IOBC / WPRS
Working group “Integrated Protection of Olive Crops”

OILB / SROP
Groupe de travail “Protection Intégrée des Olivaires”

Proceedings of the meeting
Comptes rendus de la réunion
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Chania (Crete, Greece)
29-31 May 2003

Edited by:
Argyro Kalaitzaki, Venizelos Alexandrakis, Kyriaki Varikou

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Preface

This bulletin contains the proceedings of the European meeting of the IOBC/WPRS Working Group “Integrated Protection of Olives Crops” that was be held in Chania, Greece May 29-31, 2003 in the Mediterranean Agronomic Institute of Chania (MAICh).

More than 110 scientists from 11 different olive growing countries attended the meeting, which included 38 oral presentations and 32 poster sessions. Scientists from different countries linked together by common problems and common aim the development of integrated control strategies for pests in olive groves, which preserve the complex of natural enemies and reduce inputs of pesticides, thereby minimizing impacts on the environment.

On behalf of the Local Organizing Committee and the Working Group I express my gratitude towards the Hellenic Ministry of Agriculture, the Institute of Olive Tree and Subtropical Plants of Chania (N.AG.RE.F) for supporting the meeting, Dow AgroSciences, Elanco Hellas and Elaiourgiki who financial support the organization of the meeting. I also thank most sincerely the Conference Centre Bureau of Maich for supporting us organizing the meeting.

During the meeting in Chania, I was elected as the new convener of the Working Group, a change that has now been ratified by the Council.

Argyro Kalaitzaki
Convener of the Working Group
Integrated Protection of Olive Crops
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List of participants

ALEXANDRAKIS Venizelos  
Institute of Olive Tree and Subtropical Plants of Chania,  
731 00 Chania  
GREECE  
Tel:0030 28210 83449  
Fax:0030 28210 93963  
E-mail:valexandr@nagref-cha.gr

ANASTASIADIS Michalis  
Centre of Agricultural Extension and Development of Kastori  
Kastori  
230 59 Kastori  
GREECE  
Tel:0030 27310 57770  
Fax:0030 27310 57828

APOSTOLOU Konstantinos  
Division of Agricultural Development  
26, Sfakion str.  
731 00 Chania  
GREECE  
Tel:0030 28210 28281  
Fax: 0030 28210 28047

ATHANASSIOU Christos  
Agricultural University of Athenes.  
75, Iera Odos Athens  
118 55 Athens  
GREECE  
Tel:0030 210 5294582  
Fax:0030 210 5294572  
E-mail:xathanas@uth.gr

BARBOPOULOU Eleni  
Institute of Olive Tree and Subtropical Plants of Chania  
Agrokipo  
731 00 Chania  
GREECE  
Tel:0030 28210 83425  
Fax:0030 28210 93963  
E-mail:e Barbopoulou@nagref-cha.gr

BELCARI Antonio  
University of Florence  
18 Piazzale delle Cascine  
501 44 Florence  
ITALY  
Tel:0039 0553 288277  
Fax:0039 0553 288277  
E-mail:antonio.belcar@unifi.it

BENTO Albino Antonio  
Escola Superior Agraria IPB  
172 Quinta Sta Apolonia Apartado  
5300-855 Braganca  
PORTUGAL  
Tel:00351 273 303392  
Fax:00351 273 325405  
E-mail:bento@ipb.pt

BESRI Mohamed  
Institut Agronomique et Veterinaire Hassan II  
Rabat Institut  
B. P 6202 Rabat  
MOROCCO  
Tel:00212 370778364  
Fax:00212 37778364  
Email:mohbersi@yahoo.fr/m.bersi@iav.ac.ma

BJELIS Mario  
Institut for Plant Protection in Agriculture and Forestry  
14A Zvonimirsova Solin  
212 10 Solin  
REPUBLIC OF CROATIA  
Tel:00385 21213575  
Fax:00385 21213561  
E-mail:zavodzb@st.tel.hr

BOURBOS Vaggelis  
Institute of Olive Tree and Subtropical Plants of Chania  
Agrokipo  
731 00 Chania  
GREECE  
Tel:0030 28210 83425  
Fax:0030 28210 93963  
E-mail:vbourbos@nagref-cha.gr
GIAGELI Anastasia
Centre Regional de la Protection des Vegetaux
Zaimi 23 street
261 10 Patra
GREECE
Tel:003 2610 271959
Fax: 0030 2610 623238
E-mail: pkpfpatr@otenet.gr

GIANNOPOLITIS C. N.
Benaki Phytopathology Institute
8 S. Delta str.
145 61 Kiphissia
GREECE
Tel:0030 210 8077590
Fax:0030 210 8077506
E-mail: bpiweed@otenet.gr

GOGAS Anastasios
25 El. Venizelou str.
245 00 Kyparissia
GREECE
Tel:0030 27610 25081
Fax:0030 27610 25081

GOUNAKI Pinelopi
Institute Olive Tree and Subtropical Plants of Chania
Agrokipio
731 00 Chania
GREECE
Tel:0030 28210 83426
Fax:0030 28210 93963

GUIDOTTI Diego
Scuola Superiore S. Anna
Via Rinacoo Piaggio
561 25 Pontebera
ITALY
Tel:0039 0 50 883442
E-mail: ascoli@555up.it

HAFEZ Mohamed Bahaa
Faculty of Agric. Alexandria Univ.
Alex.Univ. Faculty of Agric. El-Shatby
Alexandria
EGYPT
Tel:0020 122208099
Fax:0020 35840909
E-mail: hishambahaa@hotmail.com

HEGAZI Esmat
Faculty of Agriculture Alexandria University
El Shatby
Alexandria
EGYPT
Tel:00203 541730
Fax:00203 590849
E-mail: eshegazi@hotmail.com

HILAL Abdelkader
National Institute of Agronomic Research
Inra Marrakech
B. P. 533 Marrakech
MOROCCO
Tel:0021 24447864
Fax:0021 24446380
E-mail: inramrk@iam.net.ma

HOSSEINEJAD Seyed Abbas
Plant Pests and Diseases Research Institute
P.O Box 1454-19395
193 95 Tehran
IRAN
Tel:0098 21243761
Fax:0098 212403691
E-mail: hoseninejad@yahoo.com

IOANNOY Aristidis
Ministry of Agriculture
Directorate of Plant Produce Protection
150 Sygrou str.
176 71 Athens
GREECE
Tel:0030 210 9212091
Fax:0030 210 3632168
E-mail: m.michalaki@minagr.gr

JARDAK Taieb
Unite de Protection des plantes et de l'environnement
Institut de l Olivier Route de l Aeroport 3000 Sfax
3000 Sfax
TUNISIA
Tel:00216 74241240 /589
Fax:00216 74241033
E-mail: jardak.taieb@iresa.agrinet.tn

KALAITZAKI Argyro
Inst. of Olive Tree and Subtrop. Plants of Chania
Agrokipio
731 00 Chania
GREECE
Tel:0030 28210 83449
Fax:0030 28210 93963
E-mail: argkalaitzaki@yahoo.com
KAPETANAKIS Evangelos
Technological Education Institute of Crete
PO Box 140
715 00 Heraklion
GREECE
Tel:0030 2810 379409
Fax:0030 2810 319282
E-mail:ekapet@teiher.gr

KARABELAS George
BASF Agro Hellas
Aigialias 48 str.
151 25 Athens
GREECE
Tel:0030 210 6859010-9
Fax:0030 210 6850830

KARAMAOUNA Filitsa
Hellenic Ministry of Agriculture
Directorate of Plant Produce Protection
150 Sygrou str.
176 71 Athens
GREECE
Tel:0030 210 2124539
Fax:0030 210 3617103
E-mail:korioan@minagric.gr

KATSOYANNOS Byron
Aristotle University of Thessaloniki
Laboratory of Applied Zoology and Parasitology
541 24 Thessaloniki
GREECE
Tel:0030 2310 998841
Fax:0030 2310 998853
E-mail:katsoy@agro.auth.gr

KAVALLIERATOS Nickolas
Benaki Phytopathological Institute Labr.of Agrig. Entomology
Delta Str. 14561
145 61 Athens
GREECE
Tel:0030 210 2128018
Fax:0030 210 8077506
E-mail:nick_kaval@hotmail.com

KLIMENTZOU Persefoni
NCSR Demokritos
Aghia Paraskevi
153 10 Aghia Paraskevi Attikis
GREECE
Tel:0030 210 6503839
Fax:0030210 6545695
E-mail:ersyk@rrp.demokritos.gr

KOLEVRIS Anastasios
National Marine Park of Zakynthos
1a Archiepiskopou Latta
291 00 Zakynthos
GREECE
Tel:0030 695029872 0030 0695042142
Fax:0030 06950281740
E-mail:tkolevris@nmp-zak.org

KONDIS Angeliki
Ministry of Agriculture
Paraliaki str. Nafplio-N.Kios
211 00 Nafplio
GREECE
Tel:0030 27520 26241
Fax:0030 27520 27629
E-mail:pkpfpn@otenet.gr

KONSTANTOPOULOU Maria
NCSR " Demokritos" Institute of Biology
153 10 Ag. Paraskevi Attikis
602 28 Athens
GREECE
Tel:0030 210 6503577
Fax:0030 210 6511767
E-mail:mkonstan@bio.demokritos.gr

KONTODIMAS Dimitris
Benaki Phytopathological Institute
2 Ekalis str Kifisia
145 61 Athens
GREECE
Tel:0030 2102128019
Fax:0030 2108077506
E-mail:dckontodimas@hotmail.com

KOULOSSIS Nikos
Aristotle University of Thessaloniki
Lab of Applied Zoology & Parasitology
541 24 Thessaloniki
GREECE
Tel:0030 2310 998836
Fax:0030 2310 998843
E-mail:nikoul@agro.auth.gr

KYRIAKOPOYLOY Pagona
A.G.S.A
6 Lydias str.
161 21 Athens
GREECE
Tel:0030 210 7231053
E-mail:gatimou@yahoo.gr
LENTINI Andrea
Universita degli studi di Sassari
Via Enrico De Nicola
071 00 Sassari
ITALY
Tel:0039 079 229361
Fax:0039 079 229329
E-mail:istent@uniss.it

LO DUCA Paola
Università degli Studi della Tuscia
38 Via Saluzzo
001 82 Roma
ITALY
Tel:0039 3339719830
Fax:0039 0761 357473

LOUPASSAKI Marianthi
Institute of Olive tree and Subtropical Plants
731 00 Chania Crete
GREECE
Tel:0030 2821 083456
Fax:0030 2821 093963
E-mail:mloupas@nagref-cha.gr

LYKOURESSIS Dionysios
Agricultural University of Athens
75 Iera Odos str.
118 55 Athens
GREECE
Tel:0030 210 5294581
Fax:0030 210 5294577
E-mail:lykouressis@aua.gr

MALANDRAKI Eleni
Division of Agricultural Development
26, Sfakion str.
731 00 Chania
GREECE
Tel:0030 28210 28281
Fax:0030 28210 28047
E-mail:emalandr@otenet.gr

MALATHRAKIS Nikolaos
TEI
Stauromenos PO Box 140
711 10 Heraklion
GREECE
Tel:0030 2810 379459
Fax: 00302810 318204
E-mail:nmal@steg.tehier.gr

MALIKOYTSASI-MATHIOUDI Maria
NAGREF
Aigialias 19 & Chalepa
151 25 Maroussi Athens
GREECE
Tel: 0030 210 8175446
Fax:0030 210 6846700
E-mail:dirbdepta@nagref.gr

MANOUSAKI Klio
Division of Agricultural Development
39, Smyrnis str.
731 36 Chania
GREECE
Tel:6973348588
Fax:0030 28210 28047
E-mail:kelly_manousaki@yahoo.gr

MARKOGIANNAKI-PRINTZIOU Dimitra
Benaki Phytopathological Institute
8 St. Delta Street
145 61 Athens Kifissia
GREECE
Tel:0030 210 2128017
Fax:0030 210 8077506
E-mail:bpiacar-bpllibr@otenet.g

MAVROTAS Costas
Dow Agro Sciences
85 Vouliagmenis Avenue, City Plaza Center,
Glyfada
166 74 Athens
GREECE
Tel:0030 210 9632496
E-mail:cmavrotas@dow.com

MAZOMENOS Vasiliou
Institute of Biology NCSR
Inst. of Biology NCSR "D" Aghia Paraskevi
153 10 Athens
GREECE
Tel:0030 210 6503556
Fax:0030 210 6511767
E-mail:bamazom@bio.demokritos.gr

METALLINOS Panajotis
Prefecture of Corfu
491 00 Corfu
GREECE
Tel:0030 26610 40119
Fax:0030 26610 89125
E-mail:U14502@minagric.gr
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution/Governmental Body</th>
<th>Address</th>
<th>Country</th>
<th>Contact Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>METZIDAKIS Ioannis</td>
<td>Institute of Subtropical Plants &amp; Olive Tree Agrokipio</td>
<td>731 00 Chania</td>
<td>GREECE</td>
<td>Tel:0030 28210 83434 Fax:0030 28210 93963 E-mail:<a href="mailto:imetzis@nagref-cha.gr">imetzis@nagref-cha.gr</a></td>
</tr>
<tr>
<td>MICHALAKI Maria</td>
<td>Ministry of Agriculture</td>
<td>150 Sygrou str. 176 71 Athens</td>
<td>GREECE</td>
<td>Tel:0034 53 026568 Fax:0034 53 026508 E-mail:<a href="mailto:m.michalaki@minagr.gr">m.michalaki@minagr.gr</a></td>
</tr>
<tr>
<td>MICHELAKIS Stelios</td>
<td>Institute of Subtropical Plants &amp; Olive Tree Agrokipio</td>
<td>731 00 Chania</td>
<td>GREECE</td>
<td>Tel:0030 28250 83710 Fax:0030 28250 83726 E-mail:<a href="mailto:phyto18@otenet.gr">phyto18@otenet.gr</a></td>
</tr>
<tr>
<td>NEOKOSMIDI Afroditi</td>
<td>NCSR &quot;Demokritos&quot;</td>
<td>Aghia Paraskevi 15310 153 10 Aghia Paraskevi Attikis</td>
<td>GREECE</td>
<td>Tel:0030 210 6503839 Fax:0030 210 654695 E-mail:<a href="mailto:afrone@rrp.demokritos.gr">afrone@rrp.demokritos.gr</a></td>
</tr>
<tr>
<td>NIKLIS Nikolaos</td>
<td>Regional Centre of Plant Prot. and Quality Control</td>
<td>Thermi Thessalonikis 570 01 Thessaloniki</td>
<td>GREECE</td>
<td>Tel:0030 210 410010 Fax:0030 210 471210 E-mail:<a href="mailto:niklis@otenet.gr">niklis@otenet.gr</a></td>
</tr>
<tr>
<td>NOURMOHAMMADI Zahra</td>
<td>National Center for Genetic Eng. &amp; Biotechnol.</td>
<td>15 Ghods st Enghelab Ave</td>
<td>IRAN</td>
<td>Tel:0098 21 6419826 Fax:009821 6419834 E-mail:<a href="mailto:marjan-n@nrcgeb.ac.ir">marjan-n@nrcgeb.ac.ir</a> <a href="mailto:marjanmm@yahoo.com">marjanmm@yahoo.com</a></td>
</tr>
<tr>
<td>ORTIZ Antonio</td>
<td>University of Jaen</td>
<td>28 C/ Alfonso X el Sabio 23700 Linares (Jaen)</td>
<td>SPAIN</td>
<td>Tel:00351 273 303392 Fax:00351 273 325405 E-mail:<a href="mailto:saps@ipb.pt">saps@ipb.pt</a></td>
</tr>
<tr>
<td>OUGUAS Yamna</td>
<td>National Institute of Agronomic Research</td>
<td>Inra Marrakech B.P. 533 Marrakech</td>
<td>MOROCCO</td>
<td>Tel:00212 4447864 Fax:00212 4446380 E-mail:<a href="mailto:inramrk@iam.net.ma">inramrk@iam.net.ma</a></td>
</tr>
<tr>
<td>PAIVA SANTOS Sonia Alexandra</td>
<td>Inst. Politec. de Braganca Escola Superior Agraria</td>
<td>172 Campus de Sta Apolonia 5301-855 Braganca</td>
<td>PORTUGAL</td>
<td>Tel:0030 2810 2224280 Fax:0030 2810 225616 E-mail: <a href="mailto:pkpfghgr@otenet.gr">pkpfghgr@otenet.gr</a></td>
</tr>
<tr>
<td>PAPADOPOULOU Anastasia</td>
<td>Centre Regional de la Protection des Vegetaux</td>
<td>Zaimi 23 street 261 10 Patra</td>
<td>GREECE</td>
<td>Tel:003 2601 224629 Fax:003 2610 623238 E-mail: <a href="mailto:pkpfpatr@otenet.gr">pkpfpatr@otenet.gr</a></td>
</tr>
<tr>
<td>PANDOUVAKIS Ioannis</td>
<td>Regional Center for Plant Protection</td>
<td>Monis Gouvernetouou 713 07 Iraklio</td>
<td>GREECE</td>
<td>Tel:0030 2810 2224280 Fax:0030 2810 225616 E-mail: <a href="mailto:pkpfghgr@otenet.gr">pkpfghgr@otenet.gr</a></td>
</tr>
<tr>
<td>PAPADOPOULOU Anastasia</td>
<td>Centre Regional de la Protection des Vegetaux</td>
<td>Zaimi 23 street 261 10 Patra</td>
<td>GREECE</td>
<td>Tel:003 2601 224629 Fax:003 2610 623238 E-mail: <a href="mailto:pkpfpatr@otenet.gr">pkpfpatr@otenet.gr</a></td>
</tr>
</tbody>
</table>
PROPHETOU ATHANASIADOU Dimitra
Aristotelian University of Thessalonica
540 06 Thessalonica
GREECE
Tel:0030 210 9630251
E-mail:prophet@agro.auth.gr

PSYLLAKIS Nikolas
16, Ioumenou Gavriil str.
731 00 Chania
GREECE
Tel:0030 28210 42928
Fax:0030 28210 54949

RAGOUSSIS Nikitas
VIORYL S.A.
36 Viltaniotis Str
145 64 Athens
GREECE
Tel:0030 210 8074681
Fax:0030 210 8074603
E-mail:nragoussis@vioryl.gr

RODITAKIS Nikos
NAGREF Plant Protection Institute Heraklion
32 Kastorias str, 2229
713 07 Heraklion
GREECE
Tel:0030 2810 343787
Fax:0030 2810 24587
E-mail:roditakis@her.frothnet.gr

RUANO Francisca
University of Granada
Dept. of Animal Biology and Ecology.
180 71 Granada
SPAIN
Tel:0034 958 243383
Fax:0034 958 243238
E-mail:fruano@ugr.es

SABATHIANAKI Maria
Division of Agricultural Development
26 Sfakion Str.
731 34 Chania
GREECE
Tel:0030 2821053517

SANTOS Sonia Alexandra
Escola Superior Agraria IPB
172 Quinta Sta Apolonia Apartado
5300-855 Braganca
PORTUGAL
Tel:00351 273 303344
E-mail:saps@ipb.pt

SERVIS Dimitris
BASF Agro Hellas
48 Aigiaillas str
151 25 Athens
GREECE
Tel:0030 210 6859010-9
Fax: 0030 210 6850830
E-mail: dimitris.servis@ basf-agro.gr

SOROOSH Mohammad Javad
Tehran Islamic Republic of Iran
2 Tabnak Ave. Evin
19395-4568 Tehran
IRAN
Tel:0098 21 2402046
Fax:0098 21 2403197
E-mail:nssoroosh@yahoo.com

SPANEDDA Antonio Franco
Università della Tuscia Viterbo
Via S. Camillo de Lellis s.n.c.
011 00 Viterbo
ITALY
Tel:0039 0761 357465
Fax:0039 0761 357473
E-mail:cpucci@unitus.it

STAVRAKIS George
Laboratories of insect attractants and traps
"Phytophyl"
Shimatari Viotia
320 09 Shimatari
GREECE
Tel:0030 22620 58670
Fax:0030 22620 58735
E-mail:nista@otenet.gr

TALHINHAS Pedro
Instituto Superior de Agronomia
Tapada da Ajuda
1349-017 Lisboa
PORTUGAL
Tel:00351 213638161
Fax:00351 213635031
E-mail:ptalhinhas@isa.utl.pt

TEIXEIRA Rita
Dgpe Edif. I. Tapada Da Ajuda
1349-018 Lisboa
PORTUGAL
Tel:00 351 21 3613243
Fax:00351 21 3613277
E-mail:ritaruivo@sapo.pt
Plenary presentation
Olive pest control: Present status and prospects

George E. Haniotakis
15-B Dionysou Ave. 14578 Ekali, Athens, Greece

Abstract: Pest control is a major concern of olive growers and its annual cost added to associated crop losses contribute substantially to the total cost of olive production.

