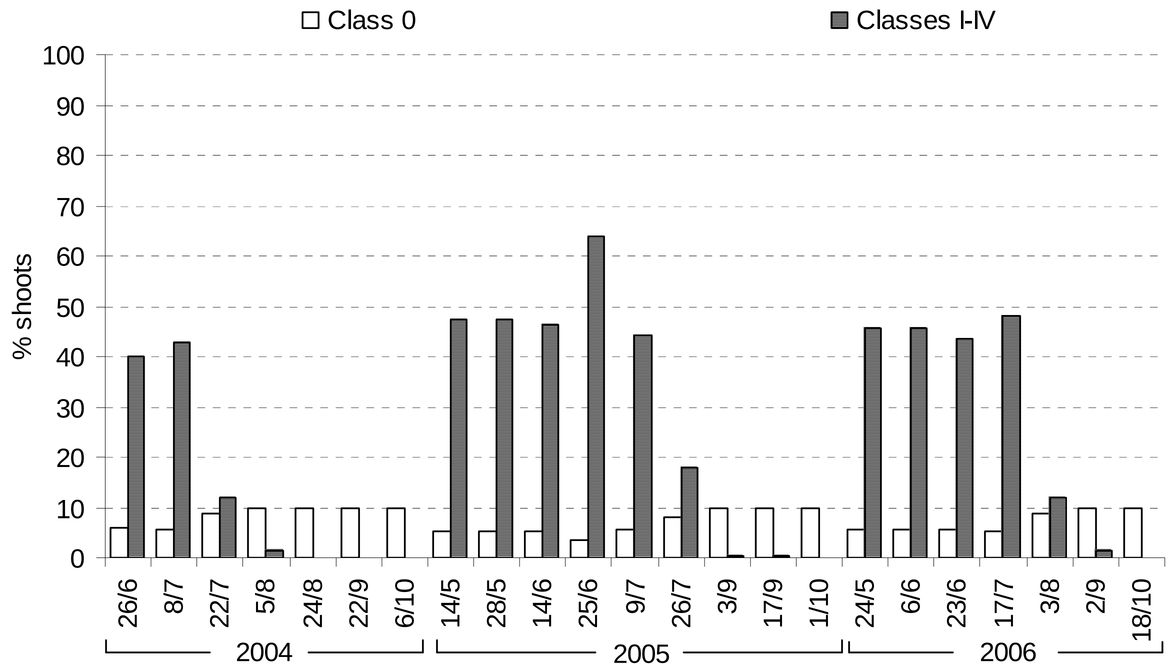

Figure 2. Infestation levels (expressed as mean percentage of shoots belonging to the Class 0 and to the 4 others) of *M. pruinosa* in 2004-2006.

Acknowledgements

The research was partially funded by the University of Catania in the framework of the University Research Project “Ricerche su insetti entomofagi in ambiente mediterraneo”. We thank Prof. V. Girolami at the Dipartimento di Agronomia Ambientale e Produzioni Vegetali (University of Padova) and Dr A. Lucchi at the Dipartimento di Coltivazione e Difesa delle Specie Legnose (University of Pisa) for providing the colonies of the parasitoid released.

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Natural parasitism of chrysopid eggs by the parasitoid *Telenomus acrobates* Giard (Hymenoptera: Scelionidae)

S. Pascual-Ruiz[1], E. Aguilar[1], M.J. Verdú[2], J.A. Jacas[1], A. Urbaneja[3]
[1] *Unidad Asociada de Entomología IVIA-UJI. Universitat Jaume I; Campus del Riu Sec; E-12071-Castelló de la Plana, Spain*
[2] *Centro Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias; Ctra. Moncada-Náquera km. 4,5; E-46113-Moncada, Valencia, Spain*
[3] *Unidad Asociada de Entomología IVIA-UJI. Instituto Valenciano de Investigaciones Agrarias; Ctra. Moncada-Náquera km. 4,5; E-46113-Moncada, Valencia, Spain; aurbaneja@ivia.es*

This is the first report of *Telenomus acrobates* Giard (Hym.: Scelionidae) as an endo-parasitoid of chrysopid eggs in citrus in Spain. The percentage of egg parasitism found on 348 eggs collected along the province of Valencia ranged from 20% on single eggs to 50% on egg batches. Although the actual impact of *T. acrobates* on natural populations of chrysopids remains unknown, further research is needed to ascertain the role of this species.

IPM and Chemical Control

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Current situation and new approaches to old challenges in citrus IPM in Israel

Y. Drishpoun
Plant Protection Department, Extension Service, Ministry of Agriculture & Rural Development, Bet Dagan, Israel; yoeldr@shaham.moag.gov.il

Israeli citriculture is an export-oriented industry. It occupies 18,000 ha, planted with 10 main citrus varieties. Pest management has been a cornerstone of the industry, in which the founders of Israeli entomology, S. F. Bodenheimer and Z. H. Avidov, were the leading figures. The current situation of pest management in citrus groves in Israel is characterized by more-or-less satisfactory biological control of most hemipteran and spider mites. However, three old enemies of the growers continue to present a management challenge: the Mediterranean fruit fly (MFF) *Ceratitis capitata*, the citrus rust mite (CRM) *Phylocoptruta oleiovora*, and mealybugs, especially the citrus mealybug *Planococcus citri*.

The recent and continuing major changes in management of MFF here include replacing Malathion in wide-baited aerial spray with Spinosad (GF/120), and launching the first operational use of sterile males. Efforts are being directed to examination of other environmentally friendly approaches that use traps as feeding stations, on the principal of lure-and-kill. The CRM receives more treatment than any other citrus pest but, up to now, the introduction of exotic predatory mites has failed to limit fruit injury, and meanwhile the acaricide arsenal is shrinking. The major problems lies in the short-term effect and low efficacy of the applied acaricides, as well as the fact that the use in Israel of the major management means, Neoron (Bromopropylate) will be banned after 2008. Three species of mealybugs – *P. citri*, *Pseudococcus cryptus* and *Nipaecoccus viridis* – cause severe injury. With the expected withdrawal of Chlorpyrifos from use in the near future, the growers may face difficulties in coping with these pests. The current trend is towards application of Confidor (Imidacloprid) to the soil, in light of the fact that in many orchards Chlorpyrifos is losing efficacy after being widely used against these mealybugs for decades. The current thinking is that use of sex pheromones for monitoring and control, together with augmentation of the natural enemies, should be tested as reasonable alternative management tactic for the future.

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Sicily IPM for Citrus Demonstration Project

Riccardo Tumminelli[1], Ernesto Raciti[1], Salvatore Calcaterra[2], Rosario Finocchiaro[3] & Luigi Pasotti[4]
[1] *OMP, Servizio Fitosanitario, Regione Siciliana, via Sclafani, 32- 95024, Acireale, Italy*
[2] *SOPAT, Ente per lo Sviluppo Agricolo, Regione Siciliana, Palagonia, Italy*
[3] *Microbios Soc. coop., Servizi per l'agricoltura, Giarre, Italy*
[4] *Servizio Informativo Agrometeorologico Siciliano, Regione Siciliana, Catania, Italy*

Abstract: The purpose of the Sicily Integrated Pest Management (IPM) for Citrus Demonstration Project was to compare the costs and efficacy of various citrus pest management practices during 2003, 2004, 2005, and 2006 years. Within the project, some of the growers depended primarily on natural enemies (*Aphytis melinus* augmentative releases) and selective pesticides (narrow range mineral oil and sugar-feeding ant population rational management) to solve their pest problems while others depended primarily upon pesticides. In this program, the County Farm Advisor of Sicily Department of Agriculture staff and pest control advisors intensively monitored all of the pests and natural enemies in 10 commercial citrus orchards in the Catania County. The growers were generously allowing us to sample the blocks and evaluate the effectiveness and costs of their various control tactics. In conclusion, this program showed to result in reduced pesticide use (from 2,6 to 0,5 treatments per year) and similar fruit quality and economic returns compared.

Key words: extension education, field training, *Aonidiella aurantii*, red scale

Introduction

Despite repeated attempts by pest control advisors and scientists to introduce various facets of biologically-based citrus IPM into the Sicily Citrus (SC) growers showed limited interest in reducing broad-spectrum pesticide use, and in the context of these treatments and the extremes of summer and winter temperatures, natural enemy effectiveness was limited (e.g., Benfatto *et al.*, 1980). A group of scientists from both southern Italy and the SC developed and tested a biologically-based citrus IPM program using methodologies and concept of Cavalloro & Prota (1983). In the mid 1990s, many growers started using twice annual (spring and summer) broad-spectrum pesticides to maintain key pest species such as mealybug, and others below economic levels. Several progressive SC growers had developed a biologically-based citrus IPM program which emphasized pest monitoring, selective pesticide use, and augmentative releases of insectary-reared beneficial insects for mealybug control (Raciti *et al*., 1997). Grower adoption of the biologically-based citrus IPM program reached a peak in 1997, with participation by 20% of SC growers to European Union (EU) subsidies.

In 2001 our lab began rearing and releasing *A. melinus* and, with funding provided by the EU, our agro meteorological system began producing degree-days data, both for control of the key pest red scale in groves (Forster *et al.*, 1995; Tumminelli *et al.*, 2007). Fortunately, conversion of SC groves to organic production resulted in the construction of a new insectary for rearing and annual release of control agents, such as *A. melinus*, with funding provided by the EU in 2007. After several years of research and evaluation, the scope of this Sicily IPM for Citrus Demonstration Project was to disseminate as a model that might be used on citrus throughout the Sicily, and grower be thus able to avoid the use of other pesticides.

Material and methods

Within the project, some of the growers depended primarily on natural enemies (*A. melinus* augmentative releases) and selective pesticides (narrow range mineral oil and sugar-feeding ant population rational management) to solve their pest problems while others depended primarily upon pesticides. In this program, the Catania County Farm Advisor of Sicily Department of Agriculture staff and pest control advisors intensively monitored all of the pests and natural enemies in 10 (1 ha each) commercial Tarocco orange similar orchards in the Catania (Mineo) county. The growers were generously allowing us to sample the blocks and evaluate the effectiveness and costs of their various control tactics. The program consisted of specific, intensive monitoring methods, intervention thresholds, and selective insecticide recommendations for each of the major arthropod pests found at that time. Key among these were use of sticky material or chlorpyrifos trunk barrier and skirt pruning for sugar ant feeding population, pyrethrum and rotenone, a botanically derived insecticide mixed with mineral oil for cotton aphids control, narrow range oil for two spotted mite, and management of red scale through augmentative releases of 25,000-50,000 lab-reared *A. melinus* parasitoids per hectare per year. *Aphytis* were released every two weeks beginning mid-February and ending mid-June each year, to total 10-20 releases of 2,500-5,000 wasps per hectare annually.

Throughout the project, yearly workshops were held to teach pest control advisors how to recognize the life stages of red scale, their parasitoids, and how to determine if biological control was successful. Field days on citrus pest monitoring were produced. In addition, yearly roundtable discussions were helds. In these discussions, pest control advisors shared information about pest pressures, monitoring methods, control tactics, and the level of success of biological control they had achieved. Data on pest densities, natural enemy levels, degree-days, and the consequences of various pest management strategies in terms of economic data were posted with our website: http://www.sias.regione.sicilia.it/ and about one hundred newsletters or SMS (about 1300 contacts).

Results and discussion

Adoption of the biologically-based citrus IPM program in the SC was slow, but was accelerated by the EU subsidies. During our Sicily IPM for Citrus Demonstration Project, growers and pest control advisors in Sicily citrus growing, often working in cooperation with extensionists, experimented and implemented reduced pesticide input pest management programs. This program was shown to result in reduced pesticide use and similar, if not higher, fruit quality and economic returns compared with the conventional broad-spectrum pesticide-based program. Although research efforts were critical, the biologically-based IPM program will not adopt without extension education of many progressive citrus growers and pest control advisors. A number of grower meetings were held and discuss progress in development of the IPM program. There is a perception that use of biological control is riskier and more difficult to employ, compared with a traditional chemical control program. For the present, many growers continue to rely on chlorpyrifos for red scale control, but we expect resistance to develop to this material.

A second problem for growers using biologically-based citrus IPM in the SC is the introduction of new (exotic) pest species. The rate of new introductions appears to be increasing because of greater movement of people and plant material between states and countries. When exotic pests enter a new region, they are often not accompanied by the full complement of natural enemies present in their native range. Thus, chemical control is often needed to maintain damage below economic thresholds until the full natural enemy complex is introduced and provides adequate control. Fortunately, the potential for biological control of this pest on bearing citrus is good. The success of the program depends on intensive sampling of pest and natural enemy populations, in order to maximize the effectiveness of soft pesticides and natural enemy populations. Developing the required level of knowledge and training needed to successfully conduct biologically-based IPM for a crop system as complex as citrus takes years of experience and input from knowledgeable pest control advisors and supportive growers.

The biologically-based citrus IPM program is both sustainable and dynamic, due to changes in pesticide registrations, pest complexes, and the introduction of exotic species. Research, extension, and management programs have to be equally dynamic to respond to those changes. For the near future, further implementation of the biologically-based citrus IPM program in the SC faces an uphill battle, because chemical pest control appears to many to be a simpler pest management solution. However, experience with citrus has shown that at best, this approach is short-lived and is more costly in the long run. All of this activity will help to increase grower adoption of biologically-based IPM methods. In the near future we‘ll adapt new technology, which showed that a high-pressure post-harvest washer was effective in removing red scale from fruit, thus the economic threshold of this key pest will be elevated.

Tab. 1 Mean number treatments, pest management cost, income and fruit damage per hectare in 10 Eastern Sicily Tarocco orange commercial citrus orchards.

Year	ha	N° broad spectrum	N° Select	Costs euro/ha			mean yield ton/ha	income euro/ha	% fruit damage
		treatment		Treat	N°Aphytis	total			
2003/04	6	2,0	0,3	400	0	400	15	5250	2
2004/05	6	3,0	0,3	501	0	501	37	12950	3
2005/06	6	2,0	0,3	400	0	400	14	4900	1
mean chemic.		**2,3**	**0,3**	**434**	**0**	**434**	**22**	**7700**	**2**
2003/04	8	1,0	1,0	389	0	389	11	3850	3
2004/05	8	1,0	1,0	389	0	389	33	11550	1
2005/06	8	0,0	1,0	186	0	186	15	5250	2
Mean interm.		**0,7**	**1,0**	**321**	**0**	**321**	**20**	**6883**	**2**
2003/04	6	0,0	0,5	125	50000	125	9	3150	1
2004/05	6	0,0	0,5	125	45000	125	30	10500	2
2005/06	6	0,0	0,5	125	25000	125	25	8750	4
mean biologically-based		**0,0**	**0,5**	**125**	**40000**	**125**	**21**	**7467**	**2**

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Integrated pest management in two citrus varieties Navel and Maroc Late in Sidi Slimane area - North Western Morocco

C. Smaili[1], D. Bouya[2]
[1] *INRA URPPV Laboratory of Entomology, Kenitra, Morocco BP 239; csmaili@yahoo.fr*
[2] *University of Sidi Mohammed Ben Abdellah, FSDM Fes, Morocco*

The development of IPM on two citrus varieties Navel and Morocco Late was carried out in Sidi Slimane area, a western north part of Morocco between 2002 and 2006. A new vision of integrated pest management was practiced and improved on a large program with pilot citrus producers. Many techniques were carried out and several thousands of parasitoid *Aphytis melinus* were released against the California red scale. The results showed that the species including *Aonidiella aurantii*, *Parlatoria pergandii*, *Lepidosaphes beckii, P. ziziphi*, *Ceratitis capitata* and snails *Theba (Helix) pisana* are the prime pests of this area. During this period, no treatments were applied against aphids, whiteflies, leafminer, mites and scale (except 2004). On the other hand, none or very little bait spraying method was executed for medfly respectively on Maroc Late and Navel varieties. The impact of all used techniques in the context of IPM was discussed. During harvest, the fruit infestation rates were tolerable economically. An adequate and methodic diagram of IPM was elaborated in order to control these principal citrus pests for this area. This new IPM strategy can be used also against Medfly in combination with the Sterile Insect Technique (SIT).

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Side-effects of insecticides on *Leptomastix dactylopii* under semi-field conditions in Italy

Gaetana Mazzeo, Pompeo Suma, Santi Longo
Dipartimento di Scienze e Tecnologie Fitosanitarie, via S. Sofia, 100 – 95123 Catania, Italy

Abstract: The side-effects of chlorpyrifos-methyl, spinosad and pyriproxyfen on *Leptomastix dactylopii* Howard, the most effective natural enemy of the citrus mealybug *Planococcus citri* (Risso), were investigated under semi-field conditions. The experiments have been conducted according to internationally approved guidelines, spraying young orange plants confined in test cage, with the insecticides at maximum field application rates. In each cage 20 *L. dactylopii* adults were released and the mortality after 24, 48 and 72 hours, the longevity, the progeny production of the survived parasitoid females and sex ratio of the progeny were assessed. Despite the fact that the tested insecticides were harmless to the parasitoid and none of them influenced the progeny production of the survived females, the longevity was negatively affected suggesting a multiple testing methods should be used when evaluating pesticide effects on beneficial arthropods.

Key words: citrus mealybug, natural enemy, pesticide, integrated control

Introduction

In IPM programs the evaluation of side effects of insecticides on natural enemies should be taken into consideration due to the activity that some chemicals have on the population growth. In the framework of the national project “Researches and experiments in Italian citriculture” the side-effects on natural enemy of citrus scale insects were investigated in laboratory and under semi-field conditions focusing on “key pests” such as *Planococcus citri* (Risso) and *Aonidiella aurantii* (Maskell) and their main parasitoids (Siscaro et al., 2006; Suma et al., 2005).

The results of trials carried out under semi-field conditions working on *Leptomastix dactylopii* Howard, the most effective natural enemy of the citrus mealybug *Planococcus citri* (Risso), are here reported. The mortality caused by some insecticides and the influence on some biological parameters were evaluated.

Material and methods

The trials were carried out in the experimental fields of the Faculty of Agriculture at the University of Catania in 2005, according to internationally approved guidelines. Young orange plants (150 cm high) were selected on the basis of shape and size; they were sprayed at maximum field application rates with the insecticides chlorpyrifos-methyl, spinosad and pyriproxyfen using tap water as control.

After drying the plants were individually confined in cages (200x50x50 cm), made of PVC tubes (ø 15mm) covered with fine mesh plastic screen. Ten 24-48 hours-old *L. dactylopii* coupleswere released in each cage. 24h after the treatment each survived female was supplied with 20 third-instar nimphs of the citrus mealybug for additional 24 hours, then the hosts were collected and isolated for the emergence of the parasitoid progeny. *L. dactylopii* females were also isolated in boxes together with new hosts in order to define the total progeny production. Mortality of adults after 24, 48 and 72h, effects on longevity, fecundity of survived females and sex ratio of the progeny were observed.

Results and discussion

In spite of the high levels of mortality (more than 50% in the case of chlorpyrifos-methyl and spinosad), all compounds have to be included in the 1st class of IOBC categories of initial toxicity because they don't affect the progeny production. Nevertheless, the longevity was significantly reduced in all treatments (Table 1).