For the Mediterranean region, the major olive producing area of the world, the following four categories of olive pests are recognized: a) Major or key pests, which cause damage of major economic importance throughout the region, require annual management and include only one species, the olive fruit fly, Bactrocera oleae Gmelin; b) Major secondary pests, which occur throughout the region, cause damage of major economic importance locally or occasionally and include the following two species, the olive moth, Prays oleae Bern and black scale, Saissetia oleae Bern. These two species were recently reduced to category b) from a) due to advances in olive pest management; c) Pests of limited or localized economic importance, which cause damage of limited economic importance locally and/or occasionally, vary with location and/or time and may include the following: Parlatoria oleae Colvee, Aspidiotus hedere Vallot, Liothrips oleae Costa, Euphylura olivina Costa, Palpita unionalis Huebn., Euzophera pinguis Hw., Zeuzera pyrina L., Rhynchites cribripennis DeStr., Phloeotribus scarabaeoides Bern; d) Pests of no economic importance, which under very rare circumstances may cause damage of limited economic importance locally. Pests of categories a) and b) only are discussed here.

Present control methods for the olive fruit fly include: a) Bait sprays, current standard control method, applied from the ground or air; b) Cover sprays; c) Mass trapping. A large variety of toxic, sticky, or liquid-containing traps are available for this purpose.

Advances in olive fruit fly control include: The improvement of crop protection level with parallel reduction of bait applications due to a better understanding of the eco-biology of the fly, modeling of its population dynamics and therefore better prognosis, trap calibration, better fly population monitoring systems, and establishment of realistic economic threshold levels; the elimination of bait applications from the air in E.U.; the production of low-cost attractants from local sources; the development of alternative control methods and their use in organic olive production, i.e., an effective mass trapping method and baits using natural pesticides, old and new.

Future prospects in olive fruit fly control include: Further improvement of crop protection levels with further reduction of pesticide use through refining, local validation and wide utilization of the existing models of population dynamics; use of new technologies for automation of field data collection and transfer to processing, and warning centers. Furthermore, new knowledge acquired through basic research currently under way in the fields of basic and applied biology, genetics, biotechnology, material science, and behavior may lead to discovery of new tools, or improvement of existing ones for the management of this pest. Current control methods for the olive moth include conventional insecticide applications in the form of sprays or dusts. Effective alternative control methods include the use of Bacillus thuringiensis (dipel), and chitin biosynthesis inhibitors (alsystin-triflumuron).

Current control methods for black scale include sprays with conventional pesticides. Alternative methods include cultural practices, biological control, and oil applications.

Key words: Olive pests, control, olive fruit fly, bait sprays, mass trapping, cover sprays
The catalogue of organisms potentially harmful to the olive tree includes over 255 species at the present time and the number is increasing with the identification of new ones, mostly mites, nematodes, and pathogenic microorganisms (see list of publications at: www.newcrops.uq.edu.au/listingoleaeuropaea.htm). More than half of them belong to the pest group and the other half to the disease-causing agents. Although only a small number (about a dozen) are capable of inflicting damage of economic importance and require management, pest control is a major concern for olive growers and its cost plus crop losses due to their activity contribute substantially to the total cost of olive production. Losses caused by parasites in general and weeds are estimated to be as high as 30% of production. Losses due to insect pests alone are estimated to be about 15%, and those due to major pests about 10%, amounting to 800 million Euros per annum (values based on IOOC figures of annual production and product prices for the 4-year period 98/99 –01/02). The annual cost of olive pest control exceeds 100 million Euros, 50% of which corresponds to pesticides (Montiel and Jones, 1992), not including the cost of the adverse side-effects of pesticide use.

For the Mediterranean region, the major olive producing area where 95% of the of the nearly 800 million olive trees of the world are grown, the following four categories of olive pests are recognized: a) Major or key pests, which cause damage of major economic importance throughout the region, require annual management and include only one species, the olive fruit fly, Bactrocera oleae Gmelin. b) Major secondary pests, which occur throughout the region, cause damage of major economic importance locally or occasionally and include two species, the olive moth, Prays oleae Bern and black scale, Saissetia oleae Bern. These two species were recently reduced to category b) from a) due to advances in olive pest management (see below). c) Pests of limited or localized economic importance, which cause damage of limited economic importance locally and/or occasionally, vary with location and/or time and may include some or all of the following species: Parlatoria oleae Colvee, Aspidiotus hederae Vallot, Liothrips oleae Costa, Euphytula olivina Costa, Palpita unionalis Huebn., Euzophera pinguis Hw., Zeuzera pyrina L., Rhynchites cribripennis DeStr., Phloeotribus scarabaeoides Bern; d) Pests of no economic importance, which under very rare circumstances may cause damage of limited local economic importance. Pests of categories a) and b) only are discussed here.

Present control methods of major olive pests

Olive fruit fly
The olive fruit fly infests olive fruit and causes damage of a quantitative and qualitative nature. Potential crop losses which can be caused by this pest in periods and at locations with conditions favoring high population densities where no control measures are taken are quoted by various authors to be as high as 80% of production with an average of 40-50%. Average crop losses, however, which usually occur with the control measures applied currently, vary between 5 and 15%, depending on the country. Present control methods for this pest include bait sprays, cover sprays, and mass trapping.

Bait sprays refer to an attractant and insecticide mix, which is sprayed directly on the foliage of the olive trees. The bait attracts and kills adults of both sexes, and therefore constitutes a preventive control method. Baits may be applied from the ground or air and consist of a food attractant (chemical or enzymatic protein hydrolyzates, ammonia releasing salts, urea), or microencapsulated sex attractant (1,7dioxaspiro [5.5] undecane) and an insecticide (organophosphate:fenthion, dimethoate, malathion, or pyrethroid:deltamethrin-decis, or natural insecticides derived from plants (pyrethrum, rotenone, and mixture of the two), or from microorganisms (spinosad), at the recommended concentrations, depending on
the amount of bait used per tree or per ha. Baits of protein hydrolyzate and organophosphate applied from the ground at 300 ml per tree contain 2% hydrolyzate and 0.3% (a.i.) insecticide, while baits applied from the air at 10 l/ha, 6% hydrolyzate and 0.9% insecticide. Hydrolyzates are produced from various local protein sources available in the olive growing countries. Flies feed on baits containing protein hydrolyzate but not on baits containing ammonia releasing alts. Although feeding is not required for fly killing, as death results from its contact with the insecticide of the bait, it enhances the killing rate. For this reason molasses is usually added to salt preparations to encourage fly feeding.

Baits were developed for application on a small part of foliage, “spot spraying”. Originally, when the baits were applied by means of small, manually operated back-carried sprayers, this was feasible. Today, however, with the shortage of farm labor and the expansion of the olive orchards, baits are applied by high-pressure sprayers, which are mounted on tractors, airplanes, or helicopters, and spot spraying is not practical. Theoretically, ground equipment spot spray one every 2, 3, or even 4 rows of trees. In actual practice, however, a large portion of the tree canopy is sprayed as tractors move between rows of trees, and the olive growers, at least in Greece, insist on spraying all rows, claiming that the efficacy of the method is reduced otherwise. Similarly, aerial bait spraying is supposed to be applied on 20-25 m wide swathes every 100 m of grove but in practice this is left to the pilot, who usually sprays the entire area due to practical difficulties in finding and remembering characteristic marking lines on the ground. For this reason, and due to their strong adverse side effects, air applications of baits containing conventional insecticides are not allowed today within the E.U. except locally and after special permission.

Bait sprays, when properly and timely applied, are very effective and constitute the current standard control method. They have, however, a number of shortcomings and scientists working in the field eagerly seek their improvement or the development of alternative methods. More specifically, requirements for high efficacy of this method are: area-wide application, due to high mobility of the adult flies, accurate timing so that fruit infestation is avoided, and prolonged residual activity of the bait. Area-wide applications require special organization and agreement by all olive growers of the area, both of which may pose difficulties. Accurate timing of applications requires accurate determination of pest population densities, which can be achieved through an effective monitoring system operated by conscientious and experienced workers, realistic economic threshold levels, and pest management coordinators with complete knowledge of fly biology and behavior under the local and seasonal conditions. The residual activity of baits used currently ranges from 3-7 days depending on prevailing temperatures, R.H. and wind velocity, not enough to cover the practically continuous emergence of adults due to the overlapping generations of this pest, or the immigration of adult flies into the protected area and therefore the need of a large number of applications for adequate crop protection. Furthermore, baits used currently are not selective and application in large areas, and on the entire or a large part of the tree foliage, often leads to severe diverse effects on olive agro-ecosystems. Guidelines for timing of bait sprays are currently available, which take under consideration: sex ratio (1:1) of flies captured in monitoring traps of the McPhail type, female fly maturity (presence of mature eggs), fruit susceptibility (pit hardening), and male response to pheromone traps for the first summer application, and daily catches in monitoring traps, fruit infestation level, local environmental conditions, olive variety, fruit load, and time of the year for subsequent applications (for details visit the sites: http://www.oliveoilnews.com/olive_fly.htm and http://www.oliveoilsource.com/olive_fly.htm). Computer models simulating pest population dynamics have also been developed for this purpose but their use is still very limited.
Cover sprays refer to conventional insecticide sprays applied on the entire foliage of all trees of the orchard. It is recommended in cases in which bait sprays have failed and fruit infestation exceeds the economic threshold levels, 8-10% for oil growing olive varieties depending on variety (fruit size). For table olive varieties the acceptable infestation level is zero. Cover sprays may be used also in cases in which bait sprays are not possible due to difficulties mentioned above. Timing of cover sprays is based on fruit infestation levels as above for oil varieties and when the first infested fruit is found in table olive varieties. Insecticides used in cover sprays are the same as those used in baits at the recommended concentrations. In the case of organophosphates at 10-15 l of solution per tree, 0.03% of insecticide is used. Although this method is very effective and curative, as it kills larvae and adults, because of its strong adverse side effects on the environment, the ecosystem, the farmer, and the consumer and its higher cost compared with the bait sprays, it is avoided by olive growers, especially those concerned with these issues, and used only in emergences.

Mass trapping refers to the use of devices designed to attract and kill adults of both sexes. Mass trapping relies on the same principle as bait sprays (attract and kill) and utilizes the same or similar tools, namely attractants and insect killing agents. Olive fruit fly traps are the result of an ongoing effort to extend the active life of the attractants and insecticides used in baits and to eliminate the adverse side effects of the conventional insecticides of the baits by avoiding their application directly on the foliage (Broumas et al. 1998 and references there in).

Traps of a great variety are available today for this purpose but to my knowledge, only one or two of them have been registered. Extension of the active life of the attractants and insecticides and therefore of the trap has been achieved by regulating the release rate of attractants or finding new ones, and by formulating insecticides for long residual activity e.g., use of U.V. screens and stabilizers. Ammonium bicarbonate has been found to be particularly suitable for this purpose and is used in most trap types, as it readily releases ammonia, the olive fly attracting molecule, in contact with air, with no need to be dissolved in water, as is the case with other food attractants. In some trap types the attracting yellow color is also added, at the expense of the beneficial fauna of olive orchards as a large number of predators and parasites are also attracted to the same color. To exterminate the attracted insects, the traps employ a number of methods such as long-lasting adhesives, treatment with long–life formulations of synthetic pyrethroids, or drowning in liquid-containing traps of the MacPhail or bottle type. Natural insecticides, already used in bait preparations, have not found their way as yet in to traps. Toxic traps with an effective life of six months, which covers the entire periods of fly reproduction and fruit susceptibility, are now available and registered, contributing significantly to the feasibility and efficacy of the mass trapping method. Traps, compared to bait sprays, have a lower attract and kill rate and therefore must be installed at least 10 days before the commencement of fly oviposition, which coincides with susceptibility of the new fruit, in order to eliminate females before fruit infestation.

As opposed to bait sprays, which exhibit a quick decline of fly populations followed by a quick recovery, traps gradually but steadily reduce fly population densities in an area. It has been shown that after about three consecutive years of mass trapping in an area, a long life-trap every other tree, each baited both with ammonium bicarbonate and a pheromone dispenser, is enough to keep fly population densities below economic threshold levels (Broumas et al. 2002). During the first two to three years of mass trapping, one trap per tree may be required for acceptable crop protection, and in some cases even local complementary bait applications may be necessary. Combination of bait sprays with mass trapping provides better olive protection than that achieved by either method alone, especially during periods or at locations with favorable conditions for the development of high fly population densities.
Trap placement inside the tree canopy is important for best results. Traps must be placed in a well-shaded place toward the center or higher of the tree canopy with a leaf-free space around them of about 30 cm. Although use of toxic traps is allowed today in organic olive growing, some growers avoid them, using sticky or liquid containing traps instead, at a higher cost as these traps have a short active life and require replacement during the olive growing season, and in certain cases even at the expense of the crop protection level.

**Olive moth**

The olive moth infests flowers (flower generation), fruit (fruit generation), and leaves, preferably young (leaf-fall generation), causing reduction of fruit setting, fruit drop, and general weakening of the trees, respectively. In cases of high population densities crop losses may be substantial if control measures are not taken. Current control methods are usually applied to the fruit generation and consist of conventional insecticide sprays or dusts: Control of the spring generation with conventional insecticides is avoided due to their catastrophic effects on the beneficial fauna of olive orchards, which are highly active in spring. Alternative methods are available and include the use of *Bacillus thuringiensis* (Dipel), effective against the flower generation, and alsystin (Triflumuron), effective against the fruit generation. The reduction of pesticides in olive orchards, the result of recent advances in olive pest management (see below), allowed the increase of natural enemies of the pest commonly present in olive orchards, including predatory Diptera, Lepidoptera, and Neuroptera, and a large number of parasitic Hymenoptera and some Diptera, which keep moth densities below economic threshold levels.

**Black scale**

Black scale insects excrete honeydew on which the sooty mold fungus, *Capnodium olaeophilum*, fungus grows. Heavy infestation reduces photosynthetic activity and consequently vigor and productivity of the tree. Continued scale feeding reduces bloom the following year. The existence of a large number of natural enemies, such as predatory Coleoptera, Lepidoptera, and Hymenoptera, and parasitic Hymenoptera, when undisturbed, keeps population levels of this pest below economic threshold levels. Furthermore, cultural practices such as appropriate pruning to provide open canopy (well-aerated) trees discourage infestation. When needed, current control measures include cover sprays with conventional insecticides and summer, or winter oils. Alternative control methods include cultural and biological control and oil sprays. Guidelines for monitoring and timing interventions for this pest are available at [http://www.ipm.ucdavis.edu/PMG/r583300511.html](http://www.ipm.ucdavis.edu/PMG/r583300511.html) and [http://www.oliveaustralia.aust.com/black_Olive_Scale.html](http://www.oliveaustralia.aust.com/black_Olive_Scale.html)

**Advances in olive pest management**

**Olive fruit fly**

The economic importance of this pest has prompted the investigation of almost every methodology available in the field of pest control for possible use with no practical results. The methods used for almost a century rely on the same principle, “attract and kill”. The reason for this is the existence of potent attractants, which are used in baits and traps and produce results difficult to meet by any other method at similar cost. Significant improvement, however, has occurred in the efficacy of these methods through progress in their basic components, namely the attractants, insecticides, and devices used. A brief history of their evolution, proves the truth of this.
Bait consisting of honey, molasses, lead arsenate and water at certain proportions and sprayed directly on the foliage of an olive tree branch was proposed by De Cillis in 1901 for the control of the olive fruit fly. Modifications concerning the ingredients and proportions of the bait, application time, size of the orchard to be protected, spraying of brooms hung on olive trees instead of foliage, which were made first by Berlese, hence the prevailing name of the method “Berlese method”, and subsequently by several other entomologists, were incorporated during the long period of its use, up to 1962 for Greece. More powerful food attractants such as protein hydrolyzates, ammonia-releasing salts, urea, and more recently, sex attractants, followed, combined with more effective wide-spectrum insecticides such as organophosphates, pyrethroids, and more recently natural insecticides.

The history of olive fruit fly traps used for control goes back several decades. In Spain traps of the McPhail type baited with food attractants were used early in the 20th century for the control of the olive fruit fly in the variety “cordal”, which is harvested while the fruit is still green. The use of this method was discontinued due to its many economical and technical problems. Use of similar traps in Greece in 1939 and 1953 for the same purpose did not produce satisfactory results. Attempts of trap use for control appear again in the pertinent literature with the discovery of visual attractants (Prokopy et al. 1975) and intensified with the discovery of sex attractants (Haniotakis, 1974, Baker et al. 1980). Extensive work on this subject has led to the development of the mass trapping method used today (Broumas et al. 2002) and continues for its refinement and cost reduction. For monitoring of the olive fruit fly different traps have been calibrated and used in different countries such as yellow sticky panels, sticky panels baited with food or sex attractants, and McPhail type traps baited with food attractants.

The advances mentioned above, combined with refinements in population monitoring, establishment of realistic economic threshold levels and accurate forecasting of population densities through a better understanding of the eco-biology of this pest, and especially the development of computer models simulating population dynamics which take under consideration the effects of all factors involved, biological (host/pest), environmental, and human interventions (cultural practices), have contributed to the accurate determination of the need and exact timing of interventions for maximum results. Significant reduction of the number of bait spray applications per year required for acceptable crop protection was thus achieved and therefore reduction of the amount of insecticides used (http://www.nf-2000.org/secure/Éclair/F162.htm) with parallel reduction of crop losses, especially during periods or at locations with conditions favoring the development of high pest population levels.

In the list of advances should be included also the development of alternative methods which are accepted in organic olive culture, namely the mass trapping method, and bait sprays with natural insecticides. The discovery of visual attractants could be also included in the list even though their use for control is limited due to lack of selectivity.

The elimination of air sprays on olive orchards within the E.U. and the drastic reduction of cover sprays constitute significant advances which contribute to environmental, ecological, consumer and grower protection as well as to the expansion of organic olive production.

**Olive moth and black scale**

Advances in the management of these pests include the development of alternative control methods mentioned above.

**Future prospects**

Future prospects are based on the prospects of a wider utilization of recent advances, the pursuance for verification and refinement of promising results of previous work, and the current research in old and new frontiers. More specifically:
For the olive fruit fly, models of population dynamics remain to be refined, validated and adjusted to local and seasonal conditions, which may culminate in a “precision” management system of this pest. Wide use of these models combined with the use of modern technologies for automated field data collection, and transfer to processing and warning centers will simplify model operation, prevent wrong decisions by non experienced control coordinators, and contribute to further reduction of the number of applications per year with parallel increase of crop protection level.

Use of available alternative control methods, restricted today mainly to organic olive production, in conventional olive production as well. Such use is possible through proper information dissemination and demonstration and will contribute to a significant reduction of their cost, especially of mass trapping through mass production of traps, as well as further reduction of conventional insecticide use.

Promising results, which need verification or refinement of the methods investigated, have been obtained for the control of this pest with: natural enemies including: parasitoids (http://www.sel.barc.usda.gov/.../diptera/tephriti/TephPara.htm, Collier and Van Steenwyk 2003); insect pathogens: Bacillus thuringiensis (Navrozidis et al. 2000, http://www.nt-2000.org/secure/Eclair/F162.htm), Beauveria bassiana (Grammatikakis/Intrachem Hellas personal communication); botanical insecticides-rotenone (Stavroulakis et al. 2001, http://www.ejb.org/feedback/proceedings/05/riassun2.html), pyrethrum or mixture of rotenone and pyrethrum (Montiel and Jones 2002); use of behavior modifying agents (Prophetou et al. 1991, Scarpati et al. 1993, Capasso et al. 1994, Lo Scalzo et al. 1994, Scarpati et al.1996, Kombargi et al. 1998); chemicals affecting pest physiology (Koveos and Tzanakakis 1993); chemicals effecting pest/symbion relationship (Tzanakakis 1984); photoactivatable compounds (BenAmoret al. 1999); releases of sterile insects-SIRT (Economopoulos et al. 1977); mating disruption with synthetic pheromones (Haniotakis 1987, and unpublished data); use of special techniques such as injection of chemical formulations into the olive tree (Navarro et al. 1992), and long-life bait formulations (Haniotakis et al. unpublished data).

Current research in the fields of molecular biology, biotechnology, genetics, material and information sciences related to the subject of pest control in general may contribute to finding new, or improving the efficacy of old, methods for this pest as well.

The unfortunate event of olive fruit fly introduction to California in 1998 has of course negative effects for local olive growers but positive effects on future prospects for its management. Large sums of money are being allocated for this purpose and many scientists are working on possible solutions to the problem. Priorities are given to the understanding of the biology of the insect under local conditions, to biological control with natural enemies, to artificial rearing of the insect for the purpose of the SIRT, and to the use of existing natural insecticides, and the discovery of new ones (more details at: http://www.danr.edu/calag/0301JFM/briefs.html).

For the olive moth, recent tests on biological control (Bento et al. 1998, Rodolfi and Campos 1998) and mating disruption with synthetic pheromones (Mazomenos et al. 1999) have shown possibilities for practical uses. The efficacy of rotenone is also being investigated.

For black scale, biological control with the parasites Metaphycus helvolus, M. bartletti, and Scutellista caerulea combined with proper pruning has shown to be effective in northern and coastal orchards of California (http://www.ipm.ucdavis.edu/PMG/r583300511.html).

Incorporation of all advances into an integrated olive pest management and further, to an integrated olive production system, will contribute to the development of a sustainable olive growing system, a generally accepted agricultural movement of today.
References


Bactrocera oleae
Analysis of spatio-temporal *Bactrocera oleae* (Diptera, Tephritidae) infestation distributions obtained from a large-scale monitoring network and its importance to IPM

Diego Guidotti, Giorgio Ragaglini, Ruggero Petacchi
Landscape Entomology Lab (LELab), Scuola Superiore S.Anna – Polo S.Anna Valdera,
Viale Rinaldo Piaggio 34, 56125 Pontedera (PI)

Abstract: *Bactrocera oleae* is the key-pest considered in the “Olive-oil quality improvement project” in Tuscany (Italy). In this region, a network of 286 representative farms has been created in 2002 for monitoring weekly olive fruit-fly infestations, and the obtained data have been used in advising farmers on *B. oleae* control. The field observations were made by the regional extension service, and data have been collected from an internet-based monitoring network implemented in the Landscape Entomology Laboratory (LELab) of Scuola Superiore Sant’Anna. In this paper, we rely on the Geographic Positioning System (GPS) to locate the monitoring farms and make use of farm-specific information to analyze the regional spatial pattern of *B. oleae* infestations. Data analysis has been performed with Arcview 8.2, and we used variograms to model autocorrelations between sample points and cross-validation to identify the most reliable index. We consider the utility of Geographic Information System for spatial analysis at the landscape (or large) scale and kriging technique to interpolate between sample points. The resultant map can be used to predict the beginning of *B. oleae* infestations.

Key words: *Bactrocera oleae*, geographic information systems, spatial analysis at landscape scale, pest management planning tool.

Introduction

Entomologists increasingly rely on the landscape ecology approach to study insect populations. Landscape ecology is an academic discipline that provides a scientific base for problem solving and decision making in land-use management (Coulson, 1993). In the last few years, there was an increasing interest to integrate the statistical analysis of spatial data into population ecology (Liebhold and Gurevitch, 2002).

The problem of pattern and scale is the central problem in ecology (Levin, 1992), in landscape ecology and in landscape entomology. Geographic Information Systems (GIS) and Spatial Data Analyses (SDA) proved suitable for addressing spatial problems at different scales in entomology (Coop *et al*., 1994). In fact, GIS and SDA at the landscape (or regional, or area-wide) scale are often used in applied insect ecology for representing the infestations of phytophagous insects and for improving the knowledge on ecological processes in agroecosystems science and forestry (Coulson *et al*., 1993; Liebhold *et al*., 1993; Berry Lyons *et al*., 2002). At the scale of regional ecosystem integration, GIS and SDA based analyses can now greatly improve policy and strategic decision-making to pest management. This development is due in part to the rapid evolution of IPM including greater consideration of the physical and biological bases underlying environmental processes (Saarenmaa, 1992). The use of GIS, when coupled with geostatistics, is also becoming an important tool for understanding processes that underlie population development (Liebhold *et al*., 1993). Geostatistics is particularly useful for the description of spatial patterns and for estimating...
values at unsampled locations. Spatial data analysis at the landscape scale of the olive fruit-fly (Bactrocera oleae (Gmelin)) infestation and environmental parameters may assist in both the management of the monitoring network set-up for the control of this pest species (Petacchi et al., 2002; Sciarretta et al., 2003) and in the improvement of the ecological knowledge.

In this paper we describe a methodology adopted to identify a reliable infestation index and to create a map of B. oleae infestation at landscape (or large) scale, Tuscany region in Italy, using geostatistics and GIS.

Material and methods

Data collection
Data of B. oleae infestations have been collected in Tuscany region from an Internet-based monitoring network implemented at the Landscape Entomology Laboratory of the Scuola Superiore Sant'Anna (Petacchi et al., 2001). Observations were made by the regional extension service. From July to October, each farm was visited weekly by advisors who collected 100 and characterized the B. oleae life stages.

A network of 283 representative farms has been created for monitoring weekly olive fruit-fly infestations and the data obtained have been used in advising farmers on B. oleae control within the framework of the “Olive oil quality improvement” project. In 2002, we performed an analysis of the data originating from all these farms. The farms have been geo-referred using a 1:10.000 Tuscany technical map available on Internet from the Tuscany GIS facility (http://www.rete.toscana.it/sett/territorio/cart/) or using GPS (Garmin 12 CX).

Infestation typology has been aggregated in infestation indexes. The main index adopted in olive fruit fly IPM is the Active Infestation, i.e. the percentage of olives infested by eggs, and alive first and second instar larvae.

Statistical analysis
STEP 1. In order to perform spatial analyses, we extract from the temporal population dynamics on each farm some indexes able to characterize different phenological aspects of the yearly infestation that can be used to synthetically define IPM strategies for each farm.

Table 1. Description of indices estimated and utilized for B. oleae infestation data analysis (YI = Young Infestation; AI = Active Infestation).

<table>
<thead>
<tr>
<th>Index</th>
<th>Index description</th>
</tr>
</thead>
<tbody>
<tr>
<td>JYI</td>
<td>Julian day with first presence of eggs and alive first instar larvae; this index represents the beginning of the infestation</td>
</tr>
<tr>
<td>jAI5</td>
<td>represent the first day in which the Active Infestation Index (eggs and alive first and second instar larvae) exceeds the 5,0% AI threshold</td>
</tr>
<tr>
<td>jAI10, jAI15</td>
<td>alike jA5 with 10,0% and 15,0% AI threshold; this index assesses the earliness of infestation by considering the risk of reaching economic threshold</td>
</tr>
<tr>
<td>AvgAI</td>
<td>Average of Active Infestation Index from 25 to 34 weeks of the year: it represents the level of infestation in the first generation of development of olive fruit fly</td>
</tr>
<tr>
<td>MaxAI</td>
<td>Maximum of Active Infestation Index from week 25 to 34 of the year: it represents the peak level of infestation in first generation</td>
</tr>
</tbody>
</table>
The two aspects considered by the indexes are the infestation period (expressed Julian days) during which infestation occurs or exceeds a determined threshold, and the infestation level (average and maximum of infestation in a determined period). The estimated indexes are reported in Table 1.

STEP 2. For each of infestation index, we carried out a general statistical analysis and estimated the semivariogram with a spherical model. Briefly, a semivariogram is a statistical model testing the degree of correlation among values measured in points separated by different distances. The semivariograms are interpolated by a function that describes the relationship between the distance of a point and the corresponding semivariogram. Interpolating semivariogram with a spherical model allows the use of the model parameters to describe the typology of spatial autocorrelation. Thereby, nugget expresses the semivariance at distance zero and corresponds to the sum of sampling error and spatial variability at distances smaller than the smallest sampling distance. Range represents the average distance within which the samples remain spatially correlated, and sill is the variance explained by the model. In these analyses, we considered the total sill as the overall variance, obtained by summing the nugget with the partial sill (variance due to spatial variation only). For further information the reader is referred to Liebhold (1993) who explains geostatistical concepts in applied insect ecology.

The ratio among the partial sill and the total sill expresses the importance of nugget effect on the overall determination of spatial variability, i.e. the amount of spatial variability detected for a single variable at a specific scale.

STEP 3. In order to test the data we performed a cross-validation of the spatial model. The spatial analysis has been performed using ArcGIS 8.2 Spatial Analysis and the partially auto-correlated indexes are represented by a counters map overlaying it with the Tuscany administrative borders.

Results and discussion

Tab. 2 reports the results of the general statistics analysis that has been carried out for each infestation index. It shows, among others, that the beginning of infestation occurs in average the first week of August (219 julian day) and the 10% threshold is exceeded, in average, during the first week of September (247 julian day).

Table 2. Results of the general statistical analysis on the infestation indexes

<table>
<thead>
<tr>
<th>Index</th>
<th>Average</th>
<th>Min</th>
<th>Max</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>jYI</td>
<td>219.4378</td>
<td>189</td>
<td>276</td>
<td>463.17454</td>
</tr>
<tr>
<td>jAI5</td>
<td>239.8224</td>
<td>189</td>
<td>303</td>
<td>700.30635</td>
</tr>
<tr>
<td>jAI10</td>
<td>247.3464</td>
<td>203</td>
<td>296</td>
<td>563.43842</td>
</tr>
<tr>
<td>jAI15</td>
<td>256.4694</td>
<td>203</td>
<td>296</td>
<td>536.4372</td>
</tr>
<tr>
<td>AvgAI</td>
<td>0.022052</td>
<td>0</td>
<td>0.148</td>
<td>0.0006388</td>
</tr>
<tr>
<td>MaxAI</td>
<td>0.051757</td>
<td>0</td>
<td>0.31</td>
<td>0.0031394</td>
</tr>
</tbody>
</table>

In Tab.3, the parameter estimations for the infestation indexes shows the beginning of the infestation index (jYI) and the average of infestation index in the 1st generation (AvgAI) and indicates that there is a good ratio between partial sill and total sill (Tab.3). This shows that the variance due to spatial variation only greatly contributes to the overall variance.
Table 3. The parameters obtained by fitting the indexes of a semivariogram with a spherical model.

<table>
<thead>
<tr>
<th>Index</th>
<th>Partial sill</th>
<th>Major range</th>
<th>Nugget</th>
<th>Partial sill/total sill</th>
</tr>
</thead>
<tbody>
<tr>
<td>jYI</td>
<td>1,443.30</td>
<td>1.4173</td>
<td>511.46</td>
<td>73.8%</td>
</tr>
<tr>
<td>jAI5</td>
<td>2,404.60</td>
<td>1.2304</td>
<td>1,299.70</td>
<td>64.9%</td>
</tr>
<tr>
<td>jAI10</td>
<td>1,654.00</td>
<td>1.0781</td>
<td>2,524.70</td>
<td>39.6%</td>
</tr>
<tr>
<td>jAI15</td>
<td>675.94</td>
<td>1.1121</td>
<td>2,478.00</td>
<td>21.4%</td>
</tr>
<tr>
<td>AvgAI</td>
<td>0.000730</td>
<td>2.121</td>
<td>0.000242</td>
<td>75.1%</td>
</tr>
<tr>
<td>MaxAI</td>
<td>0.002349</td>
<td>1.5307</td>
<td>0.001575</td>
<td>59.9%</td>
</tr>
</tbody>
</table>

The spatial prediction of the date for exceeding the several infestation thresholds presents greater nuggets and reduced partial sill than the other predictions. The earliness of infestation seems to be little influenced by local effects. This could be explained by the fact that variables are not influenced by pest management strategies adopted at farm level. On the contrary, the exceeding of particular levels of infestation depends on the economic threshold adopted by farmers. In Tuscany, a threshold of 10% of active infestations is commonly adopted.

The analysis of linear regression coefficients between values predicted and observed shows that the Young Infestation index presents performs best in cross validation (Tab.4).

Table 4. Results of the cross-validation analysis (RMSE = Root Mean Square Error; RMSE std.= Root Mean Square Error Standardized; MAE = Mean Absolute Error)

<table>
<thead>
<tr>
<th>Index</th>
<th>Mean Error</th>
<th>RMSE</th>
<th>RMSE Std.</th>
<th>MAE</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>jYI</td>
<td>-0.1444</td>
<td>25.1064</td>
<td>0.9816</td>
<td>14.9983</td>
<td>0.579072</td>
</tr>
<tr>
<td>jAI5</td>
<td>0.05352</td>
<td>38.6332</td>
<td>0.9629</td>
<td>28.6564</td>
<td>0.515253</td>
</tr>
<tr>
<td>jAI10</td>
<td>0.0885</td>
<td>48.5311</td>
<td>0.9553</td>
<td>40.8867</td>
<td>0.212166</td>
</tr>
<tr>
<td>jAI15</td>
<td>0.06514</td>
<td>51.3145</td>
<td>0.9289</td>
<td>43.4928</td>
<td>0.310479</td>
</tr>
<tr>
<td>AvgAI</td>
<td>-0.00013</td>
<td>1.96%</td>
<td>1.114</td>
<td>1.29%</td>
<td>0.400739</td>
</tr>
<tr>
<td>MaxAI</td>
<td>7.29E-05</td>
<td>4.01%</td>
<td>0.9515</td>
<td>2.62%</td>
<td>0.454528</td>
</tr>
</tbody>
</table>

Considering both the semivariogram analysis and the cross-validation analysis, the day of beginning of infestation displays the highest R² and hence, a stronger spatial structure than the other variables. As a consequence, we consider jYI as the most reliable index and use it for mapping.

By spatial interpolation through kriging we obtain a prediction map of jYI that we can combine with a contour map showing Tuscany administrative borders (Fig. 1). In Tuscany, the regional map of the B. oleae start infestation is a useful pest management planning tool and can be used in the deployment of pest management resources.

This analysis clearly demonstrates where B. oleae infestation starts, and furthermore, shows the influence of the sea as well as a rather complex response surface in interior areas of the Tuscany region. Much of this complexity probably resulted from other geographically distributed variables such as changes in climate, fruit quality and quantity as well as land-use.

In the further analysis we are considering a study on the spatial relationship between B. oleae infestations and agro-meteorological indices derived from developmental rate
summation methods. The aims will be to validate a model that can guide farmers in initiating monitoring procedures.

Our final aim is to transfer the SDA validated method in a Decision Support System (DSS). The DSS will assist farm advisors in locating representative olive groves where monitoring of *B. oleae* infestation can efficiently be done and from where IPM relevant information can be easily transferred to other farms.

![Start of infestation (Young Larvae Index >0)](image)

**Figure 1:** prediction map of jYI (Young Infestation Index) observed on monitored farms and expressed in Julian days. The thematic points represent the location of monitoring farms.

**Conclusion**

The data quality and the choice of an appropriate index to compare the spatial autocorrelation of several variables are the most important features of the *B. oleae* large scale monitoring network. The adoption of adequate methodology for data evaluation and the testing of several validation methods in spatial data analysis are prerequisites for the analysis.

In Tuscany, the regional map of the beginning *B. oleae* infestations represents a useful pest management planning tool and can be used in the deployment of pest management resources.

In this article, particular attention was given on the practical application of Spatial Data Analysis within the framework of the regional *B. oleae* Monitoring Network. A
A comprehensive description of the methodology, results obtained and their implications is under preparation.

Acknowledgements

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References

Olive fruit fly (Bactrocera oleae, Gmelin) pheromone determination in biological samples, by an enzyme-linked immunosorbent assay (Elisa)

Afroditi Neokosmidis1,2, Evangelia Livaniou1, Christos Zikos1, Maria Paravatou-Petsotat1, Valentine Ragoussis2, Nikitas Ragoussis3 and Gregory P. Evangelatos1
1 NCSR “Demokritos”, RRP Institute, Aghia Paraskevi, 153 10 Greece
2 University of Athens, Department of Chemistry, Division of Organic Chemistry, Panepistimiopolis Zographou, 157 71, Greece
3 VIORYL S.A., Research Department, Kato Kifissia, 145 64 Athens, Greece

Abstract: The olive fruit fly is the major pest of the olive, in all the Mediterranean countries and recently is introduced in California. Considerable efforts have been made worldwide, for the development of alternative control methods of this noxious insect, using its pheromone, in order to eliminate, or at least to reduce, spraying with toxic chemical insecticides. For the effective application of the pheromone, there is a need of a rapid and accurate technique to measure the quantity of the pheromone in the dispensers, in the environment and in biological samples. Until today pheromones, have been determined by gas chromatography (GC), since they are low molecular weight volatile compounds. However, these determinations are expensive, difficult to perform and time consuming for the sample preparation. We have recently developed a competitive enzyme-linked immunosorbent assay (ELISA), for monitoring and reliably quantifying the pheromone of the olive fruit fly, in biological and environmental samples. This assay uses a rabbit polyclonal anti-pheromone antiserum (which has been raised against a suitable pheromone hapten, conjugated to the carrier protein keyhole limpet hemocyanin), in combination with standard pheromone solutions (0.08-10 µg/ml) and a biotinylated pheromone derivative appropriately immobilized onto avidin pre-coated ELISA microwells. Horseradish peroxidase – labeled goat anti-rabbit IgG (second antibody) and ABTS (chromogenic substrate) were also used in the ELISA assay. The described technique is, to our knowledge, the first pheromone enzyme-immunoassay so far reported. In preliminary experiments, the ELISA displacement curve was used for determining the olive fruit fly pheromone in insect samples. More specifically, abdomens or glands obtained from six-day old virgin female olive flies were homogenized in the presence of phosphate buffer containing an appropriate quantity of organic solvent and centrifuged. The supernatants were collected and used as unknown samples in the ELISA assay. Confirmation of the results was made by analysis of the same samples in parallel by the conventional GC analytical method. The results will be presented in details. Further research is currently in progress, including the determination of the olive fruit fly pheromone in environmental samples.

Key words: Bactrocera oleae, pheromone, olive fruit fly, enzyme-linked immunosorbent assay (ELISA)

Introduction

The olive fruit fly Bactrocera oleae Gmelin, is the major pest of the olive cultivation in the Mediterranean area and has recently been introduced in California. The over-use of chemical insecticides for the control of this insect and the consequent damage to the environment and to the public health is a serious problem. Recently, considerable efforts have been paid worldwide, for the development of alternative control methods, which exploit biological factors (natural enemies, predators, bacteria, viruses, etc) or biochemical factors (hormones, pheromones etc), in order to keep the insect population under control. From all these methods
the most promising results are obtained by the use of the insect’s own pheromone. The more widely used technique is the mass trapping, combining a pheromone dispenser and a food attractant on an entomotoxic surface. Although unsuccessfully several applications were made, of the sexual confusion technique, in which pheromone is diffused in the environment at quantities enough to make matting disruption. For the successful application of the above methods, a prerequisite is the ability to measure rapidly and accurately the amount of pheromone which is present in the dispensers, in the environment or in the insects. These analyses will allow to determine the exact amount of synthetic pheromone required, for a more efficient attraction to the traps (mass trapping) (Broumas et al., 2002; Haniotakis et al., 1986) or to compete successfully the natural pheromone released by the insects (matting disruption) (Carde, 1990). Until today pheromone levels have been determined by gas chromatography (GC) (Jones, 1999), since the pheromones are volatile compounds of low molecular weight.

The determinations are, however, time consuming and difficult to perform because: a) the sample collection and treatment is difficult, since the samples must be free of all the high molecular weight compounds that coexist with the pheromone and may contaminate the chromatography columns, b) every sample is measured separately and with different protocol, depending on its origin, and c) every measurement takes at least 30min and many repetitions are necessary in order to avoid random mistakes.

The immunoassay methods, which are based on the use of specific antibodies, are the most appropriate techniques for analyzing large number of samples in the minimum time, with high sensitivity, precision and accuracy. Until today no insect pheromone immunoassay has been developed, because it is extremely difficult to raise specific antibodies against these low molecular weight compounds.

The objective of the present work was to apply an immunoassay for the determination of the olive fruit fly pheromone (Bactrocera oleae, Gmelin) in biological samples. The development of an immunoassay (Gosling, 1996) for this pheromone, which will guarantee high sensitivity and accuracy and in addition, provide the capability of analyzing many samples in short time, may be an invaluable tool for the successful application of alternative methods against this insect pest.

Materials and methods

Preparation of the Immunoassay Reagents
Conjugation of Pheromone-Hapten I to the Carrier Protein KLH: The carrier protein (10 mg) was dissolved in 0.1N HCl (1mL). Synthetic (+)-δ-[3-(1,7dioxaspiro[5.5]undecane)]propionic acid 2 (pheromone-hapten I) (3 mg), in water (100 µL), and 1-ethyl-3-(3-dimethyl-amino-propyl)carbodiimide hydrochloride (EDC) (25 mg), in water (100 µL), were added to the above solution. The pH was tested and then adjusted to 5.0. The solution was left to react, under stirring, for 5-6 h, at room temperature and then overnight at 4ºC. Next morning the solution was dialyzed (M.W. cut off: 6,000-8,000) against water for 48 h. After dialysis, the solution was transferred from the dialysis membrane to a tube, appropriately diluted with saline to a concentration calculated as 200 µg KLH/mL, divided into 0.5 mL aliquots and kept at -35ºC.