Table 1. Side-effects of pesticides on adult parasitoids, % mortality and reduction in parasitism compared to the control (RP%), sex-ratio of the progeny produced by the surviving females and their longevity (days).

Thesis	Mortality 72 h after treatment (% ± sd)	Progeny/female (average ± sd)	RP%	progeny sex-ratio (M:F)	Longevity days (average ± sd)	Class*
Control	-----	23.97 ± 1.30 a	-----	1.3:1	26.76 ± 6.35 a	-----
Pyriproxyfen	48.75 ± 12.08	22.03 ± 2.92 a	8.09	1.2:1	19.52 ± 8.49 b	1
Chlorpyrifos - methyl	57.50 ± 14.90	18.31 ± 2.67 a	23.61	0.97:1	14.35 ± 7.52 b	1
Spinosad	72.50 ± 17.03	24.32 ± 3.09 a	0	2.1:1	16.40 ± 7.89 b	1

% Mortality is corrected using Abbott's formula (Abbott, 1925); RP is the reduction in the parasitism rate compared with the control; *Evaluation categories of initial toxicity, IOBC classification: 1, harmless (<30%); 2, slightly harmful (30-79%); 3, moderately harmful (80-99%); 4, harmful (>99%) (Hassan et al., 1994; Sterk et al., 1999); Means followed by the same letter were not significantly different at P< 0.05 (LSD test).

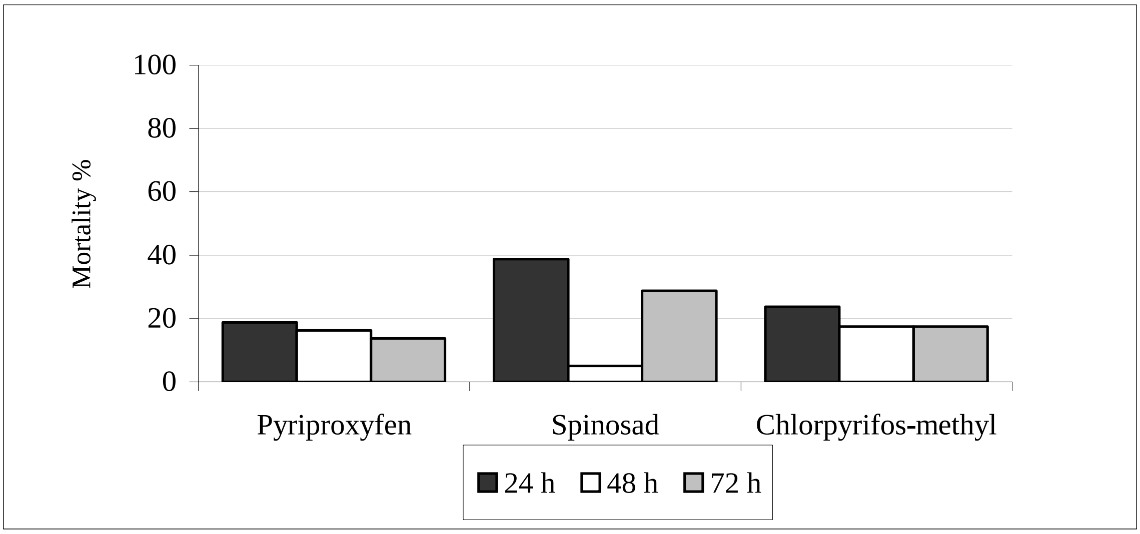

Figure 1: Mortality (%) caused by the tested insecticides on *L. dactylopii* adults 24, 48 and 72 hours after spraying.

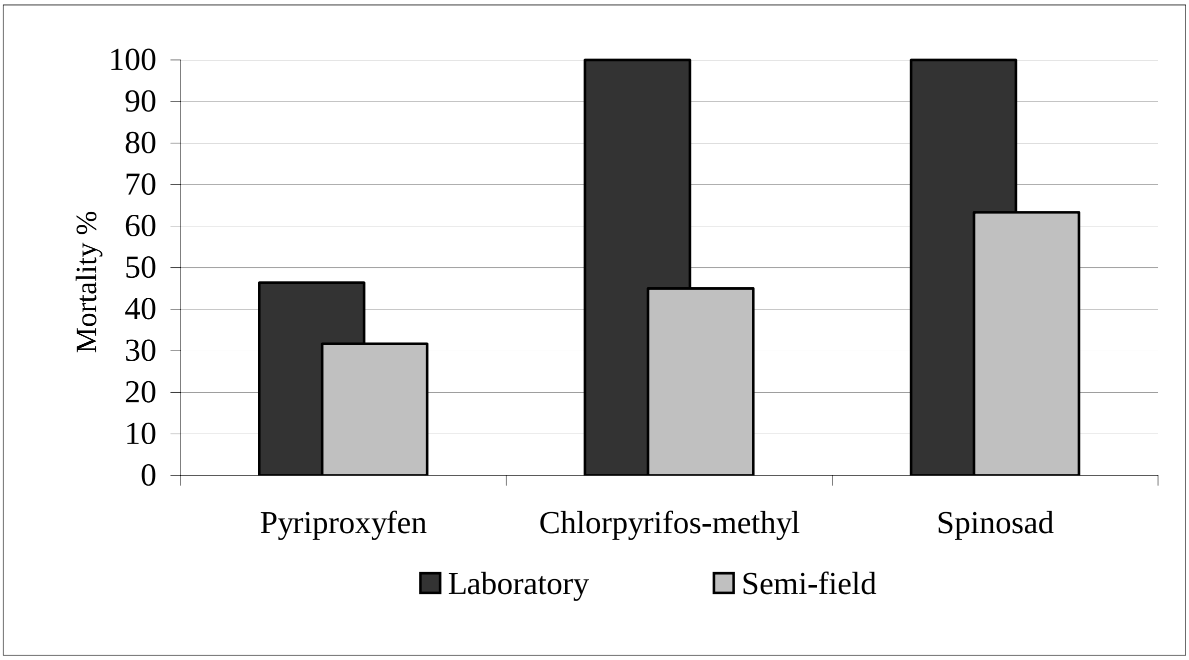

Figure 2: Comparison between total mortality on *L. dactylopii* females in laboratory (Siscaro et al., 2006) and in semi-field conditions.

The levels of mortality were higher during the first 24 h of exposition for all products and between them, the value reached by spinosad was higher than those of the other tested compounds (Figure 1). Comparing the mortality recorded under different conditions, chlorpyrifos-methyl and spinosad in laboratory tests caused 100% of mortality (Siscaro et al., 2006) but in semi-field conditions both molecules showed a lower level of toxicity (Figure 2), due to this value they were defined as belonging to the 1st class of initial toxicity (Table 1).

It is interesting to note how molecules like spinosad that in laboratory trials determined 100% mortality, in our experimental conditions killed only 63% of the tested parasitoids and didn't affect the rate of parasitism. The results obtained suggest that, also for naturally occurring insecticides, multiple testing methods should be used in evaluating pesticide effects on beneficial arthropods in order to obtain more accurate indications on their compatibility with IPM programs.

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Response of larval *Ephestia kuehniella* (Lepidoptera: Pyralidae) to individual *Bacillus thuringiensis kurstaki* toxins and toxin mixtures and effect of delta-endotoxin ratio in *Bacillus thuringiensis* crystals

S. Tounsi, M. Dammak, S. Jaoua
Laboratory of Biopesticides, Centre of Biotechnology of Sfax, P.O.Box: K, 3038. Sfax, Tunisia

Bacillus thuringiensis, a gram positive soil bacterium, is of scientific and agricultural interest, also with specific reference to citrus, due to its production of insecticidal cytoplasmic protein crystal inclusions during sporulation. Depending upon the subspecies, inclusions may be composed of one or more delta-endotoxins, which are variously toxic to larvae of Lepidoptera, Coleoptera and Diptera (Höfte and Whitely, 1989). Delta-endotoxins, encoded by *cry* genes, differ qualitatively and quantitatively in their toxicity for different insect species (Poncet *et al.*, 1995; Hughes *et al.*, 2005).

To determine the optimal ratio of *Bacillus thuringiensis* delta-endotoxins Cry1Aa, Cry1Ac and Cry2Aa bioinsecticide formulations, and for development of novel biopesticides based on *Bacillus thuringiensis*, the toxicities of these three proteins, individually and in combinations, have been determined against the Mediterranean flour moth, *Ephestia kuehniella*. While *Bacillus thuringiensis* crystals containing a mixture of Cry1Aa, Cry1Ac and Cry2Aa displayed toxicity with an LC_{50} and LC_{95} of 109.7 ng and 463.0 ng of toxin per mg flour, respectively, when used individually or in combination, Cry1Aa, Cry1Ac and Cry2Aa showed significantly lower activity (Tounsi *et al.*, 2005). However, when *Bacillus thuringiensis* Cry1Ac or Cry2Aa crystal contents were increased, the recombinant crystals were two to three folds as toxic as the wild-type crystals against *Ephestia kuehniella*, demonstrating the importance of delta-endotoxin ratio in the conception of optimal *Bacillus thuringiensis* bioinsecticide formulation (Tounsi *et al.*, 2006).

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Functional diversity and distribution of the insect pests and their auxiliary fauna in relation to an insecticidal treatment with the ZOLONE in an orchard of orange trees in the Central Mitidja (blidean Atlas, Algeria)

Z. Djazouli[1], L. Allal-Benfekih[1], A. Mahamat-Salah[2]
[1] *Département d'Agronomie, Faculté agro vétérinaire, Université de Blida, DZ 09000, Algérie.*
[2] *Département de Biologie, Faculté agro vétérinaire, Université de Blida, DZ 09000, Algérie.*

Destruction of the predatory and parasitic zoocenose by the pesticides causes a fast resurgence of the insect pest populations which one proposes to eliminate. This experimentation has the aim to show the impact of the phytosanitary treatments on the faunistic diversity of an orchard of orange trees variety Thomson Navel in the Mitidja region (Blibean Atlas, Algeria). The product used for this study is the ZOLONE 35 EC. It is a general purpose insecticide acting on a great number of pests even in period of flowering. We studied the population dynamics of the principal listed insect pests and determined their type of distribution. After treatment, we analyzed the faunistic availability. The Diptera insects have a high specific richness of 74 per-cent followed by the Homoptera order with 13 per-cent, Hymenoptera and finally the Araneida and the Coleoptera represented by an identical percentage of 3 per-cent. The population densities of the target and non target fauna decrease. The distribution of *Phyllocnistis citrella* and *Dialeurodes citri* becomes regular during January and December. In addition, we observed a total absence of *Toxoptera aurantii* colonies and an important lowering of auxiliary fauna such as the ladybirds and the spiders during a one month period and half which followed the treatment. We supposed that this redimensioning of the densities of *D. citri* and *P. citrella* populations is due primarily to a translaminary purpose of the ZOLONE added by a shock action on the mobile and resistance forms of *T. aurantii,* of the spiders and the ladybirds. The analysis of variance shows that the dosing quantities of soluble and water-soluble proteins seem not to be influenced by the application of the ZOLONE. However, these quantities can affect the biological parameters of the species from where variation in the type of distribution.

Diseases

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Seasonal variation in the population level of *Fusarium* spp. in citrus nurseries in Southern Italy

A. Khlij[1], T. Yaseen[2], A.M. D'Onghia[2], G. Cirvilleri[3], A. Ippolito[1]
[1] *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, Bari, Italy*
[2] *CIHEAM/Mediterranean Agronomic Institute - Bari, Via Ceglie 23 – Valenzano(BA), Italy*
[3] *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via S. Sofia 100, Catania, Italy*

Fusarium spp. are generally classified as soil-borne fungi causing various vascular wilts and root and stem rots of cultivated plants. The main species isolated from citrus, associated with dry root rot and symptomless infection on rootlets is *F. solani* (Mart.) Appel. & Wremend. Snyd. & Hans. This fungus is ubiquitous in citrus groves and nurseries, attacking feeder roots under stress conditions. In this work, the seasonal variation of *Fusarium* species in four citrus nurseries located in Basilicata, Calabria and Sicily (Southern Italy) is evaluated. Soil and root samples are collected in September, December, March and June from the rhizosphere of different rootstocks. The number of propagules of *Fusarium* spp. is assessed using the soil dilution plate method with a *Fusarium* selective medium. Isolates are grouped according to their morphological characteristics and classified using the DNA Sequence-Based method by amplifying and sequencing two genomic regions (betatubulin (*benA*) and translation elongation factor 1-alpha (*tef*) genes). In all nurseries, *F. solani* is the predominant species followed by *F. oxysporum*, and with a very low frequency of *F. proliferatum*. The propagules of *Fusarium* spp. show the lowest values in December, then they increase in March and reach a peak in June. The population of *Fusarium* spp. fluctuates according to the rootstocks, being generally high in Troyer citrange, Carrizo citrange and Volkameriana lemon seedlings.

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Quantitative detection of *Phytophthora nicotianae* zoospores and chlamydospores by real-time Scorpion PCR

T. Yaseen[1], L. Schena[2], F. Nigro[3], A. Ippolito[3]
[1] *CIHEAM/ Mediterranean Agronomic Institute, Via Ceglie 23 - 70010, Valenzano (BA), Italy*
[2] *Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea di Reggio Calabria, Feo di Vito - 89124, Reggio Calabria, Italy*
[3] *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari, Via Amendola 165/A - 70126 Bari, Italy*

The genus *Phytophthora* includes many highly plant pathogenic species. Among these, *P. nicotianae* has a very wide host range including citrus, on which it causes mainly feeder root rot and gummosis. *Phytophthora* disperses by means of swimming zoospores and survival structures, such as oospores and chlamydospores. A rapid, accurate and sensitive detection and quantification of *Phytophthora* spp. propagules for managing the disease in the field and in nurseries is mandatory. In this work rapid DNA extraction protocols from zoospores and chlamydospores were developed to yield DNA with a purity and quality suitable for PCR assay. Using these protocols the detection limit in the first round real-time Scorpion PCR with primers PnB5-Pn6 Scorpion, specific for *P. nicotianae*, was 1 pg μl^{-1} of DNA, 100 zoospores and a single chlamydospore. By combining the protocols with a double amplification (nested Scorpion-PCR) using primers Ph2-ITS4, amplifying DNA from the main *Phytophthora* species (first round), and primers PnB5-Pn6 Scorpion it has been possible to increase the sensitivity, enabling the detection of a single zoospore, as well as 1 fg/µl of genomic DNA from pure culture of *P. nicotianae*. The analyses of DNA extracted from zoospores or chlamydospores and DNA extracted from mycelium showed a high and significant correlation between the concentration of pathogen propagules and the real-time PCR cycle threshold. The developed assay has potential for a rapid and accurate quantitative detection of *P. nicotianae* chlamydospores and zoospores in different matrices.

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Seasonal variation in *Phytophthora* spp. in citrus nurseries in Southern Italy: preliminary results

A. Salama Eid[1], G. Cirvilleri[1], T. Yaseen[2], A. M. D'Onghia[2], A. Ippolito[3]
[1] *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via S. Sofia 100, Catania, Italy*
[2] *CIHEAM/Mediterranean Agronomic Institute - Bari, Via Ceglie 23 – Valenzano(BA), Italy*
[3] *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, Bari, Italy*

Phytophthora root rot, due to *Phytophthora nicotianae* Breda de Haan [syn. *P. nicotianae* Breda de Haan var. *parasitica* (Dast.) Waterh.] and *P. citrophthora* (Smith and Smith) Leonian, is the most widespread and severe citrus disease in Southern Italy. The pathogen is generally present in nurseries, where soil pots containing the survival propagules are putative responsible for their spread in new orchards. This investigation aimed at evaluating *Phytophthora* species and the population dynamics on feeder roots and in the rhizosphere of citrus seedlings of nurseries located in Basilicata, Calabria and Sicily (Italy). Soil and root samples were collected at a three-month interval from September to June from Sour orange, Alemow, Volkameriana lemon and Carrizo citrange rootstocks. The number of propagules of *Phytophthora* spp. and the percentage of infected feeder roots were determined by using the plate dilution method with selective media. *Phytophthora* isolates were grouped according to their morphological characteristics and identified on the basis of the ITS regions of the rDNA. In three nurseries, *P. nicotianae* was the only species detected; both pathogens, *P. nicotianae* and *P. citrophthora*, were recovered from the fourth nursery (Basilicata). Seasonal variation in pathogen propagules in feeder roots and soil pots fluctuated according to the nursery, the rootstocks and the environmental conditions, showing higher values in springtime. The results are briefly discussed in relation to the rootstocks and to the disease management.

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Application of *Metschnikowia fructicola* for the integrated control of postharvest diseases of citrus in commercial packinghouses

P. Di Primo[1], M. Coniglione[1], M. Lazare[2], M. Keren-Zur[2], A. Bercovitz[2], D. Blachinsky[2], A. Husid[2], V. Bonaccorso[3]

[1] *DECCO-CEREXAGRI ITALIA srl, a company of United Phosphorus Limited (UPL) Group, Bivio Aspro Zona Industriale 95040 Piano Tavola (Catania), Italy; pietro.di-primo@cerexagri.com*

[2] *Agro Green-The Biological Division of Minrav, Ashdod, Israel 77101, Fax: +97288633120; info@agrogreen.co.il*

[3] *Agronomist*

Green mold of citrus, caused by *Penicillium digitatum* is one of the most economically important post harvest diseases of citrus worldwide. The common commercial treatment used for preventing this disease is the application of a fungicide, *e.g.* Imazalil and/or Thiabend-azole. Pesticide residues on citrus fruits are a major concern to consumers and to the fruit packing industry. Public demand to reduce pesticide use, encouraged by greater sensitivity to environmental and health-related issues, has prompted in recent years the development of biological control alternatives against postharvest diseases of various fresh commodities. “SHEMER” is a biofungicide based on the yeast *Metschnikowia fructicola* registered on citrus in Israel. The effectiveness of SHEMER, in controlling *P. digitatum* and *P. italicum* of citrus fruits during postharvest storage, was evaluated in commercial packing houses. SHEMER was applied to the citrus fruits by spraying or dripping the yeast suspension through a nozzle or dripping system fitted on the line on a bed of brushes. In all trials, treatment with SHEMER significantly decreased the level of decay in stored orange, mandarin and grapefruit fruits when compared with the non-treated control. The use of SHEMER offers a feasible control treatment which: (1) is effective against a wide range of pathogens; (2) is easily implemented in organic, conventional and integrated control systems; (3) enables a significant reduction in toxic chemicals input; and (4) offers an additional tool for resistance management strategies,. A review is presented of the results obtained to date in the commercial test trials, product application technology, and future outlook.

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New or re-emerging fungal Citrus diseases in the Mediterranean

Francesco Maria Grasso[1], Patrizia Bella[1-2], Salvatore Grasso[1] and Antonino Catara[1]
[1] *Dipartimento di Scienze e Tecnologie Fitosanitarie, Facoltà di Agraria, Università degli Studi di Catania, Via S. Sofia 100, 95123 Catania. Italy*
[2] *Parco Scientifico e Tecnologico della Sicilia, Z.I. Blocco Palma I, 95100 Catania. Italy*

Abstract: In recent years, the spread to new Mediterranean areas of citriculture with its new cultural practices, new citrus varieties and a changing climate, has led to the need to cope with new or re-emerging fungal plant diseases. The most notable are ‘greasy spot’ and ‘alternaria spot’. A few papers have been published on this topic, but little attention has been given to them. For the last five years, many Italian orchards have been conspicuously dropping mature leaves affected with greasy spot to their undersides, which may develop groups of peritecia carrying asci which are morphologically similar to the *Mycosphaerella* genus. Potato agar cultures of the symptomatic mesophyll slowly grow greenish-brown colonies, bearing erratically multiseptate conidia, similar to the genus *Cercospora*. Some citrus species are more susceptible and may require appropriate spraying once the biological cycle of the fungus is defined. Only one out of four Alternaria diseases occurs frequently – the mandarin Alternaria brown spot, which is becoming more and more diffuse in many cultivars in Italy and Spain, damaging the leaves and fruit of mandarin hybrids despite frequent chemical spraying. Septoria spot is less common in Sicily and Calabria, where symptoms occur on the fruit and leaves of lemon and bergamot. Anthracnose is an old disease affecting citrus twigs, leaves and fruit and is caused by a primary fungus coloniser of injured and senescent tissue in the field and usually does not require spraying.

Key words: greasy spot, *Mycosphaerella* sp., *Alternaria alternata* pv. *citri*, *Septoria* spp., *Colleto-trichum gloesporioides*

Introduction

In recent years, the spread of citriculture to new areas, new cultural practices, the introduction of new cultivars and changes in climate, have led to a fight against new or (re)-emerging fungal diseases requiring unusual repeated treatments. The most significant are greasy spot (*Mycosphaerella* sp.) and Alternaria spot (*Alternaria alternata* (Fr.) Keissl. pv. *citri*). Septoria spot (*Septoria* spp.) is less common in Sicily and Calabria, where symptoms occur on the fruits and leaves of lemon (*C. limon* Burm.) and bergamot (*C. bergamia* Risso et Poit.). *Colletotrichum gloesporioides* Penz. is the agent of an old disease affecting citrus twigs, leaves and fruits. The economic significance of these diseases, their diagnosis and control measures are reported in this paper.

Material and methods

During surveys carried out in many citrus orchards at different times of the year, diseased leaves, twigs and fruits were collected and laboratory tested to make a diagnosis based on symptoms and laboratory investigation: moist chambers, isolation on artificial medium of probable microrganisms, their identification and pathogenicity.

Results and discussion

Our research ascertained the presence of the following diseases which are here reported on the basis of their frequency.

***Greasy spot* – Mycosphaerella sp.**

This disease caused by a fungus of the genus *Mycosphaerella* (Grasso *et al*., 2005), produces leaf and fruit lesions and defoliates trees, resulting in lower yield and fruit size. The spots appear yellow initially, then turn dark and appear slightly raised and greasy. With severe infection, leaves may turn yellow and drop prematurely. Affected leaves are mainly those located close to the soil. Symptoms are initially observed in early summer, like single or grouped black spots and become more marked in autumn-winter, including the leaf drop. Affected leaves show dark spots, mainly located along the lower veins and edges, and small (less than 5 mm in diameter) chlorotic and dark blotches on the upper sides (Fig. 1A). The fruit symptoms reported in Florida (Timmer et al., 2000) and Japan (Tanaka & Jamada, 1952) are unheard of in Italy. Sweet orange [*C. sinensis* (L.) Osbeck], lemon, grapefruit (*C. paradisi* Macf.) and Fortune mandarin (*C. clementine* x *C. reticulata*) are the most susceptible. In Italy, the disease has been reported since 1938 (Ruggeri, 1935), and was subsequently associated to ‘Greasy Spot’ (Grasso & Catara, 1982) even if its parasitic aetiology has only been recently confirmed as *Mycosphaerella* (Fig. 1B). In Japan this disease is attributed to *M. horii* (Tanaka & Jamada, l952) and in Florida to *M. citri* (Whiteside, 1970).

***Alternaria brown spot* – Alternaria alternata *pv.* citri**

Alternaria brown spot attacks young fruit, leaves and twigs producing small brown-to-black spots surrounded by a yellow halo after a 24 - 36 hr incubation period (Fig. 1C). The leaves may drop or the entire shoot may die (Fig. 1D). On fruits, symptoms include light brown, slightly depressed spots to circular dark brown blotches. Infected young fruits often fall and the mature fruits are unmarketable due to lesions, resulting in important economic losses (up to 80 %) (Fig. 1E). The causal agent was originally described as *Alternaria citri* Pierce and later renamed *A. alternata* pv. *citri* (Pegg, 1966). In Italy, on Fortune mandarin, the disease was reported by Bella *et al*., 2000. Two main pathotypes are described: "tangerine" and "rough lemon" according to the host plant (Peever *et al*., 1999).

***Septoria spot* – Septoria spp.**

Septoria spot (*Septoria* spp.) symptoms include both small rusty spots and large depressed areas on fruits, and small (few mm in diameter) depressed and round brown spots with a dark halo and an inner clear area on the leaves (Fig. 1F). Lemon (Grasso & La Rosa, 1983) and bergamot (Agosteo, 2002) are the most affected citrus species.

***Anthracnose* – Colletotrichum gloesporioides**

Antrachnose is commonly found on mal secco affected trees. Symptoms include very small pin (acervuli, fruiting bodies) in concentric rings on the twigs (Fig. 1G), dry, depressed, rounded and dark areas on the fruits, and dark gray or brown necrotic areas, of variable size (5 mm or more) with clear cut edges on the leaves. The causal agent is *Colletotrichum gloeosporioides*, a weak pathogen, that occasionally is responsible for heavy yield losses (Grasso, 1981) (Fig. 1H).

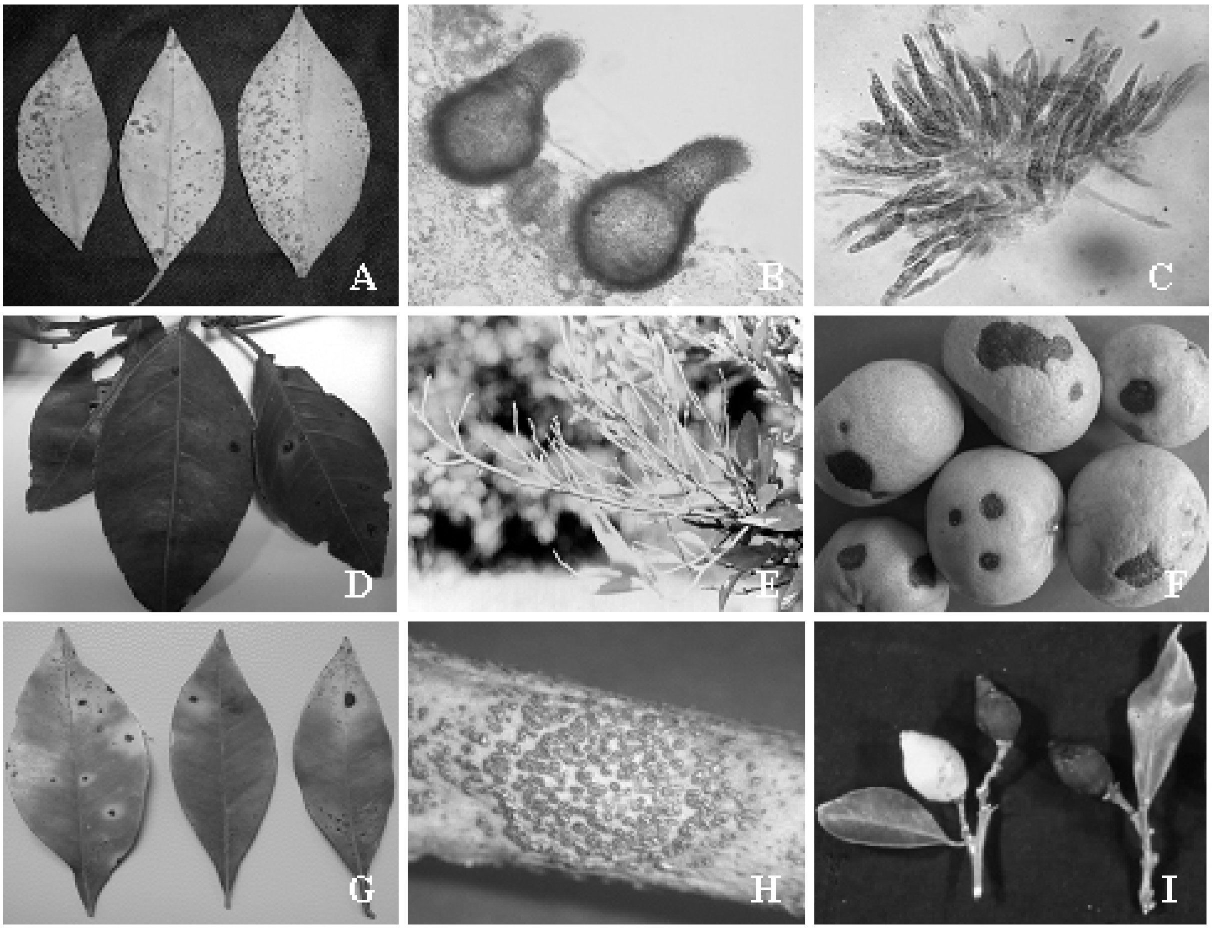

Figure 1. (A) Greasy Spot on sweet orange leaves; (B) Peritecia of *Mycosphaerella* sp.; (C) Asci of *Mycosphaerella* sp.; (D) Alternaria Brown Spot on sweet orange leaves; (E) Twig dieback caused by *Alternaria alternata* pv. *citri*; (F) Alternaria brown spot on Fortune mandarin fruits; (G) Septoriosis on sweet orange leaves; (H) Acervula of *Colletotrichum gloesporioides* on lemon twig; (I) Anthracnose on young lemon fruits.

Conclusions

In recent years, these diseases have shown significant outbreaks in many citriculture areas. *Mycosphaerella* sp. and *Alternaria alternata* both need more than one chemical spraying. The distinctive character of each disease, based on the evaluation of both leaf and fruit symptoms, are crucial in defining spray timing. Fungicides like Fenbuconazole and Propamocarb are successfully utilized in the U.S.A. as well as copper compounds and/or mineral oils. Some of them are currently being evaluated in Italy, but copper compounds are currently the only ones allowed by Italian pesticide regulations to prevent the spread and infection of pathogens. Since the disease is strictly related to climate, the treatments may in some circumstances be ineffective. In highly humid conditions the choice of resistant cultivars may be essential for quality fruit citriculture in the Mediterranean area. The symptoms of the four diseases are summarized in table 1.

Table 1. Symptoms description for a differential diagnosis.

Symptoms	Greasy spot	Alternaria brown spot	Septoria spot	Anthracnose
Leaves				
Side of leaves	both	both	both (up)	both
Type of lesion	raised	flat	raised	flat
Yellow halo	+	+	+	–
Color of spot	yellow-brown	brown	black	grey -brown
Size of lesion (mm)	1-4	variable	1-4	variable
Necrosis along the vein	–	+	–	–
Leaf drop	+	+	+	–
Twigs				
Lesion on twigs	–	+	–	+
Size of lesion (mm)	/	1-10	/	variable
Fruits				
Type of lesion	–	depressed	depressed	depressed
Colour of lesion	–	dark speck to large black lesions	light tan with reddish brown margin	brown
Size of lesion (mm)	–	1-10	1-2	15
Fruit drop	–	+	–	–

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Effectiveness of acetic and peracetic acid to control Penicillia agents of postharvest decay of citrus

C. Oliveri, A. Bonaccorsi, V. Coco
Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via S. Sofia 100, 95123, Catania, Italy; Fax: 0957147287; c.oliveri@unict.it

Six isolates of *Penicillium digitatum* and seven of *P. italicum* recovered from citrus fruit and the air and surfaces in a packinghouse were characterised for pathogenicity on different fruit species as well as for spore germination and sensitivity to imazalil, benomyl, acetic (AA) and peracetic acid (PAA). Nine strains from rotten fruit were highly pathogenic to oranges, lemons, apricots, pears and grapes, whereas both the reference strains as well as two strains isolated from surface and air showed different hosts and aggressiveness. Conidia germination was also highly variable between them, showing a percentage from 2-46% after six hours. In plate agar tests, two isolates of *P. italicum* and three of *P. digitatum* were resistant to benomyl (>100 ppm) and imazalil (>1 ppm). In order to evaluate the effectiveness of AA and PAA as alternatives to fungicides, further tests on conidia germination were performed at different rates of a.i. (0.1, 0.5 and 3%). Regardless of strain, six hours after treatment with 3% AA conidia germination was inhibited completely, whereas the other concentrations affected germ tube elongation or only partially inhibited germination. PAA was much more active than AA: the lowest PAA concentration (0.1%) totally inhibited *P. digitatum* conidia germination, but one isolate showed some germ tube starting at 0.1% concentration. Citrus fruit treated with AA 3% showed reduction of decay incidence comparable to fungicide sprays. Dipping was effective for suppressing fungal decay but peel browning was recorded. When fruits were sprayed with 2% AA and dried at 35°C, no browning was observed. PAA application at the concentration of 2 or 3% was not phytotoxic. Results confirm that Penicillia isolates vary widely in pathogenicity, germination energy, resistance to common fungicides, and that acetic and peracetic acid are very effective in controlling agents of citrus postharvest disease and could be a powerful weapon to be exploited.

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Host-pathogen interaction phenotype in citrus seedlings inoculated with *Phoma tracheiphila*

M. Russo[1], F.M. Grasso[1], G. Licciardello[1,2], V. Catara[1]
[1] *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, via S. Sofia 100, 95123 Catania, Italy*
[2] *Parco Scientifico e Tecnologico della Sicilia, Blocco Palma I, Zona Industriale, Catania, Italy; vcatara@unict.it*

The citrus Mal secco disease causal agent *Phoma tracheiphila* is an established quarantine pathogen in several Mediterranean countries which produces severe damage and restricts commerce. From the symptoms of naturally infected or experimentally inoculated plants it would seem all citrus are susceptible but their phenotypes present different modifications. Therefore, an investigation was carried out to identify the possible relationship between colonisation of the fungus and phenotype response. Seedlings of lemon ('Femminello' S.Teresa e Siracusano), mexican lime, 'Hamlin' sweet orange, Troyer citrange and *Poncirus trifoliata* were inoculated in the root and leaves with a suspension of fungus phialoconidia (10^6 conidia ml^{-1}). Disease severity was evaluated by their symptoms (2 empirical scales with values 0 – 4). Fungus concentration within tissues was evaluated by real-time PCR. For the leaf inoculations, all the species except *P. trifoliata* showed chlorotic haloes and/or vein chlorosis at the inoculation point, 7 days after inoculation. The percentage of infection after 14 days after inoculation ranged between 11% for *P. trifoliata* and 61% for Siracusano lemon. The mean disease index varied between 0.66 in *P. trifoliata* and 2 for S. Teresa lemon. For root infections, the first infection symptoms were observed 14 days after inoculation in *P. trifoliata* and 21 days after in the other species. The mean disease index after 28 days, varied between 1.2 for Hamlin sweet orange and 2.7 for S. Teresa lemon. The fungus was detected by real-time PCR at different concentrations both in the leaf and root tissues even before any symptoms were evident. The fungal DNA concentrations obtained by reaction varied approximately by 0.1 – 1.0 ng.

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Colonization of *Fusarium solani* in Troyer citrange seedlings

S. Spina[1,2], V. Coco[1], A. Gentile[3], A. Catara[1,2], G. Cirvilleri[1]
[1] *Dipartimento di Scienze e Tecnologie Fitosanitarie- Sezione Patologia Vegetale, Università degli Studi di Catania,Via S.Sofia 102,95100 Catania, Italy;*
[2] *Parco Scientifico e Tecnologico della Sicilia S.c.p.a, Viale V. Lancia Z.I., Catania;*
[3] *Dipartimento di Ortofloroarboricoltura e Tecnologie Agroalimentari – Sezione Arboricoltura, Università di Catania, Via Valdisavoia, 5, Catania.*

Abstract: A research was carried out to investigate whether the association with the *rol*ABC genes of *A. rhizogenes* could modify the susceptibility of Troyer citrange to *Fusarium solani*, the causal agent of dry root rot. *F. solani* strain 1A was inoculated in cuttings and leaves of Troyer citrange seedlings modified by *rol*ABC genes and wild type (WT). Interveinal chlorosis of leaf, wilt and defoliation, observed both on *rol*ABC and WT were more severe on transgenic line. Two months after root inoculation with the pathogen, root weight was significantly reduced in *rol*ABC seedlings, but no root rot was recorded. *F. solani* was always reisolated from artificially inoculated cuttings, midribs and roots without differences between wild types and transgenic lines. The pathogen was never recovered from not inoculated plant tissues. Cell-free fungal culture filtrates induced leaf wilt and defoliation within 10 days from inoculation, without any significant difference between *rol*ABC and WT shoots. Scanning electron microscopy (SEM) of infected roots, carried out at 3 and 6 days after inoculation, showed the penetration and colonization of the hyphae. Evidence is presented that *F. solani* infects Troyer citrange tissues without differences between wild type and transgenic lines in the early phase of colonization and in absence of visible specific disease symptoms. The tests here described could be used to evaluate rootstocks sensitivity to the pathogen.

Key words: pathogen inoculation; SEM; transgenic plants

Introduction

Traditionally the sour orange was the sole citrus rootstock used in Italy, but since 1970s it is going to be replaced with Troyer and Carrizo citranges for their resistance to the citrus tristeza virus. However, in 1990s a tree decline developed into a devastating form in some citrus groves in which citrange rootstock was used (Polizzi *et al.,* 1992; Ippolito and De Cicco, 1995). From the discoloured wood of the trees affected were constantly isolated colonies of *Fusarium solani* (Mart.) Sacc., which is a common species in the citrus rhizosphere. Isolates of the fungus can be categorised as mild, intermediate and severely pathogenic, with the latter causing extensive root rot and scorching symptoms associated with the production of phytotoxins (Strauss and Labuschagne, 1995).

The damage caused in citrus orchards by *F. solani* is probably underestimated. The fungus is able to establish a symptomless infection in Troyer citrange roots and, under stress conditions, it is responsible of a severe root rot, called “dry root rot” to distinguish it from the most common *Phytophthora* foot rot. Dry root rot cankers in fact, unlike *Phytophthora*-induced lesions, usually do not ooze gum. The most conspicuous symptom above the ground is a fatal collapse of the tree; the leaves wilt suddenly and in a few days dry up, remaining attached on the tree. Normally, however, the course of the disease is chronic and symptoms resemble those of other root rots (e.g. *Phytophthora*, *Armillaria*, rodent damage, etc.). The symptoms include twig dieback, chlorosis of main leaf-veins, yellowing and drop of the leaves (Fawcett, 1936; Klotz *et al.*, 1967; Bender *et al.*, 1982; Menge, 1988).