Immunization
Female New Zealand, two-month old white rabbits were used for raising polyclonal antibodies. Routinely, the conjugate of pheromone-hapten I to KLH dissolved in saline (quantity of conjugate corresponding to 100 µg KLH / 0.5 mL) was thoroughly emulsified with an equal volume of Freund’s complete adjuvant. The emulsion was subcutaneously
injected on the back of the rabbit. The first boost was given 7 weeks after the first immunization, while the following ones every 4 weeks in the same manner. On the 13th day after each boost, a blood sample was drawn from the marginal ear vein of the rabbit to check the titer of the polyclonal antibody. Boosts were given 7 times. The blood sample was centrifuged (2,000 g) and then left to stand in order to separate the antiserum from the blood cells. The antiserum was divided into 0.5 mL aliquots, an equal volume of glycerol was added to each aliquot and the aliquots were stored to -35°C.

**Conjugation of Pheromone-Hapten II to Biotin**
The amine derivative, (±)-δ-[3-(1,7-dioxaspiro[5.5]undecane)]butylamine 3 (pheromone-hapten-II) (5 mg, 0.022 mmol), in DMF (100 µL), was added to a solution of the long chain succinimidyl ester of biotin (12.24 mg, 0.022 mmol), in DMF (100 µL). The mixture was stirred overnight at room temperature. End of the reaction was confirmed by pH determination (change from alkaline to neutral). The crude product was dissolved in CHCl₃ and washed successively with NaHCO₃ 5 %, HCl 0.1N and H₂O (twice). Then the solvent was removed under reduced pressure, the pure product was dissolved in a mixture of H₂O : EtOH, 8 : 2 (v/v), and lyophilized.

**Evaluation of the Anti-Pheromone Antisera**
ELISA microwells were coated with 100µl/well of avidin, 10 µg/ml, in a carbonate buffer pH 9.6 and allowed to stand overnight at 4°C. On the following day, the plate was washed twice with PBS pH 7.4 and thoroughly tapped dry. Sites not coated with avidin were blocked with 200 µl/well of blocking buffer [0.05 % (v/v) Tween 20 in PBS pH 7.4 which contained 2 % (w/v) BSA]. 100µl of biotinylated pheromone (conjugate pheromone-hapten II – biotin), 1µg/ml in PBS pH 7.4, were added to the wells and the plate was incubated at room temperature for 1 h. Then the wells were washed three times with washing buffer [0.05 % (v/v) Tween 20 in PBS pH 7.4]. After incubation at room temperature for 1 h, the plates were washed as described above. Then, 100 µl of each anti-pheromone antiserum diluted (1:20,000 – 1:1,000) with dilution buffer [0.05 % (v/v) Tween 20 in PBS pH 7.4, which contained 0.2 % (w/v) BSA] were added to the wells, and the plate was incubated at 37°C for 2 h. The plate was washed 3 times with washing buffer and then, 100 µl of a second antibody, horseradish peroxidase-labeled, were added, and the plate was incubated for 2 h at 37°C. Afterwards, the plate was washed 3 times with washing buffer, and 100 µl of a suitable enzyme substrate [solution of 2,2’-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (1 mg/ml) / H₂O₂ (0.003 %), in citric / phosphate buffer solution pH 4.4] were added to each well. The plate was left for 30min at room temperature for color development and the optical absorption was measured at 405 nm. Various blank microwells were included in each run of the ELISA titer curve, in which either the avidin coating solution, or the biotinylated pheromone (conjugate pheromone-hapten II – biotin), or the specific anti-pheromone antiserum was omitted.

**ELISA Displacement Curve**
ELISA microwells were coated with 100 µl/well of avidin, 10 µg/ml, in a carbonate buffer pH 9.6 and allowed to stand overnight at 4°C. Next day, the plate was washed twice with PBS pH 7.4 and thoroughly tapped dry. Sites not coated with avidin were blocked with 200 µl/well of blocking buffer [0.05 % (v/v) Tween 20 in PBS pH 7.4 which contained 2 % (w/v) BSA]. Then 100 µl of biotinylated pheromone (conjugate pheromone-hapten II – biotin), 1 µg/ml in PBS pH 7.4, were added to the wells and the plate was incubated at room temperature for 1 h. Then the wells were washed three times with washing buffer [0.05 % (v/v) Tween 20 in PBS
pH 7.4]. After incubation at room temperature for 1 h, the plates were washed as described above. 50 µl of standard synthetic pheromone solutions (product of Vioryl SA), in concentrations ranging from 50 µg/ml to 20 ng/ml (or 50 µl of unknown samples for analysis) and 50 µl of specific anti-pheromone antiserum (pool of the antisera with best titers), diluted 1:5,000 with dilution buffer [0.05 % (v/v) Tween 20 in PBS pH 7.4, which contained 0.2 % (w/v) BSA], were added to each microwell in triplicate and they were incubated at 37°C for 2 h. After washing 3 times the plate with washing buffer, 100 µl of a second antibody, horseradish peroxidase-labeled, were added, and the plate was incubated for 2 h at 37°C. The plate was washed 3 times with washing buffer, and 100 µl of a suitable enzyme substrate [solution of 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (1 mg/ml) / H₂O₂ (0.003 %), in citric / phosphate buffer solution pH 4.4] were added to each well. The plate was left for 30min at room temperature for color development and then the optical absorption was measured at 405 nm. Various blank microwells were included in each run of the ELISA displacement curve, in which either the avidin coating solution, or the biotinylated pheromone (conjugate pheromone-hapten II – biotin) or the specific anti-pheromone antiserum was omitted. The concentration of the pheromone, which was present in the unknown samples, was determined using the displacement curve (standard curve) of the immunoassay method.

**Sample preparation**

Olive fruit fly insects were a generous gift of the Chemical Ecology and Natural Products Laboratory of the Institute of Biology of NCSR “Demokritos”. Whole abdomens or insect glands were removed from six-day old virgin female olive flies at different time intervals (early morning, afternoon). Then they were homogenized in the presence of phosphate buffer pH 7.4 containing an appropriate quantity of organic solvent (DMF, 0.5 %) and centrifuged (1,500 g). The supernatants were collected, diluted to an appropriate volume of phosphate buffer pH 7.4 containing DMF, 0.5 % (10 insects / 2.5 mL) and used as unknown samples in the ELISA assay.

**Results and discussion**

The development of the immunoassay system for a pheromone has to overcome a number of specific difficulties. In the case of the olive fly, the molecule to assay is a compound of low molecular weight (MW 156), whose direct administration to rabbits is not expected to give rise to any humoral immune response. To elicit antibodies against this molecule, it is necessary to synthesize an analogue bearing an active group, through which it can be linked (as a hapten) to an immunogenic carrier protein, such as keyhole limpet hemocyanin. The conjugates prepared (the immunogens) are then capable of triggering an immune reaction in the host animals leading to the development of specific antibodies against the hapten. On the other hand, due to its low molecular weight the molecule to assay cannot be directly immobilized onto ELISA microwells. Alternatively, a suitable derivative containing an active group can be synthesized, linked to a biotin-moiety and then indirectly immobilized onto avidin pre-coated microwells. Actually, two suitably substituted derivatives (Figure 1) of the compound 1,7-dioxaspiro[5.5]undecane (pheromone of the insect *Bactrocera oleae*) were synthesized. Details concerning the synthesis of these compounds will be published elsewhere.

The carboxyl-derivative (±)-β-[3-(1,7-dioxaspiro[5.5]undecane)]propionic acid 2 (pheromone-hapten I) was used for the preparation of a suitable pheromone-immunogen after its conjugation with the carrier protein KLH (Keyhole Limpet Hemocyanin). The conjugation reaction was performed according to the well established and widely used carbodiimide
method (Erlanger B.F., 1980). The KLH-conjugate was subsequently used for immunizing (Vaitukaitis J.L., 1981) New Zealand white rabbits, aiming at the development of specific anti-pheromone polyclonal antibodies.

Figure 1. The olive fruit fly pheromone (1), the pheromone-hapten I (2) and the pheromone-hapten II (3).

The amino derivative (±)-δ-[3-(1,7-dioxaspiro[5.5]undecane)]butylamine 3 (pheromone-hapten II) was used for the preparation of a suitable “coating antigen”, i.e. of a pheromone derivative, which could be immobilized onto ELISA plates. More specifically, this amine derivative was appropriately biotinylated, using an active biotin ester as biotinylating reagent, and then indirectly immobilized onto ELISA microwells, which had been pre-coated with the glucoprotein avidin (as known avidin recognizes and binds biotin and biotinylated molecules with great specificity and high chemical affinity) (Bayer E.A. & Wilcheck M., 1996).

**Evaluation of the anti-Pheromone Antisera**

Seven different antisera, corresponding to seven consecutive bleedings of the immunized animals, were tested as described in the experimental section. According to the results obtained, the titers ranged from 1:3,000 to 15,000. A pool of selected antisera, showing highest titer values, was used in the ELISA displacement curve experiments (titer of the pooled antiserum = 1:10,000 (Figure 2).
Figure 2. ELISA titer curve obtained with a pool of selected anti-pheromone antisera (●). Pre-immunized rabbit serum was used as a negative control (■).

Figure 3. A typical ELISA displacement curve used for determining pheromone concentrations in biological samples (insects extracts).

Table 1. ELISA and GC accuracy studies.

<table>
<thead>
<tr>
<th>Spiked concn. (ng/ml)</th>
<th>Measured concn. (ELISA) (ng/ml)</th>
<th>Recovery (%)</th>
<th>Measured concn. (GC) (ng/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1500</td>
<td>1410</td>
<td>94</td>
<td>1570</td>
<td>105</td>
</tr>
<tr>
<td>800</td>
<td>824</td>
<td>103</td>
<td>805</td>
<td>101</td>
</tr>
<tr>
<td>150</td>
<td>165</td>
<td>110</td>
<td>144</td>
<td>96</td>
</tr>
</tbody>
</table>

**ELISA Displacement Curve**

A typical ELISA displacement curve, using standard solutions of pure synthetic olive fly pheromone, is shown in Figure 3.

As shown in Figure 3, the displacement curve has a functional concentration range between 10 µg/ml and 80 ng/ml of pheromone.
Blind spiked samples were prepared and measured using pure synthetic olive fruit fly pheromone. The results shown in Table 1 indicate a good accuracy of the method since the measured values match very well the spiked concentrations. The same samples were also, measured by GC leading to very similar results (Table 1).

**Analysis of Biological Samples**
The previously described ELISA displacement curve was used for determining the concentrations of the olive fruit fly pheromone in biological samples and especially in insect extracts prepared as it is described earlier.

| Table 2. ELISA determination of pheromone in unknown biological samples (insect extracts). |
|----------------------------------|----------------------------------|
| **Unknown sample**               | **Pheromone (µg)**               |
| Extract of abdomens obtained from 39 insects (collection time: early morning) | 2.44 |
| Extract of glands obtained from 30 insects (collection time: early morning)   | 1.58 |
| Extract of glands obtained from 11 insects (collection time: afternoon)        | 0.38 |
| Extract of heads obtained from 39 insects (collection time: early morning)     | n.d.  |
| *n.d.: non detectable*                                                      |

| Table 3. Recovery of endogenous pheromone in serially diluted unknown samples. |
|----------------------------------|-----------------|-----------------|
| **Samples**                      | **Measured pheromone concn. (µg/mL)** | **Recovery (%)** |
| Unknown sample A                 | 9.51            | –               |
| 1:2                              | 5.57            | 117             |
| 1:5                              | 1.68            | 88              |
| 1:10                             | 0.81            | 85              |
| 1:20                             | 0.48            | 102             |
| Unknown sample B                 | 4.73            | –               |
| 1:2                              | 2.13            | 90              |
| 1:5                              | 0.84            | 88              |
| 1:10                             | 0.51            | 107             |
| Unknown sample C                 | 0.42            | –               |
| 1:2                              | 0.25            | 119             |

According to the above preliminary results obtained each insect seems to contain 0.4 - 2.4 µg of pheromone. Lower pheromone levels were determined when insect glands were extracted and measured instead of whole abdomens, but this finding should be confirmed by additional data. In the samples collected in the afternoon, the pheromone levels measured were substantially lower than those measured in samples collected in early morning. This finding seems to be reasonable, since the insects treated in the afternoon might have released
part of their pheromone. Extracts corresponding to the insect heads seem to contain negligible amounts of pheromone.

The accuracy of the immunoassay measurements was evaluated by linear recovery or endogenous recovery studies (recovery of endogenous pheromone in serially diluted unknown samples) as well as by exogenous recovery studies (recovery of exogenous synthetic pheromone added to unknown samples).

Table 4. Recovery of exogenous synthetic pheromone added in unknown samples.

<table>
<thead>
<tr>
<th>Measured pheromone concn. (µg/ml)</th>
<th>Expected pheromone concn. (µg/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.85</td>
<td>1.93</td>
<td>96</td>
</tr>
<tr>
<td>1.19</td>
<td>1.06</td>
<td>112</td>
</tr>
<tr>
<td>0.84</td>
<td>0.74</td>
<td>114</td>
</tr>
<tr>
<td>0.84</td>
<td>0.80</td>
<td>105</td>
</tr>
<tr>
<td>0.51</td>
<td>0.47</td>
<td>108</td>
</tr>
</tbody>
</table>

According to the recovery results obtained (Table 3, Table 4) the immunoassay measurements show very good accuracy.

Collection and analysis of more biological samples using the above described enzyme-linked immunosorbent assay are now in progress. It is expected that extended use of this analytical technique will help the development of alternative, ecologically safe methods for the control of olive fruit fly.

Acknowledgements

The authors thank the Chemical Ecology and Natural Products Laboratory of the Institute of Biology of NCSR “Demokritos” and especially Dr. V. Mazomenos, Head of the Laboratory, for the generous offer of the olive fruit fly insects.

References


Contribution to the biological olive agriculture.
Efficient control of the olive fruit fly by the ECO-TRAP®

Nikitas Ragoussis
VIORYL S.A. Research Department, Kato Kifissia, 145 64 Athens, Greece,
e-mail: nragoussis@vioryl.gr

Abstract: The ECO-TRAP is a pheromone-based trap in the form of a green paper envelop 15 X 20 cm containing ammonium bicarbonate as food attractant. The olive fruit fly, Bactrocera oleae Gmelin, is attracted by both the food and sex attractant to the surface of the trap, which is impregnated with deltamethrine, a powerful contact insecticide, and is exterminated. The active ingredients of the ECO-TRAP are allowed in the Biological Agriculture of the olive, according to the 2001/1991 and the additional 1488/97 and 473/2002 EC regulations. The ingredients of the ECO-TRAP are active for all the active period of the olive fruit fly. The application of the ECO-TRAP at the proper time provides an efficient control of the insect, keeping the fly population in low level and the final infestation in lower or at least the same level with either bait sprays (hydrolyzed proteins-dimethoate) or cover sprays (dimethoate). The use of the ECO-TRAP was launched in Greece in 1993 and it has very soon been propagated in all the Mediterranean countries. Evidence for the negligible influence of the ECO-TRAP in the beneficial entomofauna of the olive grove has been shown by experiments in Italy and Greece. Official registrations of the ECO-TRAP have been obtained in Greece, Italy and Turkey and the procedure for the registration in Spain has already started. The advantages of the ECO-TRAP are the simple and safe application for the farmer, the efficient control of the olive fly, the protection of the beneficial insects of the olive grove and the protection of the environment in general.

Key words: Pheromones, Bactrocera oleae, Biological Agriculture, Oliviculture, Mass Trapping.

Introduction

The olive fruit fly Bactrocera oleae Gmelin is the most important pest of olives in the Mediterranean area causing great economic damages to the production, if efficient control measures are not applied. Current control methods rely on the use of broad spectrum insecticides in bait or cover sprays.

After the identification and the synthesis of the sexual pheromone of the olive fly in 1980 (Baker et al., 1980; Mazomenos & Haniotakis, 1981), great efforts have been made in all the Mediterranean countries, in order to develop efficient alternative control methods based on the pheromone, which could reduce or even eliminate the dispersion of toxic insecticides. Special consideration has been given on the mass trapping technique, due to the availability of a variety of food, visual and pheromone attractants (Haniotakis & Skyrianos 1981; Jones et al., 1983).

The mass trapping technique using pheromone traps for the control of the olive fly started in Greece after 1980 when the pheromone of the insect was discovered. The traps that were used that time, designed by Dr. Haniotakis, were plywood rectangles of natural brown color, impregnated with a solution of deltamethrine, sugar and glycerin in which a capsule of the pheromone and a small plastic envelop with ammonium bicarbonate were attached (Haniotakis et al., 1986a, Haniotakis et al., 1991). These traps achieved an acceptable protection against the olive fly, applied at small to medium size orchards, using traps prepared in the
laboratory. The massive production of the traps for large-scale applications did not give at that time reliable results, due to the deficiency of the active ingredients.

A major improvement on the pheromone traps was the introduction of the paper as entomotoxic surface in 1990 and in 1993 the ECO-TRAP appeared for the first time in its final form. A green paper envelope 15 X 20 cm, lined inside by a polyethylene film for tightness. The outer surface of the envelope is impregnated with 15 mg of deltamethrine and treated with a stabilizer in order to prevent the rapid degradation of the insecticide by the daylight. The density of deltamethrine on the trap is 30 µg/cm². An amount of 70 gr. of ammonium bicarbonate is contained in the envelope. Decomposition of this salt at ambient temperature liberates ammonia that is a powerful food attractant for the olive fly. A pheromone dispenser, containing 80 mg of synthetic racemic (±)1,7-Dioxaspiro[5.5]undecane. The racemic compound has a long range male attractant activity due to the (S)-(+)enantiomer which is the natural pheromone of the insect and an aggregation pheromone activity of the (R)-(−)-enantiomer which it is also aphrodisiac and attracts males and females (Haniotakis et al., 1986b). The pheromone is incorporated into a special type wax that guarantees a slow release of the active compound.

The pheromone dispenser, the deltamethrine on the paper surface and the quantity of ammonium bicarbonate, guarantee the activity of the trap all over the active period of the insect (Ragoussis, 2002).

The trap is placed approximately in the middle of the canopy of the olive tree, in the shade, without coming in contact with leaves or branches. The rate of the application depends on the nature of the olive grove, the variety of the plants and the presence of the olive fly in the area. In general a trap every other tree is placed at the beginning of the active period of the insect, in normal plantations (100-150 trees per hectare) of trees of medium size, 3-5 m high. In a grove of large size trees (more than 5 m high) and a longer than 8 m distance between trees, one trap every tree is necessary. Following the development of the olive fruit fly population in the beginning of September a second application of traps is made if considerable increase of the insect’s population or increase of the live infestation is observed.

The mass trapping is a preventive method and therefore the traps have to be placed in the grove shortly before the emergence of the first generation of the insect and before the olive fruit becomes susceptible to be infested by the olive fly. This stage can be practically identified by the hardening of the olive stone.

Systematic evaluation of the mass trapping technique using the ECO-TRAP has started since 1993 in Greece and in Italy by state authorities or research institutes and actually the use of the ECO-TRAP is extended in all the Mediterranean area, Greece – Turkey – Israel – Jordania – Tunisia – Spain – Portugal – France and Italy.

Three points will be analyzed regarding the applications of the ECO-TRAP

• Effectiveness of the ECO-TRAP applications.
• Legal status of the ECO-TRAP
• Influence of the ECO-TRAP to the environment
  1. Impact of the ECO-TRAP to the beneficial entomofauna.
  2. No dispersion of the insecticide to the environment

Results and discussion

Effectiveness of the ECO-TRAP applications
The evaluation of the method is based, on the olive fly population density, expressed by the catches per five days or per week in either McPhail traps or yellow sticky traps with a pheromone dispenser, and on the fruit infestation level. These data are compared to the population
density of the olive fly and the fruit infestation in the neighboring areas where bait sprays or cover sprays were applied. Fruit infestation is expressed as total infestation; live and dead eggs, L1, L2, L3, pupae, exit holes and stings infested by the fungus *Camarosporium dalmatica* (Macrophoma), (Broumas 1994).

**Greece:** A typical example of ECO-TRAP application was made for four successive years from 1996 to 1999, in 40 km to the North of Athens in Tanagra Voeotia to an homogeneous, 300 hectares area (45,000 trees). The results are compared to those of two neighboring areas, one of 270 hectares at Shimatari and one of 235 hectares at Arma, two locations where bait sprays were applied. In all the experimental area the same variety “megaritiki” is cultivated and the trees are of medium size planted in a density of approximately 150 trees per hectare.

In all four years the control of the olive fly was similar and even better in the ECO-TRAP area than in the conventional bait sprays protected groves. The total fruit infestation in November, just before the beginning of the harvest, was always lower in the ECO-TRAP area than to the control area (Fig. 1). In 1999 even in the ECO-TRAP area the infestation was the double than the acceptable level of 10% but it was three times lower than in the control area where three bait sprays were applied (Broumas *et al.*, 2002).