F. solani is known as one of the components contributing to citrus decline. The fungus, in fact, often acts in association with other pathogens such as the citrus nematode *Tylenchulus semipenetrans* and *Phytophthora*, resulting in increased disease severity compared to the individual pathogens acting alone.

Studies conduced by Menge and Nemec (1997) have demonstrated that all citrus rootstocks are susceptible and none is immune to *F. solani* infections, and these results support the field observation made by Klotz (1973) in California that all common citrus rootstock cultivars were susceptible to dry root rot caused by *F. solani*. Control measures against Fusarium root rot comprise elimination of conditions that cause stress in the trees and use of resistant rootstocks (Labuschagne *et al.*, 1996). Biological control practices and use of antagonistic microorganisms (Nemec *et al.*, 1996; Lim *et al.*, 1991; Cirvilleri *et al.*, 2005) may be considered as promising strategies against citrus root diseases both in greenhouse and open field.

Taking into account the difficulties of conventional breeding, genetic transformation appears to be a promising technique for citrus, allowing specific characters to be inserted into good genotypes without affecting other traits (Peña and Navarro, 1999). Recently, Troyer citrange plants have been transformed with *rol*ABC genes from *Agrobacterium rhizogenes* to modify the growth habit of plants (Gentile *et al.*, 2004; La Malfa *et al.*, 2004). The *rol*ABC genes are involved in phytohormone balance of the plants altering plant morphology, inducing dwarfing, increased rooting, altered flowering, wrinkled leaves and/or increased branching (Christey, 2001), and increasing the sensitivity of transgenic tissues to both cytokinins and auxins (Estruch *et al.*, 1991a,b). These phenotypic and physiological alterations may have potential applications for plant improvement and significant effects on plant-pathogen interactions (Cirvilleri *et al.*, 2005).

There are only a few published reports of any pathogen resistance modification of plants transformed with genes involved in phytohormone balance. Fladung and Gieffers (1993) observed an increase of susceptibility to *Alternaria alternata*, *Botrytis cinerea* and *Erwinia carotovora* subsp. *atroseptica* of *rol*C transgenic potato leaves, whereas tubers displayed an enhanced resistance to pathogens and a positive correlation of the infection level with glucose, dry matter and starch content. Bettini *et al.* (2001) found a higher resistance to *Fusarium oxysporum* f.sp. *lycopersici* in tomatoes plants harbouring *rol*ABC genes, suggesting that physiological modifications induced by the *rol* genes, and especially the endogenous hormone balance, can positively affect the plant defense response. Balestra *et al.* (2001) found increased susceptibility to *Pseudomonas syringae* pv. *syringae* and *P. viridiflava* of *rol*ABC kiwi plants, strictly correlated with a raised nitrogen content in the leaves.

The aim of our research was to investigate whether the association with the *rol*ABC genes of *A. rhizogenes* could modify the susceptibility of Troyer citrange plants to *F. solani*. For the purpose, various inoculation methods were used to performe pathogenicity tests in plant cuttings, leaf-midribs and roots. In all the assays, colonization of *F. solani* strain 1A in the inoculated tissues as well as symptoms development were monitored. Moreover, culture filtrate of the pathogen was used to evaluate the phytotoxic activity on cuttings of Troyer citrange WT and *rol*ABC. Scanning electron microscopy (SEM) observations of infected roots were also carried out.

Materials and methods

Plant material and growth conditions

Wild type (WT) and transgenic *rol*ABC Troyer citrange plants were used in these studies. Seedlings were planted in 2L plastic pots in a pasteurized (80°C for 1h) soil:sand:peat (1:1:1) medium. Plants were grown in a conditioned greenhouse at a temperature of 24±2° C during the day and 18±2° C during the night. Natural light was reduced by 25% covering the greenhouse with a black net.

Preparation of inoculum

Fusarium solani 1A used in this study was isolated from symptomatic Troyer citrange roots in Sicily, single-spored, cultured on potato-dextrose agar (PDA, Oxoid) at 25° C for 7 days, and identified according to Balmas *et al.* (2000). Conidial suspension in sterile distilled water (SDW) was adjusted to a concentration of 10^6 conidia ml^{-1}.

Inoculation of plant cuttings

Shoots 15cm long with 5 to 8 leaves, taken from three-year-old WT and *rol*ABC plants were plunged in 10ml of conidial suspension of *F. solani* 1A and kept at an average temperature of 26° C for 14 days after inoculation. The experiments were carried out five times on ten shoots of each line at time. Shoots plunged in SDW were used as a control.

Colonization of *F. solani* was monitored on longitudinal cross-sections of inoculated *rol*ABC and WT cuttings. Three sections 5mm long (lower, middle, upper) were surface sterilized for 30'' in a 10% sodium hypochlorite solution, rinsed twice in SDW, plated on PDA enriched with streptomycin sulphate (25mg ml^{-1}, Sigma) and 25% lactic acid, and incubated at 25° C in the dark. Results were recorded as percentage of pathogen colonized tissue sections. Not inoculated cuttings were used as control.

Inoculation of leaf-midribs

Shoots 15cm long, with 5-8 leaves, taken from three-year-old WT and *rol*ABC plants, were plunged in SDW and leaves midribs wound-inoculated with *F. solani* 1A conidial suspension. Cuttings were kept at an average temperature of 26°C for 14 days. The experiments were carried out five times on thirty leaves of each line at time.

F. solani was monitored on portions of leaf midribs from inoculated *rol*ABC and WT leaves. Two sections of 5mm x 3mm (inoculation point and 1cm upper) from inoculated *rol*ABC and WT midribs were surface sterilized, rinsed twice in SDW, plated on PDA enriched with streptomycin sulphate and 25% lactic acid, and incubated at 25° C in the dark. Results were recorded as percentage of pathogen colonized tissue sections. Not inoculated leaves were used as control.

Data analysis

In all replicated experiments, disease severity ratings of inoculated cuttings and midribs were recorded 14 days after inoculation based on a 1 to 5 scale, where 1= no symptoms; 2= light symptom development (interveinal chlorosis/partial defoliation; 1-20% foliage affected); 3= moderate symptom development (interveinal chlorosis/partial defoliation; 21-50% foliage affected); 4= heavy symptom development (yellowing of the leaves/partial defoliation; 51-80% foliage affected); 5= severe symptom development (wilt/browning/total defoliation; 81-100% foliage affected). Data were converted to percents of mid-values (Hartman *et al.*, 1997), where severity rating of 1= 0%, 2= 10%, 3= 35%, 4= 65% and 5=90%. Percentage values were converted in arcsen values, before performing analysis of variance (ANOVA) by COSTAT software. Means were supported by least significant difference at P= 0.05.

Inoculation of roots

Three-month-old WT and *rol*ABC Troyer citrange seedlings, grown in steamed soil-sand-peat (1:1:1) medium, were inoculated by dipping the roots in a conidial suspension of *F. solani* 1A. Seedlings dipped in SDW were used as control. All plants were replaced in their pots and maintained in a greenhouse at a 18/26° C night/day temperature regime under normal daylight photoperiod (14h light; 10h dark). Pots were thoroughly watered after planting and left standing for 30 min to allow access water to drain. Plants were watered every second day. The experiments were carried out twice on ten seedlings of each line at time.

Two months after inoculation, random feeder root samples were collected (two replicates for each plant) and dilution series were plated using a Spiral Plater Eddy Jet (IUL Instruments, Barcelona) on PDA enriched with streptomycin sulphate and 25% lactic acid. Fungal colonies were counted, microscopically examined and the number of total fungi and *F. solani* propagules determined. Mean values of cfu g^{-1} of fresh weight were converted in log values, before performing analysis of variance (ANOVA) by COSTAT software. Means were supported by least significant difference at P= 0.05.

Plant height, root weight and root length were also evaluated and symptom severity ratings on the foliage were recorded as described above. Root rot was visually estimated.

Inoculation with culture filtrates

F. solani 1A was grown in Czapek-Dox medium ($NaNO_3$, 3g/l; K_2HPO_4, 1g/l; $MgSO_4{\bullet}7H_2O$, 0.5g/l; $FeSO_4{\bullet}7H_2O$, 0.01g/l; Sucrose 30g/l; Yeast extract, 1g/l) to stimulate toxins production (Tatum and Baker, 1983). Cultures were incubated in the dark at 25° C on a reciprocal shaker (67 oscillations min^{-1}) and after 7 days were strained through four layers of sterile cheesecloth. Filtrates were centrifuged for 30 min at 2800 rpm, passed through a Millipore filter (HA, 0.45 micron pore size) and stored at -20° C.

For phytotoxicity bioassay, cell-free culture filtrates were diluted 1:1 with 1mM KH_2PO_4. Cuttings of three-months-old WT and *rol*ABC Troyer citrange seedlings were plugged in 5ml of 1:1 diluted culture filtrate and incubated at an average temperature of 26° C under a 12h photoperiod. The experiments were carried twice on ten cuttings of each line at time.

Symptom severity ratings were recorded 3 to 10 days after inoculation as described above. Percentage values were converted in arcsen values, before performing analysis of variance (ANOVA) by COSTAT software. Means were supported by least significant difference at P= 0.05.

Scanning electron microscopy (SEM)

Infection process of the pathogen into inoculated root system was observed at 3 and 6 days after inoculation. Root pieces, taken from inoculated and not inoculated WT and *rol*ABC seedlings, were fixed in a 2% glutaraldehyde solution in 0.1M sodium cacodylate buffer (pH 7.4) with subsequent rinsing (20 min) in the same buffer. The samples were dehydrated in a series of 50% 70% 90% and 100% ethanol, for 20 min at each concentration, critical-point dried in CO_2, mounted on standard copper stubs with silver paint, sputter-coated with gold and examined with a scanning electron microscope (DSM 940A, Zeiss) (Labuschagne *et al.*, 1987).

Results

Inoculation of plant cuttings and leaf midribs

F. solani 1A was re-isolated from sections (lower, middle, upper) of stem tissue and from portions of leaf midribs (inoculation point and 1cm upper) without significant differences (P=0.05) between *rol*ABC and WT lines (Tab. 1). *F. solani* 1A was never recovered from not inoculated cuttings and leaves (data not shown).

Interveinal chlorosis, wilt and defoliation occurred on inoculated cuttings and leaves of *rol*ABC and wild type Troyer citrange within 14 days after inoculation. Disese severity ratings were significantly different (P=0.05) between transgenic and WT plant cuttings and leaves, with *rol*ABC line more sensitive to the pathogen than the WT (Tab. 2). Control cuttings and leaves of *rol*ABC and WT lines never showed any symptom (data not shown).

Table 1. Percentage of infected cuttings and leaf midribs segments in *rol*ABC and WT Troyer citrange lines in replicated growth-chamber experiments 8 days after inoculation with *F. solani* 1A.

		Infected tissues (%) [x]	
Inoculated tissues	**Portions**	***rol*ABC**	**WT**
Cuttings	Lower	87.5 a	93.8 a
	Middle	62.5 a	56.3 a
	Upper	56.3 a	50.0 a
Leaf midribs	Inoculation point	75.0 a	83.5 a
	Upper	30.0 a	48.5 a

[x] Values on the same line followed by the same letter do not differ significantly from one another according to Student-Newman-Keuls test (P=0.05). The test was performed on previously transformed data arc sen √ %. Values are means of 5 experiments.

Table 2. Disease severity ratings of *rol*ABC and WT Troyer citrange cuttings and leaf midribs in replicated growth-chamber experiments 14 days after inoculation with *F. solani* 1A.

	Disease severity ratings (%) [x]	
Inoculated tissues	***rol*ABC**	**WT**
Cuttings	37.4 b	26.8 a
Leaf midribs	64.1 b	10.0 a

[x] Values on the same line followed by the same letter do not differ significantly from one another according to Student-Newman-Keuls test (P=0.05). The test was performed on previously transformed data arc sen √ %. Values are means of 5 experiments.

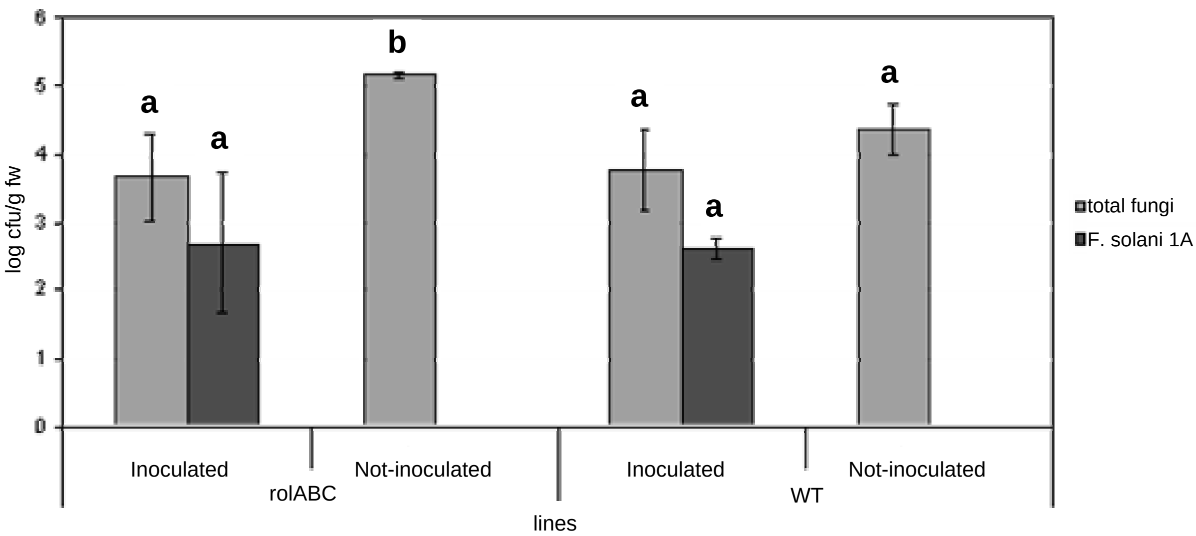

Figure 1. Total fungi and *F. solani* 1A population on roots of *rol*ABC and WT Troyer citrange seedlings in replicated growth-chamber experiments 2 months after root-inoculation with *F. solani* 1A. Values are means of eight replications (two replications for each plant of each line for two experiments). Student-Newman-Keuls test (P=0.05) was performed on previously transformed data arc sen √ %.

Inoculation of roots

F. solani 1A was consistently re-isolated from inoculated roots without significant difference between *rol*ABC (log 2,70) and WT (log 2,61) lines (P=0.05). Population of total fungi was reduced in roots inoculated with the pathogen. *F. solani* 1A was never recovered from not inoculated roots (Fig. 1).

Reduction of stem height, root length and root weight was observed two months after *F. solani* 1A inoculation, without significant differences between *rol*ABC and WT lines. Root weight was significantly reduced only in *rol*ABC seedlings. Wilting and defoliation symptoms were significantly more severe on WT than on *rol*ABC seedlings (Tab. 3). No root rot was recorded.

Table 3. Mean values of stem height, root length and root weight and mean severity ratings of *rol*ABC and WT Troyer citrange seedlings in replicated growth-chamber experiments 2 months after root-inoculation with *F. solani* 1A.

Plant line	***rol*ABC**		**WT**	
	Inoculated	*Not-inoculated*	*Inoculated*	*Not-inoculated*
Stem height (cm) [x]	6.5 a	10 a	8.3 a	8.2 a
Root length (cm) [x]	8.1 a	10.5 a	11 a	13.7 a
Root weight (mg) [x]	34 a	133 b	27 a	41 a
Disease severity ratings (%) [x]	12.5 a	0	62.5 b	0

[x] Values on the same line followed by the same letter do not differ significantly from one another according to Student-Newman-Keuls test (P=0.05). Values are means of 2 experiments.

Inoculation with culture filtrates

Wilt and defoliation occurred on inoculated cuttings of *rol*ABC and WT Troyer citrange within 10 days after inoculation, without differences (P=0.05) between *rol*ABC and WT (Fig. 2). Control cuttings did not show any symptom (data not shown).

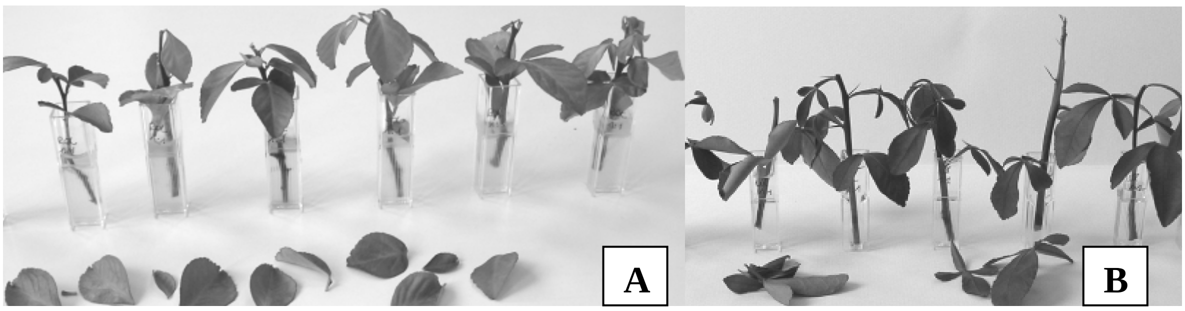

Figure 2. Phytotoxicity of culture filtrates of *F. solani* 1A on Troyer citrange *rol*ABC (A) e WT (B) plant cuttings, 10 days after inoculation.

Scanning electron microscopy (SEM) of infected roots

SEM observation of colonization by the fungus did not show any difference between *rol*ABC and WT Troyer citrange roots 3 and 6 days after root inoculation (Fig. 3A, B, C, D). Penetration into and exit from the root occurred at random. Some hyphae could be seen growing superficially along the root surface.

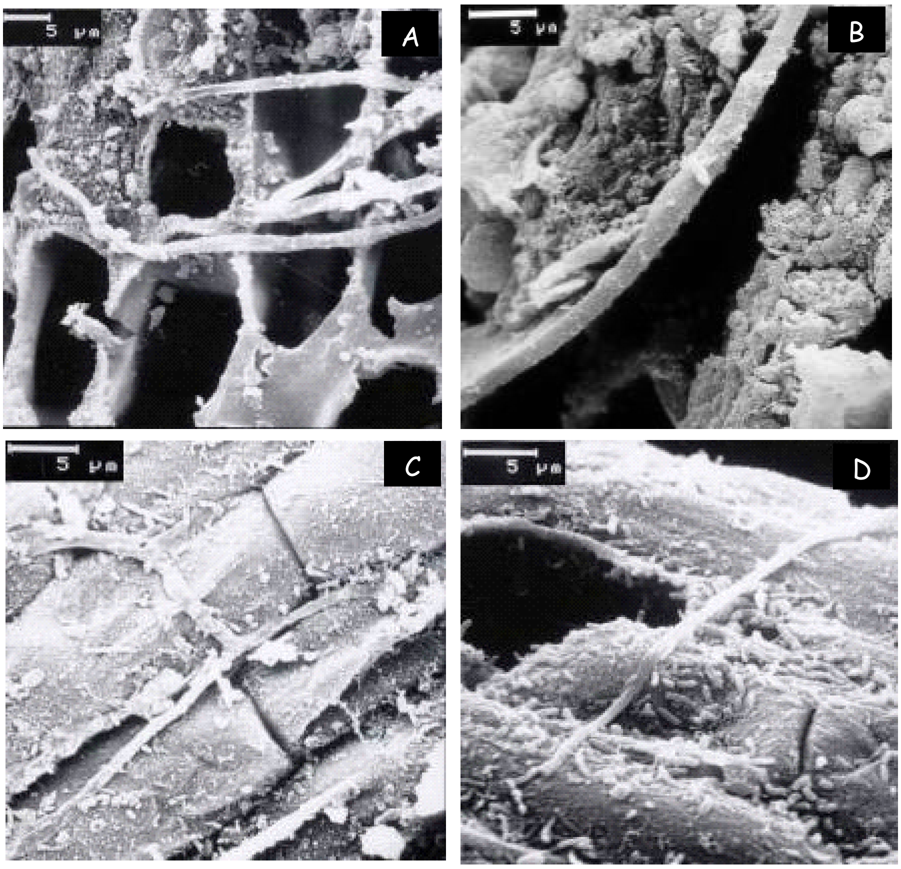

Figure 3. Scanning electron microscopy of citrus roots colonization by *F. solani* 1A. Hyphae colonize the surface of feeder *rol*ABC (A,B) and WT (C,D) roots 3 (A,C) and 6 (B,D) days after the inoculation.

Discussion

Citrus species are subjected to many biotic stresses and the development of disease-resistant plants represents a high priority goal for breeding programme. This is particularly important for Troyer citrange, seriously damaged by *Fusarium solani* infection (Peña and Navarro, 1999). *F. solani* isolates vary in pathogenicity from non-pathogenic to severely pathogenic. In the absence of stress, mild isolates of the fungus is able to colonize healthy roots establishing a symptomless infection (http://www.up.ac.za/academic/fabi/citrus/rootrot.html).

Moreover, *F. solani*'s often reported inability to produce visible disease symptoms in pathogenicity tests (Graham *et al.*, 1985; Dandurand and Menge, 1993) can possibly be ascribed in part to the methods of inoculation. Root inoculation with *F. solani* conidia has been reported and root rot and wilting of plants have been observed 36-60 h after inoculation (Nemec *et al.*, 1986). In soil inoculated with conidial suspensions, root length and root weight were reduced, but no root rot was recorded (Nemec *et al.*, 1981; Dandurand and Menge, 1993). Plants developed wilting and scorching symptoms on their shoots as well as severe root rot using millet seed inoculum of *F. solani* (Strauss and Labuschagne, 1995).

In the present study, pathogen was re-isolated from the inoculated tissues in all the experiments, without significant differences between *rol*ABC and WT, this suggesting that the colonization ability of *F. solani* is not influenced by *rol*ABC gene expression.

Moreover, chlorosis of main leaf-veins, yellowing and drop of the leaves were observed after plant cuttings and leaves inoculation with *F. solani* 1A. In these experiments, significant differences between *rol*ABC and WT lines were recorded with both methods of inoculation, being *rol*ABC more sensitive to the pathogen than the WT.

Reduction of root growth following *F. solani* inoculation has been reported in literature (Nemec *et al.*, 1981; Dandurand and Menge, 1993). Also in the present study, in roots inoculation assay, root weight of *rol*ABC and WT plants was reduced, compared to the not inoculated plants. Moreover, even if root rot symptoms have not been observed, foliage symptoms were recorded, with the WT being more sensitive than the *rol*ABC lines.

Our results indicate that *F. solani* symptom expression as such should not only be dependent on the stress conditions but also on the inoculation methods.

We evaluated the effect of a culture filtrate of *F. solani* 1A on transgenic and WT Troyer citrange cuttings and we observed wilt symptoms, similar to those observed after inoculation of conidia of the fungus and no difference was recorded in symptom development between *rol*ABC and WT lines. Baker *et al.* (1981) reported the production of a variety of phytotoxins by *F. solani* isolates from citrus roots. Naphthazarin toxins are known to have a significant effect on growth of citrus seedlings (Janse van Rensburg *et al.*, 1996) and on growth of roots (Baker *et al.*, 1981). Toxic effects on citrus include also veinal chlorosis, leaf wilt and vessel plugging (Nemec *et al.*, 1988).

It is evident from SEM observations that intact citrange Troyer roots are readily infected and colonized by *F. solani*, even in absence of visible symptoms on roots and on plants. The histopathological observations made in this study are in agreement with those by other researchers in naturally infected (Nemec, 1978) and in artificially infected citrus trees (Labuschagne *et al.*, 1987). Labuschagne *et al.* (1987) produced evidence that *F. solani* establishes a symptomless infection in roots of rough lemon plants, colonizing the epidermal, cortical and vascular tissue without any root rot occurring.

Over all, the research showed that the integration into Troyer citrange plants of *rol*ABC genes, which alter hormone sensitivity and plant morphology, does not significantly affect the susceptibility of young seedlings of Troyer citrange to *F. solani* after root inoculation and culture filtrate test.

Acknowledgements

This work was supported by Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Progetto PON N°12839 "Innovazione tecnologica per il miglioramento delle produzioni e dei processi agroalimentari nelle PMI" cofunded by EU and PRIN prot.2004078990 "Regolazione dello sviluppo e dell'habitus vegetativo di piante coltivate attraverso metodologie transgeniche: valutazione agronomico-molecolare delle interazioni ecofisiologiche ed impatto ambientale".

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IOBC/wprs Bulletin Vol. 38, 2008
pp. 317-324

New phytosanitary scenarios for Mediterranean citriculture post *Citrus tristeza virus* dispersal

Antonino Catara[1,2], Serena Rizza, [1,2], Matilde Tessitori[1]
[1] *Department of Phytosanitary Sciences and Technologies, University of Catania;*
[2] *Laboratory Phytosanitary Diagnosis and Biotechnologies, STP Sicilia, Catania.*

Abstract: Being the most popular rootstock in the Mediterranean, the substitution of sour orange with other rootstocks would seem inevitable to circumvent the devastating effects of *Citrus tristeza virus* (CTV).This will temporarily solve the problem, but the emergence and/or introduction of more virulent CTV strains or more efficient vectors than current ones should also be considered. Moreover, the risk of introducing *Candidatus* Liberibacter species and its relative vectors should not be underrated as should not the risk from *Citrus tatter leaf virus* which is particularly harmful to plants grafted on trifoliate orange and citrange. From South America the risky diseases are ‘blight’, ‘variegated chlorosis’, ‘sudden death’ and ‘leprosis’. Yet to be clarified is the emergence of other virus strains already present in some Mediterranean countries such as ‘yellow vein clearing’ and ‘chlorotic dwarf’ in Turkey, assumed to be associated to vectors, or ‘leaf blotch’, seed transmitted. Problems related to viroids, often masked by the use of sour orange, have to be reconsidered in the case of susceptible rootstocks and because of the recent discovery of new pathogenic viroids. Overall, the introduction of trifoliate and citrange as well as alemow and rough lemon has generated new problems which are frequently underestimated. More notable in loam soils is the “dry root rot” disease associated to *Fusarium* spp., affecting severely the citranges.This new condition strengthens the need to enforce international cooperation among entomologists and plant pathologists to plan valuable strategies to avoid new entries of virus strains and the displacement of pests and diseases, and to define integrated programs of control.

Key words: *Citrus tristeza virus*, Liberibacter, tatter leaf, blight, variegated chlorosis, yellow vein clearing, chlorotic dwarf, citrus leaf blotch, dry root rot.

Introduction

The Mediterranean is the largest citrus producer in the world, about 17 millions tons annually, which is approximately 49% of the global market. Half the yield is for the fresh market, 13% is juice, and 30% is exported to Europe, the world’s number one consumer. Each country’s citriculture has its own peculiarities as regards variety, composition and phytosanitation, but some of them are common and risky.The most widespread is sour orange rootstock (90%), well known for its susceptibility to the destructive citrus tristeza disease, which has encouraged a change to other rootstocks tolerant to the *Citrus tristeza virus* (CTV). Nevertheless, the experience of some countries has shown that introducing trifoliate and citrange as well as alemow and rough lemon has generated often underestimated new problems and nor should the risk be neglected of the introduction of unsuspected strains of CTV or other viruses or bacteria and/or their vectors which could cause the spread of very harmful diseases either to specific rootstocks or to all rootstocks.

This review analyzes the current situation and the potentially dangerous pathogens and vectors to exclude from the Mediterranean and/or preventing the movement. The need for a strong effort of cooperation among entomologists and plant pathologists in Mediterranean countries is emphasized.

The tristeza disease threat to citrus production in Mediterranean countries

For more than one hundred years, citrus tristeza continues to be the most serious citrus disease. Over 100 million plants have died because of CTV, which is now reported in all the citrus Countries. Its ability to develop epidemics through aphids and the severity of the symptoms induced in some conditions has stimulated the search for diagnostic tools and technologies to differentiate the virus strains, as well as to improve the knowledge of the biology and ecology of the virus and its vectors. But the predicament is still unanswered and may give rise to further complications if not faced in a timely and structured way.

CTV has been known in the Mediterranean for over 50 years, both in imported plants and through propagation material introduced from other countries, followed, after some time, by severe epidemics. The epidemics that afflicted Spain, Israel, and recently Italy were widely reported. No case of 'CTV-stem pitting' has ever been reported.

After the shocking epidemics of the sixties, Spain developed intensive research into the dispersal of the disease and virus strains. No doubt the contribution of the Spanish researchers was outstanding and appreciated worldwide. Many different strains were characterized and specific diagnostic tools were developed. After a large eradication program and a change in rootstock, citriculture continues to thrive and the Mediterranean still leads the world. But the virus persists and needs very special surveillance.

In Italy, surveys over the last five years have shown that CTV is diffused in nearly all the citrus areas with variable incidence (Catara and Davino, 2006), with disastrous results when trees are grafted onto sour orange. SSCP analysis has demonstrated that the present population in Apulia is comparable to the mild T-30 strain from Florida, whereas Sicilian isolates are close to the Californian isolates SY568 and SY107 (Davino *et al.*, 2005). Other investigators have for the first time reported some isolates referable to CTV-SY South American strains BaraoB, Val-CB and C271-2 in Sanguinello orange trees grafted on sour orange with symptoms of inverse pitting (Rizza *et al.*, 2007). A genotype of CTV dissimilar to any reference has been recently characterized in Apulia (Barbarossa and Savino, 2006).

Until now, the disease has been spread throughout the Mediterranean by the cotton aphid. But recently, the brown citrus aphid has appeared in some north-western areas of the Iberian Peninsula, not extensively citrus cultivated (Ilharco *et al.*, 2005), which is justifiably provoking fears of greater risks to the citriculture of the entire Mediterranean.

Symptomatology

Citrus tristeza symptoms vary according to the virulence of the strain, the susceptibility of the host, the scion/rootstock combinations, the co-presence of biologically different strains in the same infected plant, environmental conditions, etc. They all share the final outcome of tree collapse, so the disease is considered disastrous for citriculture (Roistacher and Moreno, 1991).

On the basis of both induced symptoms and molecular marker analysis, the CTV strains may be classified as:

- 'mild' (or T30-like), which give asymptomatic infections or slow decline;
- 'stem-pitting' (or VT-like), inducing wood pitting of the trunk, branch or stems of sweet orange and Duncan grapefruit;
- 'seedling yellows' (or T3-like), it causes yellowing in sour orange and dwarfing in sweet orange grafted on sour orange;
- 'decline' (or T36-like), causing quick decline in sweet orange trees grafted on sour orange.

Strain identification and characterization

Progress in strain differentiation and its application to the epidemiology of citrus tristeza disease has been reviewed in many papers (Noblest *et al*., 2000; Hilf *et al*., 2005; Garnsey *et al*., 2005). Nowadays, the more effective systems for the characterization of the virus strains employ: i) monoclonal antibodies in serological tests; ii) gene amplification through RT-PCR and RFLP analysis or molecular hybridization with specific oligonucleotides or SSCP analysis; iii) bidirectional RT-PCR; iv) multiple molecular markers.

Each system concurs in tracing the profile of the strain, but not always being able to predict biological impact, the biological tests still remain fundamental in determining strain virulence.

Epidemiology

Transmission through propagation materials is fundamental in the spread of the virus over long distances (Roistacher and Moreno, 1991). *Toxoptera citricidus* (the brown citrus aphid) and *Aphis gossypii* (the cotton aphid) are the most active species capable of transmitting the virus and play a crucial role in the spread of the disease in any given area. Both acquire the virus in the alimentary canal during an effective alimentary activity (usually at least one hour) and transmit it to other plants during a new feeding phase (at least six hours) (Raccah *et al*., 1989).

In presence of *A.gossypii,* disease levels rise from 5 to 95% over 8-15 years, whereas in the presence of *T. citricidus* it reaches the same levels in 2-4 years (Gottwald *et al*., 1996). The greater mobility of *A. gossypii* gives a punctiform distribution of the infected trees, while *T. citricidus* gives uniform specks (Gottwald *et al*., 2000).

Recent epidemiological studies in California demonstrate that, in areas where an eradication program is active, infections are contained (0.09-0.7%), while in others the annual increment can reach 3.6% (Yokomi and Deborde, 2005).

Managing the disease

Controlling the disease is feasible with an appropriate program. Tolerant alternatives to sour orange, adapted to the various soil and weather conditions, are a viable strategy, provided their susceptibility to other pathogens is taken into account. It should be noted that in many cases, substitution of sour orange by citrange developed new unexpected damage by dry root rot, a disease caused by *Fusarium* spp, mostly *F.solani,* until few years ago disowned or of secondary importance in the Mediterranean region, which severely affect trifoliate, citrange and similar hybrids, as well as other rootstocks, whereas sour orange is rather tolerant.

Cultural, biological, chemical, guided and integrated management strategies for brown citrus aphid control have been suggested (Halbert and Brown, 1996). The pre-inoculation of young plants with purposely selected mild strains may be useful to induce mechanisms of cross protection which could help delay the disastrous effects of the disease of some decade above all, and has given discreet success in the event of CTV-SP. The transfer of the resistant genes found in some species like trifoliate orange to cultivated varieties has limitations regardless of method and still appears far from reliable.

Dangerous infectious diseases not present in the Mediterranean region

Many pathogens and diseases are found outside the Mediterranean and pose a real threat if introduced. Some of them are mostly dangerous to rootstocks other than sour orange - others are destructive regardless of rootstock. Therefore, the spread of CTV resistant/tolerant rootstocks like trifoliate orange, citrange and alemow, as well as rough lemon, as alternatives to sour orange in the fight against the tristeza disease, could cause new problems if appropriate phytosanitary measures are not taken.

The most widespread is citrus ‘blight’, once known as ‘Young Tree Decline’, which has given rise to serious epidemics as a result of substituting sour orange with rough lemon. Today it is present in Brazil, Argentina, Venezuela, South Africa and Australia. The symptoms are first noticed in 6-10 year-old trees as progressive canopy decline with small discoloured leaves, the withering of new shoots and delayed blossoming. As the disease progresses, the main roots and hair roots die causing the death of the plant. All the main citrus cultivars are susceptible, regardless of rootstock. Even trifoliate orange and citrange Carrizo are more susceptible than sour orange. A virus has been isolated from infected trees (Bovè, 2005).

Citrus tatter leaf virus (CTLV) introduced into the United States in plants of Meyer lemon from China, is also found in Japan, whereas it is not in the Mediterranean. On sweet orange plants, grapefruit, Mandarin and lemon grafted on *P. trifoliata* and its hybrids it causes reduced size and bud-union disorder. Sour orange is tolerant.

Among the diseases not dependent on rootstock, the most serious threat is from ‘huanglongbing’ (HLB), nowadays severely affecting citrus cultivation in Florida and Brazil. Originating from China, it is known in Africa as ‘greening’, in Taiwan as ‘likubin’ and in the Philippines as ‘leaf mottling’. Infected plants have yellow leaves with chlorotic spots, canopy yellowing, defoliation and stem wilting. The fruits are small and oval, showing a persistent green (hence ‘greening’). Seeds are aborted and taste sour. The etiological agent is a phloematic bacterium (α-Proteobacteria), not cultivable in vitro, with three known species: *Ca*. Liberibacter africanus, *Ca*. L. asiaticus and *Ca*. L. americanus. It infects all the species and cultivars of Citrus, its hybrids and some correlated species, like *Murraya paniculata* (Bové, 2006). It is graft transmissable and by means of two Psyllids: *Trioza erytreae* - present in Africa, Yemen, Madagascar and Reunion - and *Diaphorina citri* - widespread in Asia and South-East Asia, India, Arabia, Central America, Florida and Iran. No management strategy is available at the moment except for using free material and controlling vectors, which is mainly effective in the nursery.

Citrus ‘leprosis’ is also caused by a virus transmitted by some mites of the genus *Brevipalpus* (*B. phoenicis, B. californicus, B. obovatus*), provoking serious economic damage in South America and, in particular, in Brazil and Florida. The most susceptible host is sweet orange. Mandarin and sour orange show much less serious symptoms.

Citrus variegated chlorosis (CVC), today one of the most important limiting factors of sweet orange in South America, induces interveinal chlorosis and tawny spots in the adaxial leaf page. The plants are reduced in size and the fruits are small, hard and acid, ripening prematurely (Ayres *et al.*, 2002). The etiological agent is *Xylella fastidiosa*, a phloem limited bacterium transmissible by graft and through sharpshooters (Krügner *et al.*, 1998).

Citrus sudden death (CSD) is a graft transmissible disease, recently noted in Brazil on sweet orange grafted on Rangpur lime (Romàn *et al.*, 2004). The leaves and stems wilt, while the fruits remain attached to the plant. The bud-union line shows extensive yellowing of the rootstock but no crease. Recent acquisitions have demonstrated the constant presence of a CTV strain and another virus associate, probably a *Marafavirus (Tymoviridae)* (Maccheroni *et al.*, 2004).

In Oman, the Arabic Emirates and Iran a serious lime disease has been found, known as ‘witches broom’ from the main symptoms, caused by *Ca*. Phytoplasma aurantifolia, transmissible by graft and likely through *Hishimonus phycitis* (Salehi *et al.*,2002). The plants die in approximately 4-5 years.

Infectious diseases already present in the Mediterranean

The risks associated to some diseases and agents reported in only some Mediterranean countries should not be underestimated because they are still no adequate answers as to why they are confined to some specific environments and/or hosts.