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**Italy:** From 1997 to 1999, 500 to 700 hectares of olive groves were protected with ECO-TRAP, in Tuscany Italy. The project was coordinated by the ARSIA Toscana and was realized by the two olive farmers associations AIPROL and OTA. The test areas where spread in the eight Tuscany regions. The last year 1999, 290 olive groves covering 734 hectares were considered as experimental area and their infestation was compared with the infestation in similar orchards in the same region, where conventional protection was made. In all applica-
tions the total infestation in the ECO-TRAP olive groves was inferior compared to that of the control groves where one to three cover sprays with dimethoate were applied. (Ricciolini et al., 2000).

A more systematic evaluation of the mass trapping technique was made in Toscana region from 2000 to 2002 in about 25 test areas. Half of the traps were placed at the end of June and the other half in autumn with a final trap/tree ratio 1/1. On the control plots on average 1 or 2 chemical treatments with dimethoate were made. The evaluation of the effectiveness of the mass trapping technique by the use of ECO-TRAP was based on the use of Relative Effectiveness Indexes. These indexes are the ratio between the yearly average infestation in control areas, where conventional chemical treatment was made, to the infestation in the test Mass Trapping technique area (Pettacchi et al., 2003). Considering the values of the relative effectiveness index on active infestation, all the three years, the ECO-TRAP trials indexes were higher to one in the large majority of the cases, 33 up to 37.

Spain: The ECO-TRAP has been also tested in Spain in several regions where efforts was made for the development of the biological agriculture of the olive, in Catalonia (1998-2001) in Aragon 1999-2001 in Extremadura and Andalusia. In all applications a positive effect of the ECO-TRAP was observed and in most of the cases a good protection of the production was obtained without any spraying with chemical insecticides.

Cyprus: The Cyprus government for two years 2000 and 2001 offered about 20.000 traps to the farmers for the control of the olive fly, in the context of a project for the promotion of the organic olive agriculture. The final result was a very efficient protection of the production. The infestation at the harvest time was only 4% in the ECO-TRAP area while was more than 12% in the area of bait sprays (Patsias A. 2002)

Legal status of the ECO-TRAP
The ECO-TRAP is officially registered, as a normal plant protection product for the oliviculture against the olive fly, in the class insecticide-ready bait (RB) in three Mediterranean countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Registration Number</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey</td>
<td>3773 / 22-05-2000</td>
<td></td>
</tr>
<tr>
<td>Greece</td>
<td>1900 / 02-04-2001</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>11107 / 29-11-2001</td>
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</tr>
</tbody>
</table>

The process of the registration of the pheromone active ingredient has also started in Spain and it is attended to be finished before the end of 2003.

The fact that the ECO-TRAP is officially registered means that the product is controlled and evaluated for the quality and the effectiveness, by the authorities of all the above countries. Furthermore the product is allowed in the biological agriculture of the olive according to the European Regulation EC 2092/1991, where it is clearly stated that “The products that are not in contact with the plant or the fruit of cultivation and do not leave any chemical residues on the crop” are allowed in the Biological Agriculture. From 1990 to 1995 several opposite opinions had been expressed, based on the presence of deltamethrine, a synthetic insecticide as an active ingredient of the trap. In 1997 an additional EC regulation (1488/97) appeared where detailed description of products like the ECO-TRAP was allowed in Biological Agriculture for a period of five years, until March 2002. In that regulation it is stated that the use of deltamethrine on a trap for the control of the olive fruit fly and Mediterranean fruit fly is allowed in biological agriculture. These five years the ECO-TRAP had a great contribution on the development of the biological agriculture of the olive in Italy and in Greece and this is one of the reasons that the last EC regulation 473/2002 extended the use of similar products for an unlimited period.
Influence of the ECO-TRAP to the environment

Impact of the ECO-TRAP to the beneficial entomofauna: One major advantage of the ECO-TRAP is the very low impact to the beneficial entomofauna of the olive groves. Two studies were made:

a) The first study was conducted simultaneously in two regions of Tuscany in olive groves, non treated by chemical insecticides (Bagnioli, 2000) A dozen of ECO-TRAP in which the ¼ of their surface was covered by a transparent sticky band, was deployed random. A dozen of similar sticky bands alone were also deployed in the same grove random. All the entrapped insects were identified and counted for 3 successive weeks. The results were shown that statistically there is not any difference on the catches of various insects on the sticky bands or on the ECO-TRAP. A difference is obvious only in the catches of the olive fly, which are much higher in the ECO-TRAP than in the sticky band alone. That means that from all the other insects, beneficial or not, only a small number is accidentally killed by the ECO-TRAP. There is not any special attraction on the toxic surface by the attractants, which are specific only for the olive fly.

b) In 2000 in Tanagra 40 km to the North of Athens in an area where the mass trapping technique with the ECO-TRAP, was applied for four successive years a sampling point was chosen in the middle of this area. In a block of four trees, 12 yellow sticky traps without any other attractant were placed, at a rate 4 traps/tree. The same number of traps and in the same rate, were placed in a block of 4 trees in Arma Voelotias in an area were bait sprays are applied all the previous years. Even though, no systematic study was made on the results of this experiment, a view on the surface of the yellow sticky traps showed that in the ECO-TRAP area the entomofauna caught was much richer than in the surface of the traps from the chemical treatment area.

No dispersion of deltamethrine in the environment: Regarding the stability of the deltamethrine on the surface of the ECO-TRAP the following experiment was done. A dozen of traps were placed in a garden and they were washed by the sprinkler for two hours, three times a day, for fifteen days. Every three days three traps were analyzed for their content in Deltamethrine and the average was noticed. The results of the analyses are reported on Fig.2.

![Decis resistance to water jet](Image)

Fig. 2. Remaining quantity of deltamethrine on the trap surface, after intensive washing by a water jet
A very small loss (0.5-1.0 mg/trap) of insecticide was observed only the first day. The following days no diminution of the insecticide was observed. In parallel the killing activity of the washed traps was tested in the laboratory and it has been proved that olive flies staying 10 sec on the trap have had 100% mortality in 24 hours (Tomazou et al., 1995)

As the ECO-TRAP is always placed inside the canopy of the tree and never is exposed directly to the rain, it is improbable to receive such a quantity of water and in such violent way. So, it is reasonably assumed that there is no any dispersion of insecticide in the environment caused by the rain or the wind.

**Conclusion**
- The mass trapping with the ECO-TRAP has been proved to be a very efficient method for the control of the olive fly, with effectiveness comparable to the conventional sprays with chemical insecticides.
- The use of the ECO-TRAP is simple and safe for the farmer.
- The application of the ECO-TRAP does not leave any residual insecticide on the fruit or the leaves of the olive tree.
- The deltamethrine on the surface of the trap is attached on the paper in a way that it cannot be washed by the rain, which means that it is not dispersed into the environment.
- The ECO-TRAP is officially registered in three Mediterranean countries (Italy, Greece and Turkey) and the procedure of registration has been started in several other Mediterranean countries. According to the 2092/1991 regulation of the European Community and the successive regulations EC 1488/97 and 473/2002, the ECO-TRAP is a product allowed in the biological agriculture of the olive.
- Actually the ECO-TRAP is maybe the most efficient tool for the control of the olive fly, allowed in the biological agriculture, which has also official registrations in three Mediterranean countries.

**References**


Economic returns of pesticide use in conventional and organic olive-growing farms in Crete, Greece

Vangelis Tzouvelekas¹, Venizelos Alexandrakis², Kyriaki Varikou²
¹ Department of Economics, School of Social Sciences, University of Crete, Greece
² Institute of Olive-Trees and Subtropical Plants, National Agricultural Research Foundation, Greece

Abstract: Empirical evidence in developing country’s agriculture suggests that the value of marginal product of pesticide use often exceeds marginal factor costs. In line to that the present paper aims to explore the economic returns of pest control inputs against olive-fruit fly Bactrocera oleae (Gmelin) for a sample of conventional and organic olive-growing farms in Western Crete, Greece. The proposed methodology captures both the biological and economic role of pesticides and permits indirect estimation of crop damage. The results suggest that the economic returns of pesticide use are indeed significant and it should be taken into account in analyzing their productivity.

Key words: Economic returns, pesticides, olive groves

Introduction

Many of the innovations introduced in the farming sector over the past few decades have involved the introduction of a special class of factors of production, the damage control inputs. Profound examples of this kind of inputs in the farming sector include pesticides, weedicides, windbreaks, sprinklers for frost protection, immunization and antibiotics in feedlots etc. Other important examples of damage control agents outside agriculture include the use of smoke alarms and sprinklers system to prevent fire, immunization to prevent diseases in population, alarm systems to prevent crimes against property, water and air purification systems. To a certain extent even national defense can be thought as an example of damage control input.

Unlike conventional factors of production (i.e., land, labor, capital) these special class of inputs do not increase farm’s potential output directly. In some cases they even decrease farm production. For instance the excess use of pesticides in the early stages of plant growth or in inadequate time period may have disastrous impact on farm produce (Pedigo et al., 1986). Instead their distinctive feature lies in their ability to increase the share of potential output that farmers realize by reducing the negative effect of the damage agents caused either from natural or human causes. In this line, a considerable amount of empirical work has been devoted in recent years on the quantitative analysis of the distinct role of conventional and damage control inputs on farm production. The first who have dealt explicitly with the appropriate specification of damage control inputs in farm production models and the subsequent measurement of their marginal productivities were Headley (1968) and Campbell (1976). Using a simple methodological approach they concluded that pesticides have been under applied in a sense that their marginal product exceeded marginal factor cost.

However, as noted several years later by Lichtenberg and Zilberman (1986) (hereafter LZ) the marginal productivities produced by Headley (1968) and Campbell (1976) model specification, were biased as they did not account for the indirect role of damage control inputs in the production process. Unlike with Headley (1968) and Campbell (1976), LZ suggested that conventional and damage control inputs should be treated asymmetrically.
They suggested that the contribution of damage control inputs to farm production may be better realized by conceiving realized output as a combination of two components: first, the maximum quantity of farm produce that it is attainable from any chosen conventional input combination and, second, the losses in farm production due to the action of damaging agents that are present in the environment like insects, weeds, bacteria etc. In addressing this issue, they introduced into the traditional production function model an output abatement or kill function capturing the abatement effort by damage control agents. Subsequently, they measured marginal productivity of damage control inputs according to their ability to reduce crop damage and not to increase directly farm output. Even since, their approach has been successfully applied by several authors including Babcock et al. (1992), Carasco-Tauber and Moffitt (1992), Lin et al., (1993) and Chambers and Lichtenberg (1994). At the same time Blackwell and Pagoulatos (1992) utilized a process model of production that accounts for state variables omitted from Lichtenberg and Zilberman theoretical specification. At the same year, Babcock (1992) based on the empirical findings reported by Carlson (1970), Noorgard (1976) and Feder (1979) introduced explicitly in the model production uncertainty.

Besides the important contribution made by LZ in measuring damage control agent’s productivity, their asymmetric functional specification has been questioned by Fox and Weersink (1995) as empirical evidence provided worldwide still reported marginal products higher than marginal factor costs. Fox and Weersink (1995) (hereafter FW) pointed out that the output abatement function suggested by LZ under any arbitrary functional specification, impose a priori a structure on the underlying biological and physical data that ensures eventually decreasing returns for damage control inputs. Cowan and Gunby (1996) in analyzing the rate of adoption for pesticides against integrated pest management strategies at the farm level, concluded that pesticide use is subject to increasing returns mainly due to the significant R&D expenditures by chemical industries and the learning effects in their application by individual farmers. However, under increasing returns the response of damage control input use to variations in prices and thus profits is not continuous as it was initially assumed by LZ. If increasing returns are allowed, a profit-maximizing farm, at the ceiling will choose either to apply damage control inputs or not as long as it obtains higher profits. This is important from a policy point of view as a specific policy aimed to reduce pesticide use for environmental conservation by imposing a tax may have substantially different effects on the levels of use of different products. Departing slightly from the traditional LZ model and maintaining weak concavity of the output abatement function, they suggested an alternative specification of the farm production model that allows for increasing returns in damage control inputs.

Along these lines, the main objective of this paper is to empirically estimate and compare the marginal productivities of chemical pesticides and biological control inputs for a sample of olive-growing farms in Western Crete, Greece. Our empirical model is based on FW theoretical foundations and on a Cobb-Douglas specification of the production function. The rest of this paper is organized as follows: the theoretical model using based on FW theoretical foundations is presented in section 2. The data employed in the empirical model and the empirical results are described in section 3. Concluding remarks follow in the last section.

Material and methods

Theoretical framework

Let assume that farm \( i \) utilizes conventional inputs (i.e., land, labor, capital) \( x_i = (x_{i1}, x_{i2}, ..., x_{ij}) \) to produce a single output \( y_i \) through a technology described by a well-
behaved production function $f(x_i; \beta)$, where $\beta$ is the vector of the associated parameters to be estimated. Apart of conventional inputs let also assume that farms are also utilizing damage control inputs (e.g., pesticides, biological control) $z_i = (z_{i1}, z_{i2}, \ldots, z_{ik})$ to prevent destruction in their potential output caused by damage agents. According to FW model specification, the effect of these damage control inputs on farm produce consists of two-stages: the first stage includes the effect of damage control input on the damage agent density and the second involves the subsequent effect of the remaining damage agent on farm output. Under this assumption farm’s $i$ realized production is obtained from:

$$
\tilde{y}_i = \{f(x_i; \beta, t)[1-h(B_i; \lambda)]\} e^{\nu_i}
$$

where $h(\cdot)$ is the output-damage function that depends on the observed level of damage agent in farm $i$ and, $\lambda$ is the associated parameter. It represents the proportion of output lost at damage agent density $B$. It is assumed that damage agent only affects the quantity of output produced and not its quality. The model can be generalized to account for quality changes (see Babcock et al., 1992). $\tilde{y}_i$ is the actual level of production for a given level of damage agent $B$, the technological constraints and conventional factors use. Damage function is assumed to possesses the properties of a cumulative distribution function and it is concave.

Even with the general assumption that the marginal damage effect of damage agent is non-negative $\frac{\partial h(\cdot)}{\partial B} \geq 0$, the sign of $\frac{\partial^2 h(\cdot)}{\partial B^2}$ is underetermined. Nevertheless, the damage function is often assumed concave. In case that the damage agent is absent $(B_i = 0)$ then $h(\cdot) = 0$ and actual output equals with $(\tilde{y}_i \rightarrow y_i)$. On the other hand, when the level of damage agent population tend to infinity $(B_i \rightarrow \infty)$, the farm output approaches a minimum level $(\tilde{y}_i \rightarrow \tilde{y}_{min})$ which, however, cannot be less than zero.

The damage agent incidence ($B_i$), depends on it’s initial population and on the abatement level of damage control input use. Equivalently, the output-damage control function can be formalized as:

$$
B_i = \tilde{B}_i[1-g(z_i; \gamma)]
$$

where $\tilde{B}_i$ is the initial damage agent incidence in farm $i$, $\gamma$ is the vector of the associated parameters and, $g(\cdot)$ is the control function which depends on the level of control inputs use $z_i$. Like the damage function, the control function it is also constrained by the (0,1) interval. If $g(\cdot) = 0$, the control agent has no effect on damage agent incidence and the level of damage agent affecting farm production is equal with it’s initial population $(\tilde{B}_i = \tilde{B}_i)$. Contrary, when $g(\cdot) = 1$ there is a complete eradication of the damage agent and farm production equals with $(\tilde{y}_i \rightarrow y_i)$. The proportion of damage agent remaining after treatment it is assumed to decrease monotonically, that is the control function is also assumed to be concave.

According to FW the curvature of the damage function relative to that of the control function is important in establishing increasing returns. Specifically they proved that increasing returns may occur whenever the following inequality holds:

$$
\frac{\partial^2 g(\cdot)}{\partial z_i^2} / \frac{\partial^2 h(\cdot)}{\partial B_i^2} < \frac{\partial g(\cdot)}{\partial z_i} \tilde{B}_i
$$

The ratio on the left-hand side of the above relation is analogous to the Arrow-Pratt coefficient of absolute risk aversion and measures the relative degree of curvature of the control and damage functions. Thus, using their own words “the less curved the control function, relative to the damage function the more likely are increasing returns for given values associated with the marginal effectiveness of the control input and untreated damage agent density” (p.36).
In order to make the above specification operational we should first assume a specific functional form for both the damage and control function. A common specification used in the relevant literature is the exponential form suggested by LZ and empirically applied by Carasco-Tauber and Moffit (1992) and Oude Lansink and Carpentier (2001) among others. Specifically both functions have the following form:

\[ h(B_i; \lambda) = 1 - e^{-iB_i \lambda} \quad (4a) \]

and

\[ g(z_i; \gamma) = 1 - e^{-\gamma z_i} \quad (4b) \]

Although the exponential specification has been mostly applied in damage control econometrics, several other functional specification having the properties of a cumulative distribution function have been suggested in the literature including: Pareto, logistic, Weibull, rectangular hyperbola, linear response plateau and square root response plateau. Lichtenberg and Zilberman (1986) and Fox and Weersink (1995) provide a thorough discussion on the properties and empirical implementation of these functional specifications.

By plugging relation (4b) into (2) we obtain the actual damage agent incident in farm production as:

\[ B_i = \bar{B}_i \left[ 1 - (1 - e^{-\gamma z_i}) \right] \Rightarrow B_i = \bar{B}_i e^{-\gamma z_i} \quad (5) \]

Substituting relation (5) into (4a) we obtain the damage control function as:

\[ h(B_i; \lambda) = 1 - e^{-i\bar{B}_i \lambda} e^{-\gamma z_i} \quad (6) \]

Then plugging (6) into (1) and taking the natural logarithms we get:

\[ \ln \bar{y}_i = f \left( \ln x_i; \beta \right) - \alpha e^{-\gamma z_i} + v_i \quad (7) \]

where \( \alpha = \lambda \bar{B}_i \). If we assume that \( f(\cdot) \) is approximated by the traditional Cobb-Douglas functional form, then relation (7) becomes:

\[ \ln \bar{y}_i = \beta_0 + \sum_j \beta_j \ln x_{ji} - \alpha e^{-\gamma z_i} + v_i \quad (8) \]

The above model is non-linear with respect to its parameters but it can be estimated via simple maximum likelihood technique using a grid search for the values of \( \gamma \) which according to LZ should lie within the \((0,1)\) interval.

Results and discussion

The data used in this study are part of a broader survey on the structural characteristics of the agricultural sector in Crete financed by the Regional Directorate of Crete in the context of the Regional Development Program 1995-99 (Liodakis, 2000). The sample consists of 23 organic and 39 conventional randomly selected olive-growing farms located in the Western part of Crete, Greece. The dependent variable in (8) is the total olive-oil production measured in kilograms. As independent variables we have included: \( a \) total labor, that is, hired and family (paid and unpaid) labor related to olive-oil production measured in working hours; \( b \) farm land devoted to olive-trees cultivation measured in stremmas (one stremma equals 0.1 ha); \( c \) total value of purchased inputs (e.g., energy) and machinery depreciation used in olive-tree cultivation, measured in euros; \( d \) the total amount of fertilizers (chemical or
organic) applied in olive-trees measured in kilograms and; (e) the total value of pesticides or biological control inputs used in both modes of production measured in euros.

Table 1. Parameter estimates of the Cobb-Douglas Production Function for the conventional and organic olive-growing farms in Crete.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Conventional Farms</th>
<th>Organic Farms</th>
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<tbody>
<tr>
<td></td>
<td>Estimate Std Error</td>
<td>Estimate Std Error</td>
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<tr>
<td><strong>Production Function</strong></td>
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<tr>
<td>Constant</td>
<td>2.3451 (0.4324)</td>
<td>1.8721 (0.6098)</td>
</tr>
<tr>
<td>Land</td>
<td>0.4092 (0.1203)</td>
<td>0.3865 (0.1324)</td>
</tr>
<tr>
<td>Labor</td>
<td>0.1763 (0.0643)</td>
<td>0.2364 (0.1072)</td>
</tr>
<tr>
<td>Intermediate Inputs</td>
<td>0.2567 (0.1174)</td>
<td>0.2809 (0.0921)</td>
</tr>
<tr>
<td>Fertilizers</td>
<td>0.0982 (0.0413)</td>
<td>0.1102 (0.0509)</td>
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<td><strong>Damage Function</strong></td>
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<td>$\gamma$</td>
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<td>0.2800</td>
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<td>$\ln(\theta)$</td>
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<td>-245.983</td>
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</tbody>
</table>

* (**) indicate statistical significance at the 1(5) percent level.

The ML estimates of the Cobb-Douglas production functions for both modes of farming are presented in Table 1 above. The value of the respective likelihood function $\ln(\theta)$ is satisfactory indicating a good fit of the relevant estimates. All the associated parameters are statistical significant at least at the 5% significance level. Land inputs seems to be the foremost important factor of production as a 1% increase in farm acreage will increase olive-oil produced by 0.4092 and 0.3865 per cent in conventional and organic farms, respectively. Intermediate inputs and labor follow in significance with the labor input being more important in organic olive-production which is a labor intensive technology.