Citrus leaf blotch virus (CLBV), originally associated with the bud-union disorder of Nagami kumquat grafted on Troyer citrange, has been shown to affect Nules clementine, and Navelina and Navelate sweet oranges grafted on trifoliate orange in Spain (Vives *et al.*, 2002). In Italy it has been reported on calamondin and Nagami kumquat grafted on Troyer citrange (Guardo *et al.*, 2007). The virus is transmitted by graft and seed different to other citrus viruses which suggests it deserves particular attention also in certification programs.

The *Satsuma dwarf virus* (SDV), long described in Japan, it is today found in Korea, China and Turkey, probably introduced through infected budsticks. Though relatively harmless, the disease arouses concern because of its assumed transmission through soil vectors. In more susceptible species, like the Satsuma Mandarin, there is a marked reduction in canopy and malformed spoon leaves.

The stubborn disease is diffused in dry warm zones, among which North Africa, the Eastern Mediterranean and the Middle East. The agent of the disease is *Spiroplasma citri*, a mollicute helicoidal, cultivable in vitro and transmitted by graft. The natural spread of this pathogen in the Mediterranean is by *Circulifer haematoceps* and *C. tenellus* (Bové *et al.*, 2002). The infected plants are reduced in size, have short internodes, thickened refolded and chlorotic leaves, irregular blooming, off-season yields, delayed ripening of stylar-end and aborted seeds.

In Turkey, 'yellow vein clearing' (Onelge, 2003) and 'chlorotic dwarf' symptoms have been found associated with different viruses. Citrus yellow vein clearing disease was originally described on lemon in Pakistan and subsequently transmitted to sour and sweet orange, Mandarin and other species (Grimaldi and Catara, 1996). Sour orange and lemon leaves show vein clearing and yellow areas, better visible on spring and fall leaves. Sweet orange shows only discoloration of the ribbings. Transmission occurs by means of infected propagation material, but vectors are suspected. Citrus chlorotic dwarf (CCD) induces rugose and goffered leaves on lemon plants, Mandarin and orange (Korkmaz *et al.*, 1994). The plants also show a reduction in size. The disease has been associated to a virus transmitted by *Parabemisia myricae*.

Viroids are well known and still surprising: after the agents of exocortis and cachexia have been identified, namely *Citrus exocortis viroid* (CEVd) and *Hop stunt viroid* (HSVd), today masked by the use of sour orange, a new viroid, citrus viroid IV, has been shown to be responsible for gummy bark in Turkey, a disease affecting sweet oranges. Although its pathogenic importance is under evaluation, its spread by mechanical tools rates it a new potential danger (Duran Vila *et al*. 2007). In the meantime, the 'useful' application of *Citrus viroid III* to obtain high density planting by dwarfing trees has been confirmed (Rizza, 2007). Moreover, it is to register positively that the susceptibility of Troyer citrange to CEV appears questionable and should be revised (Davino *et al*, 2005; Rizza and Catara, in publication).

Conclusions

The available evidence demonstrates that CTV is still the most dangerous citrus pathogen in the Mediterranean. CTV infections have various origins. Slow natural selection between efficient and compatible vector isolates and biotypes began before the arrival of the disease in the citrus groves. This could explain the long asymptomatic phase and absence of CTV-SP strains (which may be already present anyway). At the moment, the virus and brown aphid vector are the most serious threat to Mediterranean citriculture. Will the certification programs of mother trees and the orchard monitoring in various countries be effective in reducing the risk of further spread of the disease? Or will trade globalization take them as seriously dangerous to the future of cultivation?

In most countries, little or nothing is known about the biological and molecular characteristics of the CTV strains, upon which to make provisional appraisals of their virulence or transmissibility by current vectors, or on the strategies to be applied. The distribution of CTV-free propagation budsticks, obtained through specific certification programs, and the substitution of sour orange with virus tolerant rootstocks represents at the moment the most effective control of the effects of the disease.

It appears the virus still needs strong agronomic and molecular efforts towards more prompt diagnostic tools and more resolute methods of control. Maybe bio-informatics technology supported by high intensity computational nets will contribute to defining its structural genome and the events that determine the degree of virulence and the dissemination of the virus (Lombardo *et al.*, 2007).

However, it is strongly suggested that an effective system of monitoring and interception of foreign strains is organized on a large scale in order to avoid involuntary introduction which may change the equilibrium of the binomial mild strain/tolerant rootstock. From the technical-scientific point of view it becomes a priority to urgently activate a Mediterranean net to share the results of research and monitoring, from sampling methodologies, to testing the biological and molecular characterization of the strains of the virus, to analysis of the biotypes of vector/s and their efficiency, to vigilance of the presence/introduction of *T. citricidus*.

Furthermore, in the absence of restrictive phytosanitary measures, the indiscriminate option for a different rootstock could not prevent new disease problems. A preventative critical evaluation of the bio-agronomic and phytopathological effects potentially involved with any change will reduce the risks. In this sense it would be opportune to monitor the evolution of the total phytosanitary scenarios of citrus cultivation in the Mediterranean to aim at being equipped to face eventual new emergences.

In fact, the introduction of new rootstocks and cultivars, not sufficiently experienced in the region, will find growers and technicians unable to face new bio-agronomic problems, with repercussions for yield quality, cultural techniques and the same agro-ecosystems. Therefore, a new management strategy seems inevitable, supported by sanitary certification of the propagation material as well as its restriction of movement, but also by programs of international cooperation, to search for holistically effective methods to combat the disease and to generate policies of continuous education and training.

Such an integrated approach should be able to reduce or delay the introduction of other pathogens which are destroying the citrus cultivation in many countries around the world and/or of those already present in the Mediterranean, even if some of them have not yet proven harmful, but their transmissibility by vectors is obvious.

Acknowledgments

This paper was produced, in part, thanks to a contribution of the MUR PRIN, prot. 2006071323.

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Incidence, distribution and diversity of *citrus tristeza virus* in two different areas of Sicily

S. Davino[1], G. Sorrentino[2], M. Guardo[2], A. Caruso[2], M. Davino[1]
[1] *Dipartimento di Scienze e Tecnologie Fitosanitarie, Univ. degli Studi di Catania, via S. Sofia 100, 95123 Catania, Italy*
[2] *CRA- Istituto Sperimentale per l'Agrumicoltura, Corso Savoia 190, 95024 Acireale, Catania, Italy*

Different strains of citrus tristeza virus are reported in the world that shown variability in symptoms expression and transmissibility by aphids vectors. Infections of citrus tristeza virus in Italy have been reported from 1995. In the 1980s, transmission trials in environmental conditions have showed that our aphid populations were not able to transmit a strain of citrus tristeza virus. In 1985 and 1988 it was shown that *Aphis citricola* and *A. gossypii* were able to transmit three mild and a severe strain of CTV. In this work the incidence, diversity and structure of CTV strains discovered in Italy were examined.

Citrus tissue samples collected on Tarocco sweet orange and other citrus species were subjected to direct tissue blot immunoassay (DTBIA) or DAS-ELISA using two CTV antibodies. Samples testing positive to immunoenzymatic tests were subjected to molecular analysis as well as RT- PCR using primers specific to CTV genotypes (p20 gene), single stand conformation polymorphism, cloning and sequencing. Results on genotypes present and spread of CTV in two different areas are reported. Our results, about infected trees percentage are similar to nonlinear predictive model estimation of CTV-spread reported in other citrus areas where the mainly vector is *A. gossypii*. The immense diffusion of citrus tristeza virus put to risk all citrus cultivation in Italy.

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Monitoring and eradication of citrus tristeza virus in Apulia region, southern-eastern Italy

A. Percoco[1], F. Valentini[2], K. Djelouah[2], D. Frasheri[2], T. Colapietro[3], A. Guario[1], A.M. D'Onghia[2]
[1] *Osservatorio Fitosanitario Regionale, Regione Puglia, Lungomare N. Sauro, 70100 Bari, Italy*
[2] *CIHEAM/Istituto Agronomico Mediterraneo, Via Ceglie 23, 70010 Valenzano (BA), Italy*
[3] *Ufficio Provinciale Agricoltura, Via Dante, 33, 74100 Taranto, Italy*

Following the Italian Ministerial Decree of 1996 for the mandatory control of CTV in Italy, the first two CTV foci were firstly discovered during the year 2002 in 2 sites of the Jonian coast of the Apulia region. The Regional Phytosanitary Service enforced the CTV monitoring programme by issuing a Regional Decree and launching a strong eradication action. From 2002 to 2007, other CTV foci were identified in the same area showing different infection rates and evidence of natural transmission by aphid vectors. After the official mapping of foci and their relevant security zones, the movement of the propagating material in these areas was stopped and a strong eradication campaign was carried out. More than 80,000 samples were collected, mainly in the CTV outbreak areas. About 100 out of 274 sampled citrus groves (average 2 Ha/grove) showed an infection rate below 30% and 547 trees were destroyed. Only 16 groves exceeded 30% and most of them were entirely eradicated. A few infected plants were also detected in the nurseries located in the CTV foci, but the whole production was destroyed. Citrus nurseries are obliged to adopt new measures for a safe production of citrus plants, meeting the certification or CAC requirements. In the last 2 years, the number of infected plants tremendously decreased in the groves where eradication had occurred.

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Indicator cuttings instead of seedlings for a rapid biological indexing of the main citrus viruses and viroids

A.M. D'Onghia, M. Meziane, R. Brandonisio, K. Djelouah
Centre International de Hautes Etudes Agronomiques Méditerranéennes/Mediterranean Agronomic Institute (CIHEAM/MAIB), Via Ceglie 23, 70010 Valenzano, Bari, Italy

The use of indicator plants for the detection of the main viruses is still compulsory. Space, time and skills needed for the production of indicator seedlings for the traditional biological indexing can be strongly reduced by using indicator cuttings in Jiffy pots. The specific indicators of citrus infectious variegation (Etrog citron, Volkameriana lemon), citrus exocortis (E. citron), tristeza (Mexican lime) and psorosis (Madame vinous sweet orange) were used as cuttings or buds grafted onto cuttings. After the inoculation with the specific pathogen source (MAIB collection) and a short IBA treatment for rooting, cuttings were kept in Jiffy pots under plastic bags at 25°C for virus detection and at 32°C for viroids. The same pathogens were tested using the traditional biological indexing. Starting from 15-20 days after inoculation, clear-cut symptoms of the tested pathogens were observed on the new emerging leaves of the specific indicators using cuttings, whereas the same results were delayed in the case of seedlings. Results of biological indexing by cuttings were confirmed by using serological and molecular assays.

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Transmission of Turkish citrus tristeza virus isolates by *Aphis gossypii* Glover (Homoptera: Aphididae) in laboratory conditions

Serdar Satar, Ulrich Kersting, Nedim Uygun
University 0f Çukurova, Faculty of Agriculture, Department of Plant Protection, 01330, Balcalı-Adana, Turkey

Abstract: Tristeza is one of the most destructive diseases of world citrus. Although Mediterranean basin have not had effective vector until two years ago, in Spain, Italy and Israel, where tristeza is transmitted by *Aphis gossypii* Glover (Homoptera: Aphididae), it now devastates thousands of citrus trees. Although the citrus production areas of East Mediterranean Region of Turkey have been infected less than 1% with tristeza and also *A. gossypii* is one of the important aphid species in citrus, no natural spread of tristeza has been determined in East Mediterranean Region of Turkey in the last two decades. In this study eight isolates from Iğdır (I-3, I-4, I-7, I-9, I-10, I-12, I-13, I-14), two isolates from Serdengeçti (S-1, S-3), two isolates from Cyprus (K-1, K-5), and five isolate from USA (SY-560, T-510, T511, T515, T519) were transmitted by *Aphis gossypii* from Madam Vinous sweet orange to Mexican lime in laboratory conditions. The Turkish CTV-isolate "Iğdır" displayed variable transmission rates from 0% to 21.5%. No successful transmission was obtained using the second isolate collected in Serdengeçti. CTV-isolates from Cyprus were transmitted by *Aphis gossypii* in a rate of 18.2%, while only one of the five American isolates was transmitted about 4.5%, which is relatively low. The transmission rate depended on the number of aphids used in the experiments, ranging from 7.7% for five individuals to 38.5 % for 50 individuals and 28.6 % for 100 individuals.

Key words: *Aphis gossypii*, Tristeza, virus transmission, Turkey

Introduction

Citrus Tristeza Virus (CTV) apparently originated throughout Southeast Asia. CTV subsequently has been introduced in the rest of the citrus growing area of the World by movement of infected material such as plant, budwood etc (Raccah et al, 1989). Natural spread of CTV has occurred subsequently by new vectors in newly introduced area. CTV is transmitted by *Toxoptera citricida* (Kirkadly), *Aphis gossypii* Glover, *Aphis spiraecola* Patch, *Aphis craccivora* (Koch) *Toxoptera aurantii* (Boyer de Fanscolombe), *Myzus persicae* (Sulzer) and *Dactynotus* (*Uroleucon*) *jaceae* (L.) (Homoptera: Aphididae) (Bar-Joseph et al, 1983; Raccah et al, 1989; Roistacher & Bar-Joseph, 1987). Although *Toxoptera citricida* becomes an efficient vector where it is present like in South America and South Africa, *Aphis gossypii* is an important vector for isolates of CTV where *Toxoptera citricida* is absent as California and Mediterranean countries (Bar-Joseph, 1978; Yokomi et al 1989). On the other hand, Introduction of *Toxoptera citricida* in Continental Portugal and Spain may change the scenario for the Mediterranean basin. The well documented example of Florida illustrates this observation (Ilharco, 2006; Halbert et al, 2004). CTV transmitted by *Aphis gossypii* killed thousands of citrus trees in the Mediterranean basin without *Toxoptera citricida* (Bar-Joseph & Loebenstein, 1973; Hermoso de Mendoza et al., 1984).

Although CTV is reported in East Mediterranean region of Turkey, few studies were done on transmission of the disease in the area. One of the studies was done by Dolar (1976). Dolar (1976) tried to show transmission of the disease by *Aphis craccivora*, *Toxoptera* *aurantii* and *Myzus persicae* which were determined on citrus during the survey at end of the 1960's. *Aphis gossypii* were not used in the study because it was not determined in the survey. Successful transmission by *Aphis gossypii* to only one plant via low absorbance value was showed by Yılmaz et al (1990).

Though sour orange is the dominant rootstock (90%) in East Mediterranean region of Turkey, CTV has been known since 1960 and *Aphis gossypii* has been an important aphid species in citrus orchard since 1990, no detailed study has been done about vector relationship between *Aphis gossypii* and CTV. Therefore the objective of this study is to determine the transmission ability of Turkish CTV isolates by *Aphis gossypii* in laboratory conditions.

Material and methods

Plants and aphid vectors

In the transmission experiment *Aphis gossypii* was used as vector insect. The colony of *Aphis gossypii* was started from a field colony found on a cotton field near Balcalı/Adana. The colony was reared on cotton, *Gossypium hirsitum* L., in the insect rearing room under the condition of 16 h daily light and 22 ± 3°C.

Madam vinous sweet orange was selected as acquisition host plant. The entire madam vinous was selected from nucellar seedlings. As Indicator or receptor plants were selected nucellar seedling of the Mexican lime. All the young citrus plants were grown in greenhouse and fertilized weekly. Five to15 plants of Madam vinous sweet oranges were grafted with inoculum tissue containing each of the isolates. The positive ones were used as acquisition host plants.

CTV isolates

In the CTV transmission experiment, 2 isolates from the East Mediterranean region of Turkey, one isolate from Cyprus and five isolates from California which was brought by Prof. C. N. Roistacher (University of California, Riverside, CA, USA), were used for showing *Aphis gossypii* transmission of the disease in the laboratory.

The first isolate from East Mediterranean region of Turkey was taken from an orange orchard in Bekirde/Mersin. It was found on 25-30 years old Jaffa orange and named in the experiment as Iğdır (I). The infected trees were found on the same row. The second isolate came from a field in the Tarsus district called in the experiment as "Serdengeçti (S)". The isolate was found on 20-25 years old Satsuma trees. The Cyprus isolates (K) were found on young citrus seedlings which were brought from Cyprus. The "American isolates" were taken from the greenhouse of Subtropical Fruits Research Center in Adana. The bar codes of the isolates were T-510, T-511, T-515, T-519 and SY-560. They were identified as mild isolates of CTV in California except SY-560 which causes severe seedling yellow on grapefruit, sour orange and Eureka lemon.

All the isolates from different districts were graft inoculated on 2 years old madam vinous seedlings. I, S and K were included as main codes. The inoculated madam vinous plants were used in the transmission experiment as acquisition host plants.

Transmission experiment

Donor plants were cut back 15-20 days before acquisition access feeding to stimulate flushes for aphid feeding. Aphids which were reared on cotton were transferred to plant cages containing young flush of inoculated madam vinous plants for acquisition access period in the illuminated temperature cabinet. After the feeding period of 24 hours at 22 ± 1°C in 16 h daily illuminated chamber, young flush of the acquisition host, covered with aphid adults and nymphs, were taken and tied to young leaves of the receptor plants. The inoculation feeding period was also 24 hours at 22 ± 1°C in 16 h daily illuminated chamber. At the beginning of the inoculation access period and at the end of the period, aphids were counted on receptor plants. Then aphids were sprayed with an insecticide. The Mexican lime inoculated by *Aphis gossypii* were kept in greenhouse 3 to 6 months for observation of symptoms and at the end of this period leaves were collected to make serological tests by Double-antibody sandwich ELISA (Clark & Adams, 1977; Bar-Joseph et al, 1979). The source of IgG for coating and preparation of alkaline phosphate conjugates were polyclonal antiserum and were obtained from Dr. S. M. Garnsey (Horticultural Research Laboratory; ARS-USDA Orlando/USA)

Effect of number of Aphis gossypii in transmission of CTV

The transmission experiment was done in the same manner but the number of aphids in the inoculation access period was 2, 5, 10, 30, 50 and 100. Only Cyprus isolate (K-5) was used in the experiment.

Results and discussion

Of 17 CTV isolates tested, 8 were transmitted by *Aphis gossypii*. Positive results by ELISA were obtained in 13 out of 224 plants in all the transmission experiments (Table 1).

The CTV isolate collected from the Iğdır graft was inoculated on 15 madam vinous sweet orange. Eight of them gave positive results and were used in the experiment as acquisition host plants. From the sub isolates of Iğdır, I-3 showed the highest transmission rate by 21.5% among all the CTV-isolates, whereas I-4, I-12 and I-13 were found as non-transmissible by *Aphis gossypii* (Table 1). Differences in transmission rate between Iğdır isolates were explained as CTV isolates may be separated by grafting. Similar results were cited by Raccah et al. (1978) and Roistacher et al. (1980). The average number of aphids in the receptor plant at the beginning of the inoculation access period was calculated as 142.3, whereas at the end it was ranging from 17.1 to 51.8. Roistacher et al. (1980) and Roistacher (1981) had also observed that the number of aphids at end of the experiment did not relate to the transmission rate.

Although the Serdengeçti isolate was tried to graft- inoculate to 5 madam vinous sweet orange, only two of them were inoculated by CTV. The transmission experiment done with these two isolates gave no transmission at all. The low number of replications in the Serdengeçti isolate may cause this negative result. Yokomi & Garnsey (1987), tried to transmit 38 CTV isolate by *Aphis gossypii* and *Aphis spiraecola*. *Aphis spiraecola* transmitted 11 of 38 isolates, whereas *Aphis gossypii* transmitted 29 of 38 isolates. Transmission was achieved with 31 of 38 isolates.

The Cyprus isolates were graft inoculated on 2 out of 5 madam vinous sweet orange plants. The isolate K-1 was inoculated in 16.7% by CTV, the isolate K-5 was transmitted to one out of 5 Mexican limes. CTV in South Cyprus were transmitted by *Aphis gossypii* and the citrus orchards of South Cyprus were infected at rates ranging from 16 to 62% with this disease (Kyriakou et al., 1993). Although the eradication program was started in South Cyprus, and the incidence of CTV was reduced to 5.8% in 4 regions of South Cyprus, the incidence of CTV was not reduced as successfully as reported in the rest of the citrus production areas (Kyriakou et al., 1996). CTV transmission rate and severity increased when compared to first years of epidemic in Florida and California (Roistacher & Bar-Joseph, 1987). An explanation for the mechanism by which the virus changes from poorly transmissible (less than 5%) to highly transmissible (100%) form has been discussed by Bar-Joseph (1978). The explanation was that highly transmissible and poorly transmissible CTV isolates may coexist in the same tree and that possibly by cross-protection, the more transmissible isolates are suppressed. After a period of time, this protection breaks down and the more transmissible isolates are more readily transmitted by vectors to non-infected trees.

The American CTV isolates were included in the experiment of transmission of Turkish CTV isolates, as control. These isolates from America had different range of severity. SY-560 and T-515 isolates which were transmitted by *Aphis gossypii* were known from previous works (Roistacher et al., 1980; Roistacher, 1981), gave the opportunity to discuss that the reason why Turkish isolates were not transmitted while the American isolates were transmitted: is it experimental mistake ? Or it is a result. All the American CTV isolates were taken from directly virus bank and all the isolates grafted on different Madam Vinous sweet oranges were accepted as one isolate for every each of American CTV isolates. The American isolates of SY-560, T-510, T511 and T519 were tried to transmit 7, 17, 17, and 23 times to Mexican Lime by *Aphis gossypii*, respectively, but they were not transmitted. Only the isolate T-515 was transmitted one times out of 23 replications by the transmission rate of 4.5 % (Table 1).

Table 1. Transmission by *Aphis gossypii* of Citrus tristeza virus isolates (CTV) collected from Iğdır, Serdengeçti, Cyprus and American isolates.

CTV-Isolates	Avg. number of aphid[1]	Avg. number of aphid[2]	Infected plant/ inoculated plant	Transmission rate (%)
Iğdır				
I-3	145.0	29.1	4/19	21.5
I-4	144.0	20.8	0/6	0.0
I-7	145.0	45.1	2/25	8.0
I-9	145.1	17.1	1/9	11.1
I-10	141.9	51.8	2/21	9.5
I-12	137.1	36.3	0/8	0.0
I-13	137.5	42.8	0/14	0.0
I-14	142.5	40.9	1/24	4.2
Mean	**142.3**	**35.5**	10/126	**7.9**
Serdengeçti				
S-1	125,0	17.3	0/4	0.0
S-3	143,5	21.3	0/6	0.0
Mean	**134.3**	**19.3**	0/10	**0.0**
Cyprus				
K-1	152.1	19.9	1/6	16.7
K-5	143.0	27.2	1/5	20.0
Mean	**147.5**	**23.6**	2/11	**18.2**
American				
SY-560	140.3	36.3	0/7	0.0
T-510	144.5	25.9	0/17	0.0
T-511	139.2	31.6	0/17	0.0
T-515	145.7	40.9	1/23	4.5
T-519	151.6	35.7	0/13	0.0

[1] Average number of Aphids at the beginning of the inoculation access period on the Mexican lime
[2] Average number of Aphids at the end of the inoculation access period on the Mexican lime

SY-560 and T-515 isolates are known as a highly transmissible isolates however, the Turkish *Aphis gossypii* population were not able to transmit the isolates though the number of the aphid in the beginning of acquisition access period were three times more than the transmission experiment of Roistacher et al., (1980) and Roistacher (1981). The source of the aphid was not a factor in transmissibility of CTV by *Aphis gossypii* and also our aphids have been already transmitted some Turkish CTV isolates. Transmissibility of CTV by *Aphis gossypii* appears to be a function of the virus isolate and host and no the aphid (Roistacher et al., 1980). The isolate T-515 were transmitted by *Aphis gossypii* in the range of 0-96% shown by Roistacher et al., (1980).

The numbers of aphid in the transmission experiment strongly affect to transmission rate and the experiments done on this subject usually used different number of aphid. Therefore the comparison of the transmission results from different countries should be based on single aphid transmission rates. The formula of the Hunt et al., (1988) was used in our result to asses single aphid transmission rate (Table 2).

Table 2. Single aphid transmission rates of Turkish and American CTV isolates calculated from the formula (HUNT et al., 1988)

CTV-Isolates	Avg. number of aphid[1]	Calculated transmission percentage for single aphid (%)[3]	Avg. number of aphid[2]	Calculated transmission percentage for single aphid (%)[4]
Iğdır				
I-3	145.0	0.16	29.1	0.81
I-4	144.0	0.00	20.8	0.00
I-7	145.0	0.06	45.1	0.18
I-9	145.1	0.08	17.1	0.69
I-10	141.9	0.07	51.8	0.19
I-12	137.1	0.00	36.3	0.00
I-13	137.5	0.00	42.8	0.00
I-14	145.0	0.06	45.1	0.18
Mean	**142.3**	**0.06**	**35.5**	**0.23**
Serdengeçti				
S-1	125.0	0.00	17.3	0.00
S-3	143.5	0.00	21.3	0.00
Mean	**136.5**	**0.00**	**19.3**	**0.00**
Cyprus				
K-1	152.1	0.12	19.9	0.91
K-2	143.0	0.16	27.2	0.82
Mean	**147.6**	**0.14**	**23.6**	**0.85**
American				
SY-560	140.3	0.00	36.3	0.00
T-510	144.5	0.00	25.9	0.00
T-511	139.2	0.00	31.6	0.00
T-515	145.7	0.03	40.9	0.12
T-519	151.6	0.00	36.3	0.00

[1] Average number of Aphids at the beginning of the inoculation access period on the Mexican lime

[2] Average number of Aphids at the end of the inoculation access period on the Mexican lime

[3] Transmission efficiency calculated at the probability of transmission by single aphid and determined by the formula "$(1\text{-}L)^{1/K}X100$" Where L= Proportion of test plant infected K=Number of Vector per test plant (Hunt et al, 1988) at the beginning of the inoculation access period on the Mexican lime

[4] Same as it is 3 but at the end of the inoculation access period on the Mexican lime

The transmission rates were changing depending on which number of aphid accepted. If the number of aphid at the beginning of inoculation access period were accepted for Igdır isolate, the transmission rate were ranged 0.0 to 0.16% but if we accept the number of aphid at the end of the inoculation access period for same isolates, transmission rate increased 3 to 5 times (Table 2). The number of aphid at the beginning of the inoculation access period in the most of the transmission experiment was generally accepted as inoculative number. Igdır isolates were transmitted 0.06 % per aphid which were higher than the ST isolate (0.04) of Israel but lower than AT (0.08%) and VT isolate (1.16%) of Israel (Raccah et al., 1976) and also lower than T-308 (0.75%), T-300 (0.75 %) and T-388 (3.0%) of Spain (Hermoso de Mendoza an et al., 1984; 1988a). Single aphid transmission rate of K-1 and K-5 CTV isolates was 2 times higher than Iğdır isolates whereas it was much lower than Cyprus isolates (2.02%) (Kyriakou et al., 1993).

Increasing number of aphid increased the transmission rate of K-5 CTV isolate but this increasing is not parallel to transmission rate of the CTV isolate. This result is similar to the result of Raccah et al., (1976) and Roistacher et al., (1984) (Table 3).

Table 3. Transmission of Cyprus CTV isolate (K-5) by 2, 5, 10, 30, 50 and 100 *Aphis gossypii* individuals.

Number of aphid[1]	Avg. number of aphid[2]	Infected plant/ inoculated plant	Transmission rate (%)
2	0,7	0/18	0,0
5	1,0	1/13	7,7
10	2,1	1/26	3,9
30	4,6	0/20	0,0
50	14,6	5/13	38,5
100	23,1	4/14	28,6

[1] Average number of Aphids at the beginning of the inoculation access period on the Mexican lime;
[2] Average number of Aphids at the end of the inoculation access period on the Mexican lime

The Turkish CTV isolates were transmitted in the laboratory by *Aphis gossypii,* which is an important vector in the Mediterranean basin and other *Toxoptera citricidus* free areas. CTV is epidemic in some Mediterranean countries but it did not naturally spread in the East Mediterranean region of Turkey, although 0.04 % of mandarins were infected by CTV in the East Mediterranean region of Turkey (Çınar et al., 1993). The transmission rates of Turkish isolates indicated that they are mild isolate as in Spain's isolates of the 1960's. It may start to spread naturally in the near future. It is a big threat for East Mediterranean Region of Turkey, because more than 90% of the citrus are using sour orange as a rootstock.

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High density citrus orchard sustainability through a non-pathogenic viroid

S. Rizza[1], G. Nobile[1,2], M. Tessitori[1], R. La Rosa[1], A. Catara[1,2]
[1] *Dipartimento di Scienze e Tecnologie Fitosanitarie, Università di Catania, Via S. Sofia 102, 95123 Catania, Italy*
[2] *Parco Scientifico e Tecnologico della Sicilia, Blocco Palma I, Z. I., 95131 Catania, Italy*

High density citrus plantings, obtained by using viroid-RNAs inoculation, have been evaluated in experimental plots in different countries. This technology is authorized in commercial orchards of New South Wales and California. A similar research has been carried out in Italy since long time and some commercial orchards have been already established. In this paper we report the results obtained after 12-yr in a commercial orchard of clementine grafted onto trifoliate orange inoculated with different combinations of citrus viroids as *Citrus exocortis viroid* (CEVd), *Citrus viroid III* (CVd-III) and *Hop stunt viroid* (HSVd). Over 4,000 trees, spaced at 2 x 3 m, were inoculated one year after planting with four different viroid isolate combinations. Management was conventional, but to avoid canopy crowding and high humidity levels, the trees were pruned yearly thus benefiting also the size of the fruit. As expected, twelve years after inoculation, all the trees containing CEVd showed bark cracking and/or scaling, whereas those with only CVd-III were healthy looking. Fifty trees were randomly selected in different plots and tested for the three viroids.

The trees inoculated with CVd-III alone had an average circumference of 37.25 cm (scion) and 57.00 cm (rootstock), whereas those inoculated with CVd-III+HSVd were 35.20 cm and 54.63 cm, those with CEVd+CVd-III were 31.87 cm and 47.00 cm, and those with CVd-III+HSVd+CEVd were 29.58 cm and 45.11 cm. Trunk size and symptom expression were largely affected by other conditions, which are now under evaluation. Yield maxed at 60 tons/ha in year eighteen (2003), and fruit quality reached high standards every year. For the first time on a large scale, the results show *Citrus viroid III* is suitable to obtain mild dwarfing of citrus trees that allows a better sustainable management of the orchard.

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IOBC/wprs Bulletin Vol. 38, 2008
pp. 337-344

Use of *lux*-marked genes to monitor antagonistic *Pseudomonas syringae* on citrus fruits

Bonaccorsi Alessandra, Cirvilleri Gabriella
Dipartimento di Scienze e Tecnologie Fitosanitarie, Sezione Patologia Vegetale, Università dgli Studi di Catania, Via S. Sofia 100, 95100 Catania, Italy. E-mail: gcirvil@unict.it

Abstract: *Pseudomonas syringae* strain 48SR2, effective as biological control agent, was genetically tagged with the promoterless *lux* operon *Tn4431* to monitor the population dynamic in *in vivo* assay. Four hundred thirteen mutants were obtained and diverse bioluminescent activities were observed according to the insertion of *Tn4431* into a wide variety of regions of the chromosome. A selected strongly bioluminescent mutant (*lux* 176) and the wild-type strain were tested for their antagonistic properties to control the post-harvest pathogen *Penicillium digitatum.* Both the wild type and the *lux*-marked strain equally reduced the growth of *P. digitatum in vitro* and the severity and incidence of citrus decay *in vivo* when the biocontrol agents were applied in wounds 24 h before challenging *P. digitatum.* The persistence of the genetically engineered bacteria on citrus wounds was monitored over the time with bioluminescence detection systems as well as by dilution plating techniques. Population sizes of both wild-type and *lux*-mutant strain were comparable. These results indicate that *P. syringae* strain 48SR2 could be considered a biological control agent for citrus green mould and that bioluminescence can be a sensitive detection method to study population dynamics and antagonistic behaviour during fruit storage.

Key words: *Pseudomonas syringae*; monitoring; *lux* genes; biological control.

Introduction

Green mould caused by *P. digitatum* is a universal postharvest disease of citrus. Currently, green mould is mainly controlled by the application of synthetic fungicide either in the dump tank water or as a spray onto the fruit. The use of chemicals is becoming increasingly restricted because of the concern for the environment and health as well as the cost of developing new pesticides to overcome resistance developed by pathogens. Biological control using microbial antagonists has been considered as a desirable alternative to the use of chemicals. Several microbial agents have shown promise as biological control agents for post-harvest diseases of fruit crops. The first commercial biocontrol product were registered in the United States and sold as BioSave 100 and 110 (EcoScience, Longwood, FLA) and Aspire (Ecogen, Langhorne, PA). Aspire has a yeast active ingredient (*Candida oleophila* I-182) that has been particularly effective in commercial trials (Droby *et al*., 1998), but is not commercially available anymore. Products that contain isolates of *P. syringae* such as *P. syringae* ESC-10 in Biosave can be a possibility, but are not commercially available in Italy. Recently, several strains of *P. syringae* were isolated from different products (Cirvilleri *et al*., 2005) and tested for activity against postharvest pathogens. Some of them proved to be very effective in reducing growth of the pathogens *in vitro* and in preliminary screening trials in artificially inoculated fruits of apple and citrus. With recent advances in the ability to manipulate biological-control agents genetically and the consequential benefits in the environmental and agricultural fields, it has become increasingly important to develop the techniques to monitor and asses the risks involved in the release of microrganisms into the environment. Bioluminescence genes from various organisms are widely utilised for gene expression analysis and tagging of plants, animals and microbes to investigate various biological questions (Greer III and Szalay, 2002). Bioluminescence is an effective reporter for detecting recombinant bacteria containing CDABE *lux* genes of *Vibrio fischeri*, which encodes luciferase and an aldehyde substrate to generate light. Bioluminescence has been used previously for gene expression studies (Cirvilleri and Lindow, 1994) and for monitoring the presence of plant pathogenic bacteria such as *Xanthomonas campestris* and *Erwinia carotovora* growth *in planta* (Shaw and Kado, 1986; Shaw *et al*., 1992), *P. syringae* in bean plants (Cirvilleri and Lindow, 1994) and *P. corrugata* in tomato plants (Cirvilleri *et al*., 2000) . The objectives of this study were (i) to transform *P. syringae* 48SR2 antagonistic strain using Tn 4431, a transposon that allows transcriptional fusions to a promotorless luciferase (lux) operon and (ii) to evaluate the use of bioluminescence detection system to monitor the released strain.

Materials and methods

Transposon mutagenesis and light measurement

P. syringae 48SR2 was selected for its antagonistic activity (Cirvilleri *et al*., 2005) and was genetically tagged with the promotorless *lux* operon *Tn4431*. Transposon *Tn4431*, which intern contains a promotorless *lux*CDABE operon of *Vibrio fischeri* and a gene conferring tetracycline resistance, was introduced into *P. syringae* 48SR2 as follows: the donor strain *Escherichia coli* HB101, that carries the "suicide " vector pUCD623, and the helper strain *E. coli* HB101 were grown at 37°C in Luria Bertani (LB) medium (Miller, 1972) containing 10 µg/ml tetracycline (Tc) and 25 µg/ml kanamicin (Km), respectively. The recipient strain 48SR2 was grown at 28°C in LB containing 100 µg/ml rifampicina (Rif). Bacterial cell in logarithmic phase (10^8 cells/ml) were washed twice in LB and resuspended to their original volume in LB. Equal amounts (200 µl) of donor and helper strains and 400 ml of the recipient strain were mixed and 50 µl were placed on sterile nitrocellulose filters on LB plates. The filters were incubated at 28°C over night and then bacteria were streaked onto LB plates supplemented with 100 µl/ml Rif and 10 µl/ml Tc. Colonies appearing after 2-3 days were restreaked several times onto King's medium B (KB) (King *et al*., 1954) supplemented with 100 µl/ml Rif and 25 µl/ml Tc. Positive transformations were identified by fluorescence under UV and then checked for light production. Light emission by *lux* fusion containing strains was quantified by a photometric assay in an Optocomp I Luminometer (MGM Instrumens, Inc.). The *lux*-mutants were grown on KB plates for 48 h and then suspended in 2 ml of peptone water and then mixed with 250 µl of freshly sonicated suspension of 0.2% n-decanal in SSC 20X buffer and placed in a cuvette. The cuvettes were quickly inserted into the Luminometer and relative light units (RLU) were measured over a 10 s period. Bacterial cell concentrations in separate aliquots were determined by measuring the optical density at 600 nm and relating it to a standard curve using known cell concentration. Light production was normalised to that produced by 10^8 cells/sample.

In vitro *antagonistic assay of lux mutants*

The parental strain *P. syringae* 48SR2 and all the *lux* mutants obtained were tested for their inhibition activity on PDA agar plate assay against *Rodothorula pilimanae*. Aliquots (20 µl) of bacterial strain suspensions obtained from 4-day-old cultures on nutrient agar (NA; Oxoid) (approximately $1x10^9$ CFU/ml) were spotted on PDA plates, using two spots per plate. The plates were incubated for 4 days at 27°C. Then plates were over sprayed with a suspension containing approximately $1x10^6$ CFU/ml of target microrganism and incubated at 27°C for

2–4 days. The presence and size of a clear zone around the colonies, indicating the inhibitory effect, was scored. All tests were repeated twice. The wild type strain, a selected strongly bioluminescent mutant (*lux* 176) and a mutant with reduced antagonistic activity against *R. pilimanae* (*lux* 341) were selected and evaluated for antimicrobial activity against *Bacillus megaterium*, *Geotricum candidum* and *Penicillium digitatum* on potato dextrose agar (PDA; Oxoid) and against *G. candidum* and *P. digitatum* on orange flavedo tissues (OFT) agar.