The underlying conventional production technology exhibits decreasing returns to scale as the relevant point estimate were 0.9404, whereas that of organic farms was found to exhibit increasing returns to scale 1.014. The null hypothesis of a linearly homogeneous production technology (i.e., $\sum \beta_j = 1$ for all $j$) is rejected at the 5% significance level, indicating non-constant returns to scale for both modes of olive-oil production (the respective value of the LR-test is 23.43 and 17.09 for conventional and organic farms, respectively). These values mean that an equiproportional increase in the use of all inputs in conventional and organic olive-oil production will increase olive-oil produced by 0.9404% and 1.014%, respectively. These findings were expected as organic olive-culture is still at an infant stage of production. The size of organic farms do not exceed the potential capabilities of the existing production technology and thus there is still considerable scope for output expansion.

The associated parameters in the damage control function are also reported in Table 1 (recall that the $\gamma$ parameter was estimated after a grid search within it’s domain). In both
samples the $\alpha$-parameter is found to be negative, as expected, and statistically significant. Specifically, for conventional farms the relevant estimate is -0.4093, whereas for organic olive farms considerably higher -0.6234. Hence, rather reasonably, it seems that biological pest control is more important than chemical damage control agents.

Using the parameter estimates of the production functions, the marginal products of both conventional and damage control inputs were calculated at the sample mean and are reported in Table 2 next. These estimates are derived from the first-order partial derivatives of relation (8) with respect to each conventional or damage control inputs:

$$\left[ MP_j = \frac{\partial \bar{y}_j}{\partial x_{ji}} = \left( \frac{\partial \ln \bar{y}_j}{\partial \ln x_{ji}} \right) \cdot \left( \bar{y}_j / x_{ji} \right) \right].$$

According to these point estimates, land input has the highest economic return in olive farming as it’s marginal product was found to be 65.3 and 54.2 kilograms per stremma in conventional and organic farms respectively.

### Table 2. Mean marginal products of conventional and damage control inputs

<table>
<thead>
<tr>
<th>Input</th>
<th>Conventional Farms</th>
<th>Organic Farms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Land$^a$</td>
<td>65.3</td>
<td>54.2</td>
</tr>
<tr>
<td>Labour$^b$</td>
<td>3.65</td>
<td>4.09</td>
</tr>
<tr>
<td>Intermediate Inputs$^c$</td>
<td>0.235</td>
<td>0.256</td>
</tr>
<tr>
<td>Pest Control$^c$</td>
<td>0.609</td>
<td>0.745</td>
</tr>
</tbody>
</table>

*Note: $^a$ in Kgs per stremma (1 stremma equals 0.1 ha); $^b$ in Kgs per working hour; $^c$ in Kgs per euro.*

Family and hired (unpaid and unpaid) labor contributes also significantly to conventional and organic olive production as it’s marginal product was found to be 3.65 and 4.09 kilograms per working hour, respectively. The fact that organic olive farming is a labor intensive technology results to that higher point estimate for the organic sample. Intermediate inputs exhibit a lower marginal product value which is quite similar across the two modes of production, 0.235 and 0.256 kilograms per euro for conventional and organic farms, respectively.

Finally, for pest management inputs (chemical or biological) the point estimates are higher than those of intermediate inputs. Specifically an additional euro spent on chemical pesticides against olive-fruit fly *Bactrocera oleae* (Gmellin) will increase conventional olive-oil production by 0.609 kilograms. Similarly, a euro spent on biological control inputs against olive-fruit fly will increase organic olive-oil production by 0.745 kilograms. Again like labor input the organic olive farms exhibit higher point value. This is not surprising considering the lower effectiveness of integrated pest management against chemical pesticides. Pesticides are more effectively used against olive-fruit fly reaching an upper threshold and thus resulting to lower economic returns.

Exploring further these estimates, in Figure 1 the value of the marginal product of pest control inputs for a wide range of application in both modes of olive-farming is presented. As it is clearly shown in this figure as the amount of pest control inputs increases, the value of their marginal product converges to a given value for both modes of production. Although in low levels of application the marginal product of biological control agents is higher than their chemical counterparts, this difference vanishes as the amount of pesticides reach a certain threshold.
Figure 1. Marginal products of damage control inputs

Conclusions

This paper based on recent advances in the econometrics of damage control inputs provides estimates of their marginal product for a sample of organic and conventional olive farms in Western Crete, Greece. The empirical model is based on the theoretical foundations suggested by Fox and Weersink (1995) that that extent the traditional model developed by Lichtenberg and Zilberman (1986) allowing for increasing returns in the use of damage control agents. The econometric estimation of the resulted model is based on maximum likelihood techniques and it can be applied to any functional specification of the production function. Finally, although our model is based on an exponential specification of both the damage and control function it can be readily extended to any other functional specification suggested in the relevant literature of damage control econometrics (i.e., Pareto, logistic, Weibull, rectangular hyperbola, linear response plateau and square root response plateau).

The empirical results suggest that organic olive farms have not exceeded the potential capabilities of their organic production technology exhibiting increasing returns to scale on the average. Land and labour (family or hired) are the foremost important factors of production for both modes of farming. Average estimates of the marginal product of pest control inputs was found to be 0.609 and 0.745 kilograms per euro spent for conventional and organic farms, respectively. This difference vanishes however as long as the level of pest control inputs increases.

References


The use of copper products to control the olive fly (*Bactrocera oleae*) in central Italy

A. Belcari, P. Sacchetti, M.C. Rosi, R. Del Piana
Department of Agricultural Biotechnology, University of Florence, Italy
E-mail: antonio.belcari@unifi.it

**Abstract:** Olive fly fitness is greatly advantaged by the presence of associated bacteria living both on the phylloplane and in specialized parts of the gut. These microorganisms play an important role both as a proteinaceous source for the adult and as elicitors of protein hydrolysis in the blind sac of the young larva midgut. Several authors have studied this association, and some have demonstrated its importance in developing alternative control strategies. The major development of organic olive growing in Italy in recent years has generated new interest in the possible application of copper products against the pest. It is well known that copper can play an important role as an oviposition deterrent, but more recently our attention has focused on the possible effectiveness of copper as a bactericide. This work reports the results of applications of copper products over a two-year period in some olive grove areas in Tuscany. In 2000, the Bordeaux mixture was sprayed at two different dosages (1 kg/100 l water or 500 g/100 l water). In 2001, copper hydroxide, oxychloride and Bordeaux mixture were compared. In the first year, results showed a good degree of effectiveness of the Bordeaux mixture; as a matter of fact the level of infestation in the control plot at harvesting was about 20%, whereas in the treated plots it was respectively 9% and 6%. In 2001, no evident differences in the relative effectiveness of the copper products were evidenced. All of them reduced the final level of olive infestation compared to the control plot.

Two years’ data were discussed in order to evaluate the effectiveness of different copper active ingredients, tested for future applications of copper products or any other product acting as a bactericide, in IPM strategies in olive orchards.

**Key words:** *Bactrocera oleae*, copper, control, IPM.

**Introduction**

The fitness of the olive fly is greatly assisted by the presence of associated bacteria living on the phylloplane and in specialised parts of the gut. These microorganisms play an important role as a proteinaceous source for the adult (Drew & Lloyd, 1987; 1989) and as elicitors of protein hydrolysis in the blind sac of the midgut of young larvae (Girolami, 1973). Several authors have studied this association in the past and some have demonstrated its importance in developing alternative control strategies (Fytizas & Tzanakakis, 1966; Tzanakakis, 1985). Recently, the great development of organic olive cultivation in Italy has generated new interest in the possible application of copper products against the pest. It is known that copper can play an important role as an oviposition deterrent (Prophetou et al., 1991), but more recently our attention has focused on the effectiveness of copper as a bactericide (Belcari & Bobbio, 1999). This work reports results obtained over a 4-year period during which copper products were applied to olive groves in Tuscany.

**Material and methods**

Research was carried out in two olive grove areas in southern Tuscany, near the coast. In 1997–98 and in 2000 the olive groves were divided into plots of about half a hectare each. In
the 2001 experimentation the groves were divided according to a randomised block design into 12 plots of 1000 square metres. The 1997–98 experiments only evaluated the effectiveness of Bordeaux mixture at normal dosage, while the 2000 experiments tested its effectiveness at normal and at half dosage. In 2001, Bordeaux mixture and two new formulations of copper, Coprantol® and Coprantol Ultramicron®, were compared.

Sprays were applied beyond the 5% threshold of active infestation (eggs, 1st and 2nd larval instars). Weather forecasts were also considered in order to avoid the risk of rain soon after treatment. However, new sprays were applied when several days of rain had occurred. The composition of the preimaginal population and the trend of infestation were evaluated weekly from a sample of 100 olives/plot and one olive/tree, randomly picked according to the methodology described by Loi et al., 1981. In all the experiments, counts were made of the eggs, the sterile ovipositions and the number of alive L1 and L2 larval instars, and the level of damaging infestation (L3 and pupae) was evaluated. Comparisons between treatments were made by analysing data with the chi-square goodness-of-fit test. The null hypothesis was for equal frequencies among treatments (Zar, 1999; Statsoft, 1997).

Results and discussion

In 1997, the experimentation was conducted at Donoratico (LI) with just one spray, applied in early September, whereas in 1998 two treatments were applied. The final level of damaging infestation for both years was lower in the treated plots compared to the infestation level of the untreated plot (Tab. 1). Table 1 reports the results of the statistical analysis carried out to determine the influence of treatments on preimaginal stages of the fly. In both years, the values for alive first larval instars (L1) and second larval instars (L2) were significantly lower in the treated plots. In the untreated plot, the number of eggs was significantly higher and the sterile oviposition values were lower.

Table 1. Frequencies of preimaginal instars and damaging infestation level of the olive fly. Results of field treatments in 1997–98

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatments</th>
<th>χ²</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blank</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. mixture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.556</td>
<td>1</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>Sterile oviposition</td>
<td>12.500</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.256</td>
<td>1</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>L1+L2 alive</td>
<td>25.130</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>67.765</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Damaging infestation</td>
<td>57.316</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>86.223</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Chi-square goodness-of-fit test

In 2000, the experimentation was carried out in an olive grove near Follonica (GR). One treatment was applied at the end of August. Table 2 shows the effectiveness of Bordeaux mixture at both normal and half dosage. Statistical analysis evidences the effects of the
treatments in all preimaginal instars. As in the previous years, Bordeaux mixture did not affect eggs and sterile oviposition; differences were present only in alive L1 and L2 and in the level of damaging infestation. There were no significant differences between the two dosages.

Table 2. Frequencies of preimaginal instars and damaging infestation level of the olive fly. Results of field treatments in 2000

<table>
<thead>
<tr>
<th>Treatments</th>
<th>χ²</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>17</td>
<td>12</td>
<td>13.98</td>
</tr>
<tr>
<td>B. mixture</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>B. mixture 1/2</td>
<td>17</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Chi-square goodness-of-fit test

In 2001, the experimentation was repeated in the same area, with a comparison of three products: Bordeaux mixture, Coprantol® (C-450) and Coprantol Ultramicron® (C-200). Due to a rainy period, each plot had to be sprayed twice. All three products were effective against young olive fly larvae; the results reported in Table 3 clearly show the reduction of alive L1 and L2 in the treated plots, and the final values of damaging infestation show marked differences compared to those for the untreated plot. The observed values show that there were no statistically significant differences between the effectiveness of the three products, although Bordeaux mixture had the greatest impact on the infestation level.

Table 3. Frequencies of preimaginal instars and damaging infestation level of the olive fly. Results of field treatments in 2001

<table>
<thead>
<tr>
<th>Treatments</th>
<th>χ²</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>15</td>
<td>7</td>
<td>11.05</td>
</tr>
<tr>
<td>B. mixture</td>
<td>6</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>B. mixture 1/2</td>
<td>6</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

Chi-square goodness-of-fit test

Copper products tested in the field in all four years of experimentation seem to act more as a symbionticide than as an oviposition deterrent. This is supported by the higher alive L1 and L2 values in the untreated plots and by the eggs frequencies similar in all treatments.
Nonetheless, experiments performed in our lab have shown a certain activity of copper as an oviposition deterrent, as already demonstrated by Prophetou et al., 1991. Additional trials need to be carried out to highlight this activity.

Copper, which is permitted by regulations governing organic olive cultivation, can therefore be used as the basis for strategies to control the olive fly. It has also proved effective in areas of the southern Tuscany where the population density of the olive fly is invariably above the economic damage threshold. The use of copper products could affect the olive crop environment, but it must be considered that there are generally fewer treatments (1–2) than in organic viticulture (6–8). Besides, the availability of new formulations with a reduced metallic copper content opens new perspectives for IPM in olive cultivation. Of course, the side effects of this active ingredient on both useful insects and soil fauna have yet to be evaluated.

References


Effects of two fungal based biopesticides on *Bactrocera (Dacus) oleae* (Gmelin) (Diptera: Tephritidae)

M. Anagnou-Veroniki, D.C. Kontodimas, A.D. Adamopoulos, N.D. Tsimboukis and A. Voulgaropoulou

Benaki Phytopathological Institute, Laboratory of Insects Microbiology and Pathology, St. Delta 8, 145 65, Kifissia, Greece, E-Mail: bpilibr@otenet.gr

Abstract: An estimation of the effectiveness of fungal entomopathogenic agents which has been provided on *Bactrocera (Dacus) oleae* (Gmelin) (Diptera: Tephritidae) has been carried out. Two fungal based insecticides have been tested for their effects on adults of the pest. The products Naturalis-L [*Beauveria bassiana* (Balsamo) Vuillemin] and Mycotal [*Verticillium lecanii* (Zimmerman) Viegas] have been mixed with sugar suspension, in order to study the effects *per os* on *B. oleae* newly emergent adults, in laboratory conditions (24°C, 60% RH and 18 hours light photoperiod). The *B. bassiana*-product caused more than 95% mortality whereas the *V. lecanii*-product only 11.3%, three days after the treatment. In addition the more effective product (Naturalis-L) has been tested in the semi-field in two olive orchards and the crop samplings showed significantly less infestation in the treated olive trees.

Key words: *Bactrocera oleae*, *Beauveria bassiana*, *Verticillium lecanii*

Introduction

The environmental, economic and social conditions in olive culture demand the development of alternative control methods for *Bactrocera oleae*. As with many of other pest, the use of microorganisms as biological control agents might be an alternative. There are few reports on the potential of bacteria (Karamanlidou *et al.*, 1991) and virus (Anagnou-Veroniki, 1994; Anagnou-Veroniki *et al.*, 1997) as biological control agents for olive fruit fly or concerning the use of entomopathogenic fungi against other important fruit flies (Castillo *et al.*, 2000; De la Rosa *et al.*, 2002; Ekesi *et al.*, 2002). The objective of this study was to measure the effect of two commercial fungal preparations of *Beauveria bassiana* (Balsamo) Vuillemin and *Verticillium lecanii* (Zimmerman) Viegas, under laboratory and semi-field conditions.

Material and methods

**In the laboratory**

The products that have been tested were:

Naturalis-L [*Beauveria bassiana* (Balsamo) Vuillemin] and

Mycotal [*Verticillium lecanii* (Zimmerman) Viegas]

To study the effects on *Bactrocera oleae* adults the products have been mixed with artificial diet composed of honey, sugar and water. The solutions were:

0.5 ml Naturalis-L (=11.5 x 10^6 conidia of *B. bassiana*) per 100 ml diet and

0.5 ml Mycotal (=4.22 x 10^6 spores of *V. lecanii*) per 100 ml diet.
Ten trials of each product have taken place in cubic cages 25 cm height under laboratory conditions (temperature: 24±1°C relative humidity: 60±2% and photoperiod: 18 hours light / 6 hours dark). Each trial has been carried out by putting 250 newly emergent adults of *B. oleae* in each of two cages with mixture diet. A cage with normal diet was the control of the trial. The cages have been observed daily for three days. Mortalities have been compared by Tukey – Kramer (HSD) test (Sokal and Rohlf, 1995) using the statistical package JMP (Shall et al., 2001). The efficacy has been calculated by Abbott’s formula (Kuratak, 1982):

\[
Efficacy = \left[ 1 - \left( \frac{\text{post - spray density in treatment}}{\text{pre - spray density in treatment}} \times \frac{\text{pre - spray density in control}}{\text{post - spray density in control}} \right) \right] \times 100
\]

In addition a trial has been carried out on *B. oleae* newly emergent adults that have been reared for three days on normal diet and then on mixed with Naturalis-L diet.

**In the semi-field**

The more effective product (Naturalis-L) has been tested in the semi-field in two trials:

**1st trial:** Two orchard (one in Oropos region and one in Kifissia region) of 220 olive trees has been sprayed (cover spray) three times (at 14/9, 21/9 and 28/9). Two untreated orchard 200m away were the control of the trial. The proportion of the infestation by *B. oleae* was measured by sampling of 200 olive-crops of 12 olive-trees of each orchard at 14/10.

**2nd trial:** An orchard (in Kifissia region) of 220 olive trees has been sprayed (cover spray plus attractant) one time (at 5/9). An untreated orchard was the control of the trial. By sampling of 200 olive-crops of 12 olive-trees of each orchard was measured the proportion of the infestation by *B. oleae*. In the time of the operation the infestation was 4.12% in the treated orchard and 3.47% in the control orchard. The proportion of the infestation was measured again at 28/9.

**Results and discussion**

The results are presented in Tables 1 and 2. In the laboratory tests the *Beauveria bassiana*-based product caused more than 95% mortality whereas the *Verticillium lecanii*-based product only 11.3%, three days after the operation.

In the semi-field trials, in all treated orchards, the proportion of the infestation on olive-crops by *Bactrocera oleae*, was significantly less than the respective in the untreated orchard.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mortality after 3 days (%)</th>
<th>Efficacy after 3 days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Beauveria bassiana</em> (1)</td>
<td>96.2 a</td>
<td>95.8</td>
</tr>
<tr>
<td><em>Beauveria bassiana</em> (2)</td>
<td>95.4 a</td>
<td>94.9</td>
</tr>
<tr>
<td><em>Verticillium lecanii</em> (1)</td>
<td>11.3 b</td>
<td>1.9</td>
</tr>
<tr>
<td>Untreated</td>
<td>9.6 b</td>
<td></td>
</tr>
</tbody>
</table>

(1) Newly emergent adults
(2) Three days old adults
Table 2. Proportion of infestation of the olive-crops by Bactrocera oleae in the semi-field trials.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pre-spraying Infestation</th>
<th>Post-spraying Infestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three sprays (Kifissia region)</td>
<td>Treated orchard</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Control (untreated orchard)</td>
<td>-</td>
</tr>
<tr>
<td>Three sprays (Oropos region)</td>
<td>Treated orchard</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Control (untreated orchard)</td>
<td>-</td>
</tr>
<tr>
<td>One spray + food attractant (Kifissia region)</td>
<td>Treated orchard</td>
<td>4.12 a</td>
</tr>
<tr>
<td></td>
<td>Control (untreated orchard)</td>
<td>3.47 a</td>
</tr>
</tbody>
</table>

Within the limitation of laboratory bioassays, our results suggest that the *B. bassiana* based product is a suitable candidate to be used for the control of *B. oleae* adults and possibly other flies. *Beauveria bassiana* products could be a suitable alternative, with the added advantage to propagate and dispersal within the natural environment. Once the mortality and efficacy in laboratory and in semi-field bioassays has been demonstrated, the next step is to develop an application method that can be used in extended field.

References


Biological control of olive fruit fly through inoculative releases of *Opius concolor* Szépl.

Gavino Delrio, Andrea Lentini, Alberto Satta
*Dipartimento di Protezione delle Piante, sez. Entomologia agraria, Via E. De Nicola 07100 Sassari; Università di Sassari, Italy*

**Abstract:** A field trial of biological control of *Bactrocera oleae* (Gmel.) by means of inoculative releases of *Opius concolor* Szépl. was performed during 1997 – 2001 in an olive grove of 30 ha in northwestern Sardinia (Italy). The total number of parasitoids released per year, based on the density of infestation of the olives and the level of parasitism determined by *O. concolor*, varied between 36 and 225 adults per plant.

The maximum parasitism rate in the years 1997, 1998, 2000 and 2001 reached, respectively, 82, 62, 77 and 100% and was null in 1999. The high variability observed in the parasitism rate can be attributed to several factors: poor quality of mass-reared parasitoids, extremely high abundance of olive fly population during years of low yield, and the higher growth rate of *B. oleae* population compared to that of *O. concolor*. The results showed that inoculative releases of *O. concolor* were sufficient to control *B. oleae* infestation in years of high yield.

**Key words:** *Bactrocera oleae*, *Opius concolor*, inoculation biological control

**Introduction**

*Opius concolor* Szépl. (Hym. Braconidae) is an important parasitoid of *Bactrocera oleae* (Gmel.), the olive fruit fly, and other tephritids. In Italy *O. concolor* is found consistently only in Sicily and southern Sardinia, and sporadically in southern Tuscany (Canale and Raspi, 2000). This parasitoid has been mass reared in insectaries and repeatedly released in some Mediterranean regions to improve biological control of *B. oleae* (Delanoue, 1962; Monastero, 1967; Stavraki, 1966; Kapatos et al., 1977).

Large-scale experiments carried out in Sicily over several years indicate that inundative releases of *O. concolor* may reduce *B. oleae* population and might be an interesting alternative to chemical control (Genduso, 1981).

In Sardinia, laboratory rearing of *O. concolor* began in 1995 and from that year on, large-scale releases have been conducted with results that are difficult to evaluate.

This work reports the results of a biological control experiment using inoculative releases of *O. concolor* carried out over 5 years in an isolated olive grove in northwestern Sardinia, with the aim of understanding the factors which influence the efficiency of the parasitoid in controlling *B. oleae* populations and infestation of the olives.

**Material and methods**

The trials were conducted in the years 1997–2001 in an isolated olive grove of about 30 ha of oil cultivars (Bosana) with a few trees of table olives in Alghero, Sardinia, Italy. No chemical insecticides were applied in the olive grove during the observation period.

The parasitoids released were mass reared on *Ceratitis capitata* Wied. larvae in the Laboratorio Allevamento Insetti Utili – Centro Regionale Agrario Sperimentale (C.R.A.S.) –
in Ussana, Cagliari, Sardinia, using the standard techniques with several modifications (Delanoue, 1962; Genduso, 1967; Brotzu et al., 1996).

Population density of the adult olive fruit flies and *O. concolor* was monitored weekly using 5 yellow traps. Infestation was estimated weekly in a sample of 200 randomly selected drupes. The number of living and dead instars of the olive fruits fly was determined, and the mature larvae and pupae of *B. oleae* were kept at 25°C to observe emergence of *B. oleae* and *O. concolor* adults. After the adult emerged the remaining pupae were dissected to determine how many contained dead larvae or pupae of *Opius*. The occurrence of other *B. oleae* parasitoids was also observed. The mean percentage of *O. concolor* parasitism was calculated from the ratio between the number of *Opius* (emerged or dead) and the total number of *B. oleae* pupae.

The releases of the parasitoids were performed using paper bags containing about 10,000 *Ceratitis* puparia with *Opius* ready to emerge hung on the trees in the centre of the grove (Marongiu et al., in press).

**Results**

The inoculative releases of *Opius concolor* began in either July or August when 2\(^{nd}\) and 3\(^{rd}\) stage *B. oleae* larvae were found on olives, and continued over the following months. The number of parasitoids released was based on the level of infestation and parasitism and ranged from slightly more than 100,000 to over 600,000 adults per year. (Table 1). The highest number of *O. concolor* released was in 1999 since no parasitism was found on oil olives.

<table>
<thead>
<tr>
<th>Month</th>
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Data on trap captures of adult olive fruit flies and their parasitoid, active infestation level (live instars) and *O. concolor* parasitism for the five years of observation are given in Fig. 1 and 2.

In 1997, a year of high yield, the *B. oleae* population remained fairly low for the whole season. Infestation level was lower than 10% in summer and began to increase at the end of October. Parasitism of *B. oleae* larvae by ectophagous Chalcidids (mainly *Pnigalius agraules* (Walk), but also *Eupelmus urozonus* Dalm. and *Eurytoma martellii* Dom.) of maximum 16% was observed in summer. The first larvae parasitised by *O. concolor* were found at the beginning of August and the rate of parasitism increased rapidly from the end of the month, remaining high throughout autumn. More *Opius* adults were captured in the traps in autumn than olive fruit flies, with a peak in October. Mortality caused by *Opius* certainly played a part in keeping olive infestation low which was only 22% at the end of November.

Olive yield in 1998 was low and climatic conditions, with moderate maximum temperatures, favoured the development of *B. oleae*. Captures of *B. oleae* were high through-
out the summer and infestation, already high in August, continued to rise, reaching 70% at the beginning of November.

The level of parasitism by ectophagous Chalcidids was low in August but rose to 14% in September. *Opius* parasitism fluctuated at around 50% for the whole autumn with a peak of 62% in October. Despite this, fewer *Opius* were captured than *B. oleae*. The action of *Opius* was insufficient to control the attack of *B. oleae* because of the low number of olives.

![Figure 1](image-url)

Figure 1. A) Yellow trap captures of adult *Bactrocera oleae* and *Opius concolor*. B) Percentage of active infestation found on oil olives and *Opius concolor* parasitism rates (Alghero, 1997-1999).

1999 was a year of high yield, characterised by very high temperatures in August (maximum temperatures 34 – 40°C for 11 consecutive days), which suppressed *B. oleae* infestation in the summer. Infestation returned in September on the table olives and in October on the oil olives, reaching a maximum of 60% in November. *O. concolor* parasitism
was found only on table olives in September and October, with a maximum of 15%. Mature larvae of *B. oleae* on oil olives were found only in November and therefore escaped attack by the parasitoids. Mean values of the number of *B. oleae* and its parasitoid captured were low.

In 2000, a year of very low yield and high populations of olive fruit flies in summer, infestation of 100% was found in August on the oil olives, with an average 10 punctures per olive. Infestation was subsequently suppressed by high temperatures that reached 35-40°C for 12 consecutive days but returned in September and rapidly affected all the olives. The olive fruits that were attacked mostly fell too early and the few olives remaining on the tree were infested and/or had emergence holes. The level of *O. concolor* parasitism became high only at the end of October and the number of adults trapped was very low.

Production in 2001 was high, with sporadic trapping of olive fruit flies in the summer months. Infestation was therefore very low in summer and progressively increased in autumn, reaching a maximum of 40% in October. This low level of infestation was certainly due in part to *Opius*, which showed high values already at the beginning of September and reached 100% in November. The number of *O. concolor* captured in the traps was also very high and in November was five times the number of *B. oleae* trapped.

![Figure 2. A) Yellow trap captures of adult *Bactrocera oleae* and *Opius concolor*. B) Percentage of active infestation found on oil olives and *Opius concolor* parasitism rates (Alghero, 2000-2001).](image-url)
Discussion

The biological control trial using *Opius concolor* showed that it is possible to reach high levels of parasitism on *B. oleae*, with maximum values of 80, 60, 80 and 100% respectively in 1997, 1998, 2000 and 2001, even with inoculative releases of a small number of individuals.

In difficult climatic conditions, such as those of 1999 when very high temperatures prevented the parasitoid from multiplying, no parasitism was obtained on the oil olives even by releasing a much higher number of individuals.

In the years when the parasitoid was effective, between 36 and 119 adults per tree were released, equal to the number used in Sicily (Genduso,1981), but fewer than in trials carried out by other authors (Jimenez, 1985; Jimenez et al., 1990). In our experiment, we observed that *Opius* increases its population by reproducing first on olives that were attacked early (mainly table olives) and later on oil olives, where its activity is at a peak in October and November. According to the definition of Eilenberg et al (2001), this type of biological control can be classified as inoculation biological control, not inundation biological control since the released organism will control the target after multiplication.

The marked variability in the pattern of parasitism rates in the years of the trial could depend on different factors, such as the poor quality of laboratory-reared parasitoids, climatic conditions, abundance of olive fruit fly populations at the beginning of summer, and olive fruit yield. As regards parasitoid quality, it has been observed that artificially reared *O. concolor* have poor flying ability, which could depend on the genetic structure of the population, reared in mass for several years, or the rearing methods employed (Delrio et al., in print). The number of *B. oleae* adults present and the yield of olive fruits are the most important factors in determining the efficacy of *Opius* in controlling the infestations. In years of low production (1998 and 2000), despite the high level of parasitism, the olives were totally damaged by the very high rate of infestation. The best results, on the other hand, were obtained in years of high yield (1997 and 2001) in which the first attack was very low and infestation increased slowly over the following months. The action of the parasitoid kept the damage down to tolerable levels on the oil cultivars, which at harvest had 20-40% of olives infested.

Ectophagous Chalcidids also contributed to the biological control of *B. oleae*. Their parasite activity was never at a high level but started earlier than that of *Opius*.

In conclusion, experiments conducted over the last few years seem to indicate that the use of *Opius* in inoculation biological control can be recommended only in years of high yield on oil olives, also as part of integrated control alongside other methods (attract and kill) allowed in organic oliviculture.

References


A forecasting model of the olive-fruit fly infestation based on monitoring of males

Paola Lo Duca¹, Antonio F. Spanedda², Alessandra Terrosi², Claudio Pucci²
¹. Dipartimento di Statistica, Probabilità e Statistiche Applicate, Università “La Sapienza”, Roma - Italy
². Dipartimento di Protezione delle Piante, Università della Tuscia, Viterbo – Italy

Abstract: The relationship between Bactrocera oleae (Gmel.) males captured by means sexpheromone traps and drupe infestation was studied. The statistical method applied was the canonical analysis. This method consisted in determining pairs of linear combinations of two groups of variables having the highest within-pair correlation. The first group of variables, that is the predictive ones, were the number of males (average/trap) captured during one week and the mean value of some climatic parameters (i.e. temperature, relative air humidity and rainfall) recorded in the same capture week. On the other hand, the subsequent infestation, detected both by picking fruits directly from the tree and by gathering dropped fruits, represented the second group of predicted variables. Infestation was subdivided into three categories: eggs + 1st instar larvae, 2nd + 3rd instar larvae, cocoons + empty cocoons + abandoned galleries. Results evinced that the linear combination of predictive variables (forecasting model) showing the best correlation with the infestation variables is the following: 

\[ Z = 0.027 M_m - 0.399 T_m + 8.71 \]

where, \( M_m \) is the average number of males / week captured by means of a sex-pheromone trap and \( T_m \) represents the mean temperature of the capture week. The others climatic factors were not taken into account as they do not improve the above model. The forecasting model will be a useful tool for defining a threshold for suitability or unsuitability of control intervention.

Key words: forecasting model, Bactrocera oleae, sex-pheromone trap

Introduction

Tools, such as forecasting models, are currently being employed with the aim of increasing effectiveness of treatment thresholds for pest control. On concerning olive fruit fly we essentially have two kinds of models: one based on the phenology of the pest and the other referred to its population dynamics. The former provides information mainly on phenological phases and on development rate during the season depending on thermal conditions of the environment (Raspi, 1999). The latter, usually more complex, has the purpose of estimating population density of the pest (by trapping females) and then forecasting level and severity of infestation (Pucci, 1993).

The olive fly sex-pheromone has been isolated from females more than 20 years ago and its use for monitoring is now quite widespread. As the environmental impact of sexpheromone based traps is irrelevant and their management is very feasible, using them for elaborating a forecasting model useful for the definition of a treatment threshold was the aim of the present work. It has been observed that olive fruit infestation not only depends on the population density of the fly adults, but also on many other factors such as the insect biology and its mobility, incidence of predators and parasitoids of the preimaginal stages, kind of cultivar, foliar density, production yields and last but not least on climatic factors (Ricci et al., 1979; Ballatori et al., 1980; Ricci et al., 1982; Pucci et al., 1982; Bagnoli et al., 1982).
The relationship between captured males of olive fruit fly and subsequent infestation of olives has been studied. The statistical method applied was the canonical analysis. This method consisted in determining pairs of linear combinations of two groups of variables having the highest within-pair correlation.

The first group of variables, that is the predictive ones, were the number of males (average/trap) captured during one week, as well as the mean value of some climatic parameters (i.e. temperature, relative humidity and rainfall) recorded in the same capture week were taken into account. On the other hand, the subsequent infestation, detected both on fruits picked directly from the tree and on dropped fruits gathered on the ground, represented the second group of predicted variables.

Material and methods

The experiment was carried out in 2002—a year featured by a severe B. oleae infestation—in an olive grove near the coast in the middle of Abruzzo Region in Central Italy. The olive grove, with a compass of 6 x 6 m is made up of 15 years old trees, mainly of Leccino cultivar (90%). For the whole period of investigation no treatments were performed.

By the third decade of July, 5 trees of “Leccino” were chosen. On each tree a sex-pheromone trap was placed tangentially to the foliage at medium height of the canopy; the exposure was South-West. The pheromon dispensers were replaced every four weeks.

Olive fly males captured were counted weekly. At the same time the olive sampling was performed by randomly picking 40 drupes per plant from the same tree chosen for flies trapping. Then olive fruits have been observed by a stereomicroscope with the aim of quantifying infestation. Furthermore, all fruits dropped during the capture week were gathered and weighted. From them 40 drupes were chosen for detecting infestation.

Infestation was subdivided into 3 categories, namely:
- I type = eggs (dead and alive) + 1st instar larvae (dead and alive)
- II type = 2nd instar larvae (dead and alive) + 3rd instar larvae (dead, alive and parasitized)
- III type = pupae (dead, alive and parasitized) + empty cocoons + abandoned galleries

The primary aim of the analysis was to study the correlation between olive infestation and number of males captured by means of sex-pheromone traps. As regards infestation assessment, 6 variables potentially suitable were chosen. They represented the “dependent variables”. Each variable corresponded to the percentage of sampled olives (at the various times for the individual tree) found to be infested by the different developmental stages of the fly. The following dependent variables were grouped into an X set:

(olives picked on the canopy)
- x1 = % infestation of I type at t time + 1 week
- x2 = % infestation of II type at t time + 2 weeks
- x3 = % infestation of III type at t time + 4 weeks

(dropped olives gathered on the ground)
- x4 = % infestation of I type at t time + 4 weeks
- x5 = % infestation of II type at t time + 4 weeks
- x6 = % infestation of III type at t time + 4 weeks

where t time was that after a whatever capturing week.

The other set of variables (Y), it means the “independent” ones, was composed by the following parameters considered at time t:
- y1 = average number of male/trap/week
- y2 = average of daily mean temperature recorded in the capturing week
- \( y_3 \) = average of daily mean relative humidity recorded in the capturing week
- \( y_4 \) = cumulated rainfall of the capturing week

In Figure 1 trends of climatic parameters recorded all over the investigation period are shown.

**Fig. 1. Trends of climatic parameters**

The working hypothesis consisted in assuming a link between the two sets of variables: \((y_1, y_2, y_3, y_4)\) and \((x_1, x_2, x_3, x_4, x_5, x_6)\). In order to detect this link from a statistical point of view, a data array per plant was made up, in which to each \( Y \) set of a week corresponded an \( X \) set determined as above. In this way, for each of the 10 sample trees it was possible to identify 10 observations of the variables concerned (from July the 23\(^{rd}\) to September the 24\(^{th}\)) thereby obtaining an array with three dimensions corresponding to: number of sampled trees, number of data recording weeks, number of analysed variables. It must be highlighted that September the 24\(^{th}\) represented the last useful date for searching correlation between the above mentioned variables, as in that week infestation attained almost 100% and maintained it until the harvest. To this array, the technique of canonical analysis was applied (Dillon & Goldstein, 1984; Coppi, 1986), which consists in determining pairs of linear combinations, respectively from the \( Y \) and \( X \) sets, having the highest possible correlation between them.

Graphs in Figure 2 show dynamics of both captured males and infestation.

**Results and discussion**

A first application of the canonical analysis to the data of this experiment proved the scarce contribution of the variable \( y_3 \) and \( y_4 \), whose inclusion into the analysis does not modify the
results obtainable with the other variables. Therefore, the analysis was limited to searching for the following linear combinations:

\[ Z = a_1 y_1 + a_2 y_2 \]
\[ W = b_1 x_1 + b_2 x_2 + b_3 x_3 + b_4 x_4 + b_5 x_5 + b_6 x_6 \]

so that \( r^2 (Z,W) = \max \).

How and in which direction can one of the two canonical variables be predicted on the basis of the values taken by the other, is especially important, as the knowledge of both the number of captured males and mean temperature of the week at any \( t \) time, would allow us – through the linear combination \( Z \) – to predict the value of \( W \), which should presumably be an indicator of the infestation expected in the immediately subsequent period.

The SAS CANCORR procedure, applied to the data of this work, suitably reduced as explained above, gave as first best solution the following pair of canonical variables:

\[ Z = 0.027 y_1 - 0.399 y_2 \]
\[ W = -0.010 x_1 - 0.001 x_2 + 0.023 x_3 - 0.681 x_4 - 1.490 x_5 + 0.802 x_6 \]

with (canonical) correlation coefficient \( r (Z,W) = 0.90 \) (see Figure 3).
Within this solution, Z accounts for 81% of the variability of the y values and for 66% of the x values. On the other hand, W explains 71% of its own set of variables (x values) and 58% of the opposite set (y values). The canonical variables involved are therefore a good statistical synthesis of the relevant groups of original variables. Moreover, the canonical variable Z
seems to be sufficient to explain the infestation variables. It is worth mentioning that the subsequent pairs of canonical variables (which are not going to deal with now) do not supply any further meaningful statistical contributions for the explanation of the assessed phenomenon, as compared with the first pairs (Z,W) already considered.

It is clear that W can be considered as an infestation indicator, especially with reference to cocoons and abandoned galleries and to the 2nd and 3rd instar larvae, which appear to be strictly linked to this canonical variable (both taking into account pendant and dropped olive fruits), causing the most severe damage. On the other hand, as it was to be expected, the relationship with the early stages (eggs and 1st instar larvae) is weaker, especially for dropped fruits. Therefore W expressed, basically, a “mature” infestation. Higher values of W are suggestive of this situation, whereas low values, on the contrary, point to the absence of an infestation due to more advanced development stages.

In Figure 4 it can be observed the agreement between increasing infestation on olives still pendant on the tree and increasing Z index values.

To make an operational use of this result, a threshold value must be determined, let us say Z*, so that when Z>Z* infestation development in the immediately following period will probably rise enough and a treatment is justified. To calculate the average value of Z at a whenever date, the general average values for the whole period of both y₁ and y₂ should be taken into account. Thus, the result appears into the formula as a fixed known term:

\[ Z = 0.027 M_m - 0.399 T_m + 8.71 \]

where:

- \( M_m \): is the average number of males weekly captured by means of sex-pheromone traps
- \( T_m \): is the average of the seven daily mean temperatures recorded in the same capture week

It must be stressed that the above formula has to be proved for other sites and for at least another year. Defining the threshold value for treatment will be our next objective.

**Acknowledgements**

We thank the Abruzzo Regional Agency for Rural Development (ARSSA) for the availability and courtesy in accompanying and satisfying all our needs in implementing this work.

**References**


Effect of temperature on the development and on other biological parameters of the parasitoid *Pnigalio pectinicornis* (Linnaeus) *(Hymenoptera: Eulophidae)*

**Argyro Kalaitzaki**¹, **Dyonysis Lykouressis**², **Stelios Michelakis**³, **Venizelos Alexandrakis**³

¹ Prefecture of Chania, Division of Agricultural Development, Chania Greece, E-mail: argkalaitzaki@yahoo.com

² Laboratory of Agricultural Zoology and Entomology, Agricultural Univ. of Athens, Iera Odos 75, 118 55 Athens, Greece, E-mail: lykouressis@auadec.aua.gr

³ Inst. of Olive Tree and Subtropical Plants of Chania, Agrokipio, 73 100 Chania, Greece, E-mail: valexandr@nagref-cha.gr

**Abstract:** *Pnigalio pectinicornis* is an indigenous polyphagous parasitoid mainly of Lepidoptera, Diptera and Coleoptera. Shipments of the species, reared from *Bactrocera oleae* and *Phyllocnistis citrella* during 1997 and 1998 were identified from Dr La Salle (CABI Bioscience UK Centre). In Greece has been found to parasitized *Bactrocera oleae*, *Prays oleae*, *Phyllocnistis citrella* and several other microlepidoptera (Neuenschwander et al., 1983, Kalaitzaki et al, 2002). The effect of temperature on the development and mortality was studied under controlled conditions when the parasitoid developed on *P. citrella* as host pest and fed on leaves of *Washington navel*. Mean total development time of immature stages was estimated at 15, 20, 25, 30, and 32.5°C. Within the limits 15–30°C development time shortened as the temperature increased. The shortest total development time of the immature stages was recorded at 30°C. Development time of females was longer than males. The highest mortality rate was found at 15°C and the lowest at 25°C. Between the different stages the larva was the most sensitive stage at all the temperatures and the pupa stage the less sensitive. Lower development threshold and thermal constant estimated to 5.25°C and 192 DD respectively for the total development period. Intrinsic rate of natural increase was highest at 20°C and lowest at 30°C. The intrinsic rate of natural increase almost tripled from 25 to 20°C and doubled from 30 to 25°C. It is concluded that having no diapause, development of *P. pectinicornis* could continue all the year under Greece climatic conditions. Is observed throughout the season but becomes abundant from August to October in the olive groves and from late May to early July in the citrus orchards. Is an effective biological control agent for *B. oleae* late in the season under our climatic conditions.

**Key words:** *Pnigalio pectinicornis*, temperature, development, biological control

**Introduction**

*Bactrocera oleae* (Gmel.) is the most important insect pest of olives in the Mediterranean area. The parasitoid complex associated with the olive fly, in the Mediterranean area, it includes the following parasitoids: *Pnigalio mediterraneus* (Ferr. & Del.), *Eupelmus urozonus* (Dalm.), *Cyrioptyx latipes* Rond., *Eurytoma martelli* (Dom.), and *Opis concolor* (Szepl.) (Neuenschwander et al., 1983). *P. mediterraneus* is a Holarctic species and polyphagous parasitoid of leafmining or gallmaking insects mainly Lepidoptera but also Coleoptera, Diptera and Hymenoptera (Schauft et al., 1998). Shipments of the species, reared from *Bactrocera oleae* and *Phyllocnistis citrella* during 1997 and 1998 were identified from Dr LaSalle (CABI Bioscience UK Centre). In Greece has been found to parasitized *Bactrocera oleae*, *Prays oleae*, *Phyllocnistis citrella* and several other microlepidoptera (Neuenschwander et al., 1983, Stavraki, 1970, Kalaitzaki et al, 1997). Although *P. pectinicornis* is important natural enemy in regulating population of several pests, there is no available data for its biology.
The objective of the current study have been to determine the influence of temperature on development and mortality of *P. pectinicornis* under controlled laboratory conditions in order to estimate the rate of development, the lowest developmental thresholds, the thermal constant for the total development period of immature stages and the intrinsic rate of natural increase of parasitoid. As host pest was used *P. citrella* fed on leaves of *Washington navel*.

**Material and methods**

**Development and immature mortality at different temperatures**

Detached infested leaves, housing stages preferred for parasitization of *P. pectinicornis* which are 3rd instars and prepupae stages of *P. citrella*, were placed in groups of ten instars per cage into small plastic jar filled with water (25 ml), and left undisturbed for 4h. Leaves were checked under stereoscopic binocular microscope at the end of the exposure time and those exhibiting hosts parasitized with 1 single egg were placed individually into a plastic petri dish (9 cm diameter and 1.5 cm high). The parasitized hosts were transferred in controlled environment room at 15, 20, 25, 30, 32.5°C, RH 60 ± 10% and 14L:10D hrs photoperiod and 10,000 Lux light intensity. The development time and the immature mortality were studied at 15, 20, 25, 30 and 32.5 ± 0.5°C on host plant sweet orange *Citrus sinensis* (L) Osbck (cv. *Washington navel*). The parasitoid development of immature stages and mortality were recorded at 12 hrs intervals until adult emergence except for the case of 32.5°C in which recording at 8 hrs interval. Eventually adults were sexed. Parasitoid development period was measured as the time from oviposition to adult emergence and includes the egg, larval and pupal period.

**Development rate, temperature thresholds (t) and thermal constant (K) of parasitoid**

In the present study the *t* and the *K* for the period of development of immature stages of parasitoid were calculated by using the regression equation *y=a+bT* were *y* was the rate of development (reciprocal of the mean development time in days or 1/D being the time in days required for the completion of a particular development stage at the temperature T in °C). The lower thermal threshold was calculated as *t* = -a/b (a=intercept and b= the slope of the above referred linear equation) and the thermal constant as *K*=1/b (DD) of the temperature development rate equation (Campbell *et al.* 1974).

**Intrinsic rate of natural increase (rm)**

The following equation was used for estimating of intrinsic rate of natural increase (rM):

\[
\Sigma e^{-r_{m}x}l_{x}m_{x}dx = 1
\]

in which *x* = is the mid point of age interval in days, *r_M* = intrinsic rate of increase, *l_x* = the fraction of the females surviving to the pivotal age *x* (the probability of a female surviving to age *x*) (developmental duration plus the time after emergence) and *m_x* = the mean number of female births during age interval *x* produced per female aged *x* and *e* is the base of natural logarithms (Birch, 1948).

**Results and discussion**

**Development time and mortality of immature stages**

Mean total developmental time varied from 23.95 days at 15°C to 8.45 days at 32.5°C. Within the limits 15–30°C development time shortened as the temperature increased. The shortest total developmental period of the immature stages was recorded at 30°C (Fig. 1). The period of the development of the pupal stage was proved to be in all cases the longest while the egg stage found to developed in a significantly shorter period (Fig. 1).
Total developmental time was shorter at males than females at all tested temperatures except at 32.5°C which females showed a non-significant shorter period of development (Table 1).

The highest mortality rate for the total developmental time (51.5%) was found at 15°C and the lowest mortality at 25°C (30.7%). Among the different immature stages the larva was the most sensitive stage at all the temperatures and the pupal stage the less sensitive (Table 2).

![Developmental time in days (mean ± SE) of egg, larval and pupal stage of the parasitoid *P. pectinicornis* at different constant temperatures. Means followed by different small letters per column of respective immature stage are significantly different (P<0.05).](image)

**Developmental rate, lower threshold and thermal constant**

The estimated lower development threshold was found to be 7.03 °C for egg development, 5.63°C and 5.36°C for larval and pupal development respectively and 5.25°C for the total period of development (from egg to adult) (Table 3).

The day degrees demanded for completion of *P. pectinicornis* development was found to be 192 degree days. The pupal stage of the parasitoid has the largest thermal constant followed by that of the larval stage. The egg stage had the lowest thermal constant (Table 3).

Taking these in account and knowing that for poikilothermic animals, lower temperature thresholds estimated from linear regressions are usually higher than actual values (Laudien, 1973) it is concluded that during the coldest months parasitoid development does not stop.
under Greece weather conditions. These findings are in agreement with those of other workers in the study of Neuenschwander et al. (1983) and of Michelakis (1986) who found that in Crete this parasitoid is the only of the complex in olive orchards in late autumn and winter.

Table 1. Development period in days (mean ± SE) of total development period of immature stages of male and female of *P. pectinicornis* on *P. citrella* at different constant temperatures and on sweet orange *Citrus sinensis* (cv. Washington navel) host plant. Means followed by different small letters in a row are significantly different (P<0.05).

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<tr>
<th>Temperature °C ± 0.5°C</th>
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<th>Female</th>
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<td>15</td>
<td>23.95 ± 0.37 a</td>
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<td>25</td>
<td>8.86 ± 0.14 a</td>
<td>9.61 ± 0.37 b</td>
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<td>30.25</td>
<td>8.65 ± 0.05 a</td>
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Table 2. % Mortality of egg, larval and pupal stage of *P. pectinicornis* reared on *P. citrella* at different temperatures on sweet orange.

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<th>Temperature °C ± 0.5°C</th>
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<th>Larva</th>
<th>Pupa</th>
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<td>15</td>
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<td>34.6</td>
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Table 3. Regression equations which are described the relationship between the rate of development of the parasitoid *P. pectinicornis* and the temperature (X) on sweet orange and the estimates of lower temperature threshold \( t=-a/b \) (in °C) and the thermal constant \( k=1/b \) (in DD).

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<tr>
<th></th>
<th>d.f</th>
<th>Regression Equation</th>
<th>( R^2 )</th>
<th>( P )</th>
<th>( t )</th>
<th>( K )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>(1,187)</td>
<td>( Y=-0.3730 + 0.0532 X )</td>
<td>0.9804</td>
<td>0.000</td>
<td>7.03</td>
<td>19</td>
</tr>
<tr>
<td>Larva</td>
<td>(1,187)</td>
<td>( Y=-0.0733 + 0.0163 X )</td>
<td>0.6440</td>
<td>0.000</td>
<td>5.63</td>
<td>61</td>
</tr>
<tr>
<td>Pupa</td>
<td>(1,187)</td>
<td>( Y=-0.0488 + 0.0091 X )</td>
<td>0.8276</td>
<td>0.000</td>
<td>5.36</td>
<td>110</td>
</tr>
<tr>
<td>Egg to adult</td>
<td>(1,187)</td>
<td>( Y=-0.0274 + 0.0052 X )</td>
<td>0.8614</td>
<td>0.000</td>
<td>5.25</td>
<td>192</td>
</tr>
</tbody>
</table>
Intrinsic rate of natural increase ($r_m$)

Temperature had a major effect on the intrinsic rate of natural increase of $P. pectinicornis$ and it was highest at 20°C ($r_m=0.073$ female eggs/female/day) and lowest at 30°C ($r_m=0.012$ female eggs/female/day). The intrinsic rate of natural increase almost tripled from 25 to 20°C and doubled from 30°C to 25°C. These data indicates that the optimum temperature for population growth is 20°C.

This eulophid parasitoid is observed throughout the season, but it becomes abundant only from August reaching a peak in October. Having no winter diapause, is the only parasitoid of $B. oleae$ in late autumn and winter that reared from olive fruits. Also $P. pectinicornis$ was reared in winter and spring from leaf mines on olive trees, which were attributed to $Prays oleae$ Bern (Neuenschwander et al., 1983).

It is concluded that, development of $P. pectinicornis$ could continue all the year under Greece climatic conditions. Is observed throughout the season but becomes abundant from August to October in the olive groves parasitized $B. oleae$ and $Prays oleae$ and from late May to early July in the citrus orchards parasitized $Phyllocnistis citrella$ (Kalaitzaki et al, 2002). The findings of this study indicates that $P. pectinicornis$ could be an effective biological control agent of $B. oleae$ during autumn and early winter under Crete climatic conditions.

References

Experiments for the control of olive fly in organic agriculture

Lentini Andrea, Delrio Gavino, Foxi Cipriano

Dipartimento di Protezione delle Piante, Sezione di Entomologia agraria, Università degli Studi di Sassari, Via E. De Nicola, 07100 Sassari, Italy

Abstract: During the years 2001-2002 several experiments were carried out in Sardinia to estimate the efficiency of insecticides of natural origin and of the mass-trapping technique to control the olive fruit fly. Rotenone never determined a containment of the insect infestation. The pesticides having a base of Azadirachtin gave contradictory results and, in most of the tests, were not efficient. Copper salts constantly determined a reduction in the infestations, even though that was significant in only one case. Traps used for mass-trapping were baited with ammonium carbonate and sexual pheromone and treated with deltamethrin (Vioryl Ecotrap) or lambda-cyhalothrin (AgriSense Attract & Kill). In mid-July, the traps were hung with a density of 100 per hectare. The two types of trap gave positive results showing, in some cases, a halving of the damage produced by the olive fly compared to the untreated plots. The techniques currently available for the control of the olive fly in organic agriculture are less efficient than chemical control and are rather unreliable. It is, therefore, necessary to keep testing the existing techniques in order to establish the best conditions for their use.

Key words: Bactrocera oleae, mass-trapping, Rotenone, Azadirachtin, copper salts.

Introduction

According to the Regulation (EEC) N° 2092/91, the only employable methods of control of the olive fruit fly in order to obtain an organic production are the use of insecticides of natural origin and the mass-trapping technique. During the years 2001-2002 several experiments were carried out in Sardinia (Italy) to estimate the efficiency of these methods.

Materials and methods

Chemical control experiments

The experiments testing insecticides allowed by EEC for organic production were conducted in oil-olive groves of cv. Semidana in the Oristano province (Central-Western Sardinia).

In the year 2001 the experiments were conducted in two olive orchards, of 4 and 6 hectares each, subdivided in plots of the same size. The plots were treated with Azadirachtin A, Rotenone, copper hydroxide and copper oxychloride. One of the plots was untreated and used as control. In the year 2002 the experiments were conducted in two other olive groves, 2 hectares each, subdivided in plots. One plot was untreated and used as control, while the others were treated with products containing Azadirachtin A, Rotenone and copper sulphate. In both years, the plants were treated twice with standard volumes using the doses suggested by the insecticide producing companies (Table 1).

The efficacy of the insecticides against the olive fly was evaluated at harvest time measuring the olive infestation levels expressed as percentage of olives with L3, pupae and exit holes. From each treatment 4 samples of 50 olives, one per tree randomly chosen, were collected. Data were analysed by analysis of variance followed by Duncan’s multiple range test for mean separation.
**Olive fly control with the mass-trapping technique**

Mass-trapping experiments were conducted with two types of trap: Eco-Trap (Vioryl) and Attract and Kill (AgriSense).

The Eco-Trap is a 15 cm x 20 cm envelope, made of light-green-coloured paper, with 15 mg a.i. of Deltamethrin on its surface. Each trap contains 70 g of ammonium bicarbonate salt, and a pheromone dispenser. The AgriSense Olive Fruit Fly Attract and Kill is a laminated card target device that is pre-treated with 15 mg of Lambda-cyhalothrin and carries both the pheromone and ammonium bicarbonate attractant lure formulations.

In 2001, the tests were carried out in two oil-olive groves using Eco-Traps only. One olive grove, of 1 ha and located in Siamanna (OR), is isolated, being 500 m far from other olive groves. The other olive grove, of 3 ha and located in Alghero (SS), is near a large area rich of olive groves. In both locations, one olive grove located nearby the experimental grove was left untreated and considered as a control. In 2002, the experiments comprised 5 oil-olive groves of approximately 15 hectares each. The efficiency of the Eco-traps was compared to that of the AgriSense traps. Each grove was divided into three plots of equal area: two were protected using either types of trap and one was kept as an untreated control plot.

The traps were hung in mid-July with a density of 100 per hectare. Evaluation of the efficacy of the traps against the olive fly was based on the olive infestation levels at harvest time expressed as percentage of olives with L3, pupae and exit holes. From each treatment 4 samples of 50 olives, one per tree randomly chosen, were collected.

Data were analysed by analysis of variance followed by Duncan’s multiple range test for mean separation.

**Results**

**Chemical control experiments**

The results of 2 years of experiments showed that the products based on Rotenone were not effective at all. The products based on azadirachtin A only in one trial out of four caused an infestation reduction, that was very small but statistically significant ($P<0.05$) (Table 1). The plots treated with the copper products had lower infestations than the control plots, but only once this effect was significant ($P<0.05$).

**Olive fly control with the mass-trapping technique**

During the year 2001, the olive groves in which the Eco-Trap was used had always lower infestations than the controls. By the end of October-mid November the percentage of damaged olives was less than half that of the control (Table 2).

During the year 2002, the olive fly infestation was very high in all experimental olive groves, except for that of Simaxis (OR). The plots with either types of mass-traps had much lower infestations than the control ones and, in same cases, the percentage of olives damaged was halved compared to the control (Table 2).

**Discussion and conclusions**

Among the products tested, Azadirachtin gave contrasting results, while Rotenone never controlled the olive fly. The best results were obtained with copper products, even though the reduction was significant only once. The efficacy of copper products was reported in other experiments (Belcari & Bobbio, 1999). Copper products had a limited negative impact on useful arthropods. However, it is still necessary to test if they leave residues in the oil.
Table 1. Olive fly control experiments with insecticides used in organic agriculture.

<table>
<thead>
<tr>
<th>Test location</th>
<th>Active ingredient</th>
<th>Insecticide</th>
<th>Dose (g/hl H2O)</th>
<th>Application dates</th>
<th>% Fruit infestation (mean ± st. dev.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donigala (Oristano, 2001) 6 hectares</td>
<td>Rotenone</td>
<td>Rotena</td>
<td>250 ml</td>
<td>August 15, September 19</td>
<td>86.5 ± 16.5 b</td>
</tr>
<tr>
<td>&amp; Azadirachtin</td>
<td>Oikos</td>
<td>150 ml</td>
<td></td>
<td>&amp; 53.5 ± 11.3 a</td>
<td></td>
</tr>
<tr>
<td>&amp; Cu hydroxide</td>
<td>Coprantol</td>
<td>300 g</td>
<td></td>
<td>&amp; 38.5 ± 3.0 a</td>
<td></td>
</tr>
<tr>
<td>&amp; Cu oxychloride</td>
<td>Neoram</td>
<td>500 g</td>
<td></td>
<td>&amp; 52.0 ± 13.9 a</td>
<td></td>
</tr>
<tr>
<td>&amp; Untreated</td>
<td></td>
<td></td>
<td></td>
<td>&amp; 82.0 ± 6.7 b</td>
<td></td>
</tr>
<tr>
<td>Cabras (Oristano, 2001) 4 hectares</td>
<td>Rotenone</td>
<td>Rotenil</td>
<td>250 ml</td>
<td>August 15, September 19</td>
<td>44.5 ± 18.8 b</td>
</tr>
<tr>
<td>&amp; Azadirachtin</td>
<td>Oikos</td>
<td>150 ml</td>
<td></td>
<td>&amp; 26.0 ± 13.9 a</td>
<td></td>
</tr>
<tr>
<td>&amp; Cu hydroxide</td>
<td>Coprantol</td>
<td>300 g</td>
<td></td>
<td>&amp; 10.0 ± 4.9 a</td>
<td></td>
</tr>
<tr>
<td>&amp; Cu oxychloride</td>
<td>Neoram</td>
<td>500 g</td>
<td></td>
<td>&amp; 14.5 ± 5.7 a</td>
<td></td>
</tr>
<tr>
<td>&amp; Untreated</td>
<td></td>
<td></td>
<td></td>
<td>&amp; 22.5 ± 9.6 a</td>
<td></td>
</tr>
<tr>
<td>Cabras (Oristano, 2002) 2 hectares</td>
<td>Rotenone</td>
<td>Rotenil</td>
<td>300 ml</td>
<td>September 4, September 25</td>
<td>48.0 ± 9.2 b</td>
</tr>
<tr>
<td>&amp; Azadirachtin</td>
<td>Oikos</td>
<td>150 ml</td>
<td></td>
<td>&amp; 31.0 ± 23.5 b</td>
<td></td>
</tr>
<tr>
<td>&amp; Cu sulphate</td>
<td>Cuproxat</td>
<td>500 ml</td>
<td></td>
<td>&amp; 11.0 ± 2.0 ab</td>
<td></td>
</tr>
<tr>
<td>&amp; Untreated</td>
<td></td>
<td></td>
<td></td>
<td>&amp; 31.0 ± 17.0 ab</td>
<td></td>
</tr>
<tr>
<td>Nurachi (Oristano, 2002) 2 hectares</td>
<td>Rotenone</td>
<td>Rotenil</td>
<td>300 ml</td>
<td>September 12, September 30</td>
<td>48.0 ± 14.6 a</td>
</tr>
<tr>
<td>&amp; Azadirachtin</td>
<td>Oikos</td>
<td>150 ml</td>
<td></td>
<td>&amp; 48.0 ± 16.0 a</td>
<td></td>
</tr>
<tr>
<td>&amp; Cu sulphate</td>
<td>Cuproxat</td>
<td>500 ml</td>
<td></td>
<td>&amp; 33.0 ± 5.1 a</td>
<td></td>
</tr>
<tr>
<td>&amp; Untreated</td>
<td></td>
<td></td>
<td></td>
<td>&amp; 50.0 ± 12.0 a</td>
<td></td>
</tr>
</tbody>
</table>

Means followed by the same letter are not statistically different at P≤0.05 (Duncan’s Test).

It would also be important to test if their efficacy can be improved by combining them with other insecticides of vegetal origin (Tsolakis & Ragusa, 2001).

The mass-trapping technique was able to reduce the populations of olive flies even in years of high infestation. Indeed, in many cases, the percentage of damaged olives at harvest did not reach the threshold of 30-40%. This allows the production of olive oil with chemical, physical and organoleptic characteristics typical of extra-virgin oils (Longo & Parlati, 1991; Parlati & Iannotta, 1993; Delrio et al., 1995). Other experiments carried out in Italy and Europe (Silvestri, 1999; Petacchi et al., 2001; Viggiani, 2001; Broumas et al., 2002) found similar results, confirming the efficacy of the technique. However, the utilisation of these traps encounters many limitations: 1) high cost of the technique; 2) low efficacy with high infestations; 3) necessity of use in large areas or in isolated olive groves.

The techniques currently available for the control of the olive fly in organic agriculture are less efficient than chemical control and are rather unreliable. At the moment, only copper products and mass-trapping seem to be useful to reduce infestations of *Bactrocera oleae*. 
Table 2. Results of mass-trapping experiments for olive fly control.

<table>
<thead>
<tr>
<th>Location &amp; Year</th>
<th>% Fruit infestation (L3, pupae, exit holes) (mean ± st. dev.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
</tr>
<tr>
<td>Alghero (Mamuntanas), 2001</td>
<td>18.0 ± 9.5 a</td>
</tr>
<tr>
<td>Siamanna (Or), 2001</td>
<td>79.0 ± 5.0 a</td>
</tr>
<tr>
<td>Siamanna (Or), 2002</td>
<td>55.5 ± 3.7 a</td>
</tr>
<tr>
<td>Alghero (Brionis), 2002</td>
<td>59.25 ± 4.50 a</td>
</tr>
<tr>
<td>Alghero (Valverde), 2002</td>
<td>56.75 ± 5.32 a</td>
</tr>
<tr>
<td>Narbolia (Or), 2002</td>
<td>61.0 ± 6.3 a</td>
</tr>
<tr>
<td>Simaxis (Or), 2002</td>
<td>28.25 ± 10.4 a</td>
</tr>
</tbody