In vivo *antagonistic assay of lux mutants*

The wild type strain and mutants *lux*176 and *lux*341 were tested for the capacity to suppress the growth of *P. digitatum* (green mould) on lemon (*Citrus sinensis* Osbeck) cv. Femminello and cv. Monachello. Before each experiment, fruits showing no visible wound were carefully hand selected and were washed with tap water, surface-sterilized by dipping for 2 min in 2% of sodium hypochlorite, rinsed with SDW and then air-dried. Bacteria were grown for 4 days at 27°C on PDA (Bull *et al*., 1997) and then were suspended in SDW and the concentrations adjusted with SDW to a cell density corresponding to $1x10^9$ CFU/ml. Fruits were wounded with a sterile needle to make two 2-mm deep and 5-mm wide wounds on their peel at the equatorial region. Aliquots (20 µl) of cell suspensions were pipetted into each wound. After 4 h or 24 h the wounds were inoculated with 20 µl of spore suspension ($1x10^6$ CFU/ml) of fungal pathogen. Control fruits were treated with SDW only. Fruits were then placed in plastic cavity packaging trays. To provide ample humidity for disease development, a wet paper towel was placed on empty cavity trays and the packaging trays were sealed in polyethylene plastic boxes and incubated at 20°C. Four fruits per each strain and time of inoculation of the pathogen were used. The number of wounds showing symptoms of infection was counted and incidence of disease (% of decayed wounds) was evaluated 5 days after the inoculation. At the same time disease severity was evaluated with an empiric scale (1= no visible symptoms; 2 = initial soft rot; 3 = presence of mycelium; 4 = sporulation). Disease severity data were converted to percentage midpoint values (Campbell and Madden, 1990), where 1 = 0%, 2 = 35%, 3 = 65% and 4 =90%. Incidence of disease and disease severity ratings were subjected to an arcsine square root transformation before running the anova. Subsequently, one-way anova was performed. The mean values were separated using the Student–Newman–Keul's mean separation test, at $P < 0.05$. The percentages shown in the tables are untransformed data.

Population studies and lux activity of* P. syringae *in fruits wounds

Wild type *P. syringae* 48SR2 and mutants 176 lux and 341lux were tested. Lemons were wounded as described previously and aliquots of 20 µl 10^9 CFU/ml cell suspensions of *P. syringae* were applied to each wound. Fruits were then placed in plastic cavity packaging trays. *P. syringae* was recovered from the wounds after incubation at 20°C for 0 (just prior to storage), 1, 2 and 7 days. Wounded tissues was removed with an ethanol-flamed 10 mm (internal diameter) cork borer, pestle in 1 ml sterile 0.05 M phosphate buffer (pH 7.0) and aliquots were plated by using a spiral plater (EDDY-JET, IUL Instruments, Königswinter, Germany) on KB+Rifampicina^{100}. The plates were incubated for 2 days at 25-27 °C. Colony counting and CFU determination were carried out according to the instructions of the manufacturer. Population densities of *P. syringae* were expressed as $\log_{10}$CFU per wound. There were four single fruits replicates per treatment, with four injury per fruits. Light production by bacterial strains on citrus was determined by method similar to that used for cultured cells except that 1 ml of undiluted tissue washing was used as a sample and 250 µl of freshly sonicated sample of 0.2% n-decanal was added to each suspension. Light production was normalized to that produced by 10^8 cells/sample.

Results

Transposon mutagenesis and light measurement

The *Tn4431* mutagenesis of *P. syringae* strain 48SR2 generated four hundred thirteen mutants. Mutants carrying *Tn4431* were selected on the basis of tetracycline and rifampicin resistance and subsequently screened for light production by luminometer measurements. Luminometer measurements of light activity were carried out with suspensions of *Tn4431* mutants harvested from selective medium (KB containing Tc ^{25} and Rif ^{100}) after 48 h growth. All the tetracycline and rifampicin – resistant exconjugants obtained emitted detectable light, indicating the presence of the transposon. In contrast, parental strain produced very little light when measured in the luminometer. Addition of n-decanal to suspension of *Tn4431* mutants immediately prior to measurement greatly increased light production (Cirvilleri and Lindow, 1994). A wide range of light-producing lux mutants harvested from KB plates was observed. The *lux*176 mutant showed the highest lux activity with a light production of 3.377.783 RLU/10^8 cells/ml (Figure 1).

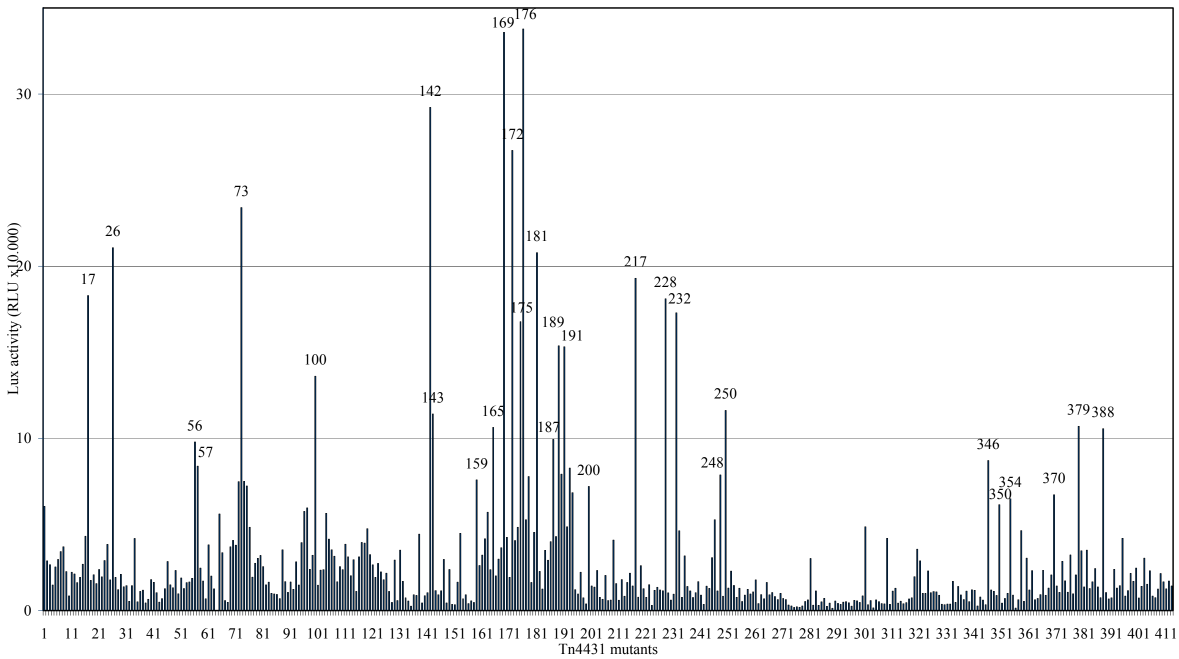

Figure 1. Light production by cells of different Tn4431 mutants of *P.syringae* strain 48SR2 when grown for 48 h in KB+Rif^{100}+Tc^{25} medium at 28°C. The number of 413 mutants producing given amounts when assayed after addition of n-decanal to the assay mixture is reported. Light production is expressed as arbitrary luminometer light units per 10^8 cells/sample when measured for 10s.

In vitro *assay of lux mutants*

In *in vitro* antagonistic activity assay on PDA all mutants were identical to the wild type strain, while one mutant (*lux*341) reduced its antagonistic activity against *R. pilimanae*, *P. digitatum* and *G. candidum* and enhanced activity against *B. megaterium*. On orange flavedo tissues (OFT) agar mutant *lux*176, as well as wild type strain 48SR2, enhanced activity against *P. digitatum* whereas mutant *lux*341 confirmed lost of antagonistic activity (Table 1).

Table 1. The effect of mutation on antimicrobial activity by strain 48SR2 of *P. syringae*.

	Antimicrobial activity (mm)							
	R. pilimanae		*B. megaterium*		*G. candidum*		*P. digitatum*	
	on PDA	on orange[a]	on PDA	on orange[a]	on PDA	on orange[a]	on PDA	on orange[a]
48SR2	25.0	n.t.	10.0	n.t.	12.0	5.0	4.0	20.0
*lux*176	25.0	n.t.	10.0	n.t.	11.0	5.5	3.0	18.0
*lux*341	15.0	n.t.	17.0	n.t.	5.8	2.0	0.0	3.0

[a] orange flavedo tissues agar

In vivo *antagonistic assay of lux mutants*

In *in vivo* antagonistic activity assay against *P. digitatum* on lemon cv. Femminello and Monachello, wild type strains 48SR2 and mutants *lux*176 equally and significantly reduced incidence and severity of citrus decay after 5 days inoculation in comparison with the control, while mutant *lux*341 significantly reduced its biocontrol activity. Biological control was consistently superior when application of the pathogen was 24 hours postponed on all the cultivar tested (Fig. a2 and b2). Also *lux*341, which lost antagonistic activity in co-inoculated experiments, was able to inhibit the growth of the pathogen in different inoculation (Figure 2).

Population studies and lux activity

Survival of the wild type and *lux* mutants was monitored over 7 days. Both wild-type and lux-mutant strains were able to establish large populations on citrus wound and their growth, followed over time with dilution plating techniques, remained similar over 1 week at 20°C. Mutants were consistently recovered from tissue sections emitting bioluminescence. Mutant *lux*176 exhibited higher level of light than *lux*341 (Figure 3).

Discussion

The data reported here confirmed that *P. syringae* 48SR2 could be considered a biological control agent for citrus green mould as previously demonstrated by Cirvilleri *et al*. (2005). A reliable, rapid system to monitor bacteria in the environment is essential, if these microorganisms are to be used for purposes such as biological control of disease-causing microorganism. Previously an efficient detection method for monitoring the colonization capacity of the antagonistic *P. fluorescens* A506 was investigated by transforming the naturally rifampicin resistant strain with the promotorless luciferase (*lux*) operon of *V. fischeri* (Cirvilleri and Caldarera, 1998). This bioluminescence-based marker system was chosen because of the stability of the *Tn4431* insertion and the absence of indigenous bioluminescent bacteria in the carposphere. Moreover bioluminescence is assayed easily and rapidly and poses little metabolic burden to host cells.

Four hundred and thirteen *lux* fusion containing strain were easily isolated on media amended with both rifampicin and tetracycline and emitted detectable light upon addition of emulsified n-decanal. Transposon *Tn4431* remained stable in all mutants in which it was expressed (data not shown). The bioluminescent ability of the mutants was evaluated both *in vitro* and *in planta*. In both cases, a wide range of light production was revealed on fruits and in culture. Light production on fruits was on average 5 fold lower than that of the same *lux* mutant strains after growth on KB agar. Since the insertion of *Tn4431* is random, the levels of light production depend on the orientation of the *lux* operon and on the transcripitional activity of the gene into which the *lux* operon is inserted. Lux fusion-containing strains evaluated in KB agar plates that showed low levels of light production indicated similarly low level of promoter activity, while the mutants exhibiting variable high levels of bioluminescence may represent fusion in which the trancripitional activity of the target gene is high.

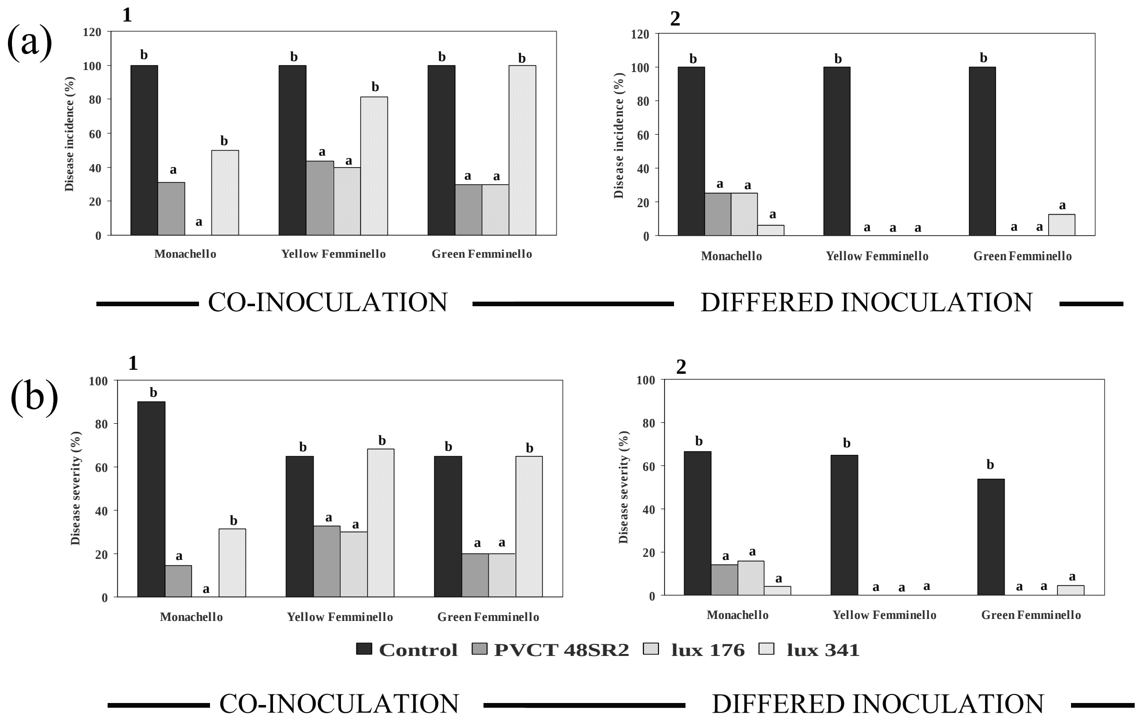

Figure 2. Incidence (a) and severity (b) of disease on lemon (*Citrus sinensis* Osbeck) cv. Femminello and cv. Monachello after inoculating with *P. syringae* 48SR2 WT, *lux*176 and *lux*341. The wounds were then treated with *P. digitatum* 4 hours (co-inoculation experiments) or 24 hours (differed inoculation experiments). Values are mean of four replicates, two replicates for experiment. (Control: wounds inoculated with *P. digitatum*).

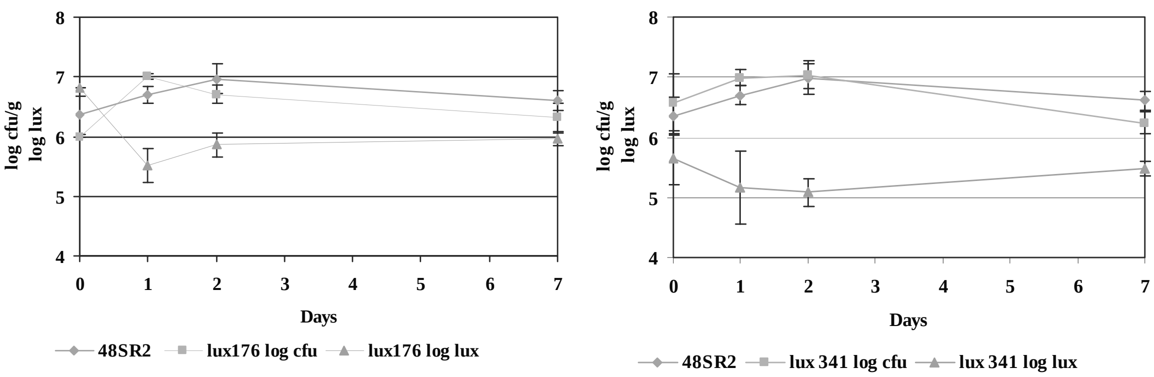

Figure 3. Population and lux activity of *P. syringae* 48SR2 and mutants *lux*176 and *lux*341.

Both wild type and mutants *lux*176 and *lux*341 survived equally well on fruits over one week. Cirvilleri and Caldarera (1998) found similar survival patterns on bean leaves of wild type and recombinant *lux* modified *P. fluorescens*. Our data show that the carposphere competence and fruit colonizing abilities of the *lux* mutants were not affected by the presence of the *lux* operon. The *lux*-mutant strains actively colonized wound fruits with population levels comparable than that of the wild type strain.

The amount of light emitted from a *lux* fusion containing strain is a function of the promoter activity of the target gene, the number of bacteria present, the different amount of FMNH2 within an individual bacterial cell, and the metabolic activity of these bacteria. The reduced amount of light produced by the mutant strains during the colonization of fruits, in spite of the high number of bacterial cells, may be related to microbial competition reducing the metabolic activity of the *lux*-mutant strains, or to low levels of nutrients on heavily colonized wound fruits.

Assessment of environmental impact and risks associated with release of micro organisms requires knowledge of microbial survival, growth and activity within the environment. The genetically engineered *P. syringae* strain 48SR2 marked with resistance to rifampicin and stable integrated *lux*-genes into the chromosome will be helpful for further studied on epiphytic survival, behaviour and biocontrol activity under various conditions. In particularly *lux*176, exhibiting higher light production relative to the other *lux*-mutants *in vitro* and *in vivo*, suggested its use for further studies on population dynamics and behaviour in postharvest, while *lux*341, that reduced its antagonistic activity *in vitro* and *in vivo*, could be used to investigate the mechanism of antagonism activity. Previous studies (Grgurina *et al*., 1996) demonstrated that a *syr*B and *syr*C mutants, still able to produce syringopeptin but not syringomicin, lacked antifungal activity against *R. pilimanae*, but showed similar (*syr*B mutant) or higher (*syr*C mutant) antimicrobial activity against *B. megaterium* than that of the parental strain.

In conclusion results indicate that *P. syringae* strain 48SR2 could be considered a biological control agent for citrus green mould and that bioluminescence can be a sensitive detection method to study population dynamics and antagonistic behaviour during fruit storage.

Acknowledgements

This research was supported by founding from MIUR-PRIN 2006-2008 “Selection of antagonistic microorganisms with high compatibility and their use to improve biocontrol performances”

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IOBC/wprs Bulletin Vol. 38, 2008
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Microbial antagonists of the citrus nematode, *Tylenchulus semipenetrans*, in Southern Italy and host-parasite rhizosphere interactions

A. Ciancio
Istituto per la Protezione delle Piante, Consiglio Nazionale delle Ricerche, Via Amendola 165/A, 70126 Bari, Italy

The citrus nematode, *Tylenchulus semipenetrans*, is widespread in all citrus areas of Italy and is commonly found worldwide on citrus crops. Studies on the biological control agents regulating the nematode density in soil have been carried out in citrus orchards in Southern Italy for more than a decade. A Gram-positive bacterium of the genus *Pasteuria* (Bacillaceae) was observed in a population of *T. semipenetrans* found at Taviano (Lecce). Models previously applied to prevalence and nematode density data obtained through population dynamics studies showed a potential of the bacterium for nematodes regulation. The antagonist, which appears a new species, develops in vermiform stages and was detected with prevalence levels usually lower than 20-25 % of juveniles. It appears highly persistent in soil microcosms since it was found, during long term studies, in the same field 15 years after its initial discovery. Other data from field surveys carried out in Sicily and Basilicata showed that *T. semipenetrans* is associated to several parasitic hypomycetes present in the citrus rhizosphere, including *Pochonia chlamydosporia* and nematophagous fungi of the genera *Arthrobotrys*, *Dactylellina* and *Monacrosporium*. This complex of species is considered a rhizosphere limiting factor for *T. semipenetrans* and, together with soil fertility, provides a potential application tool in nematode population management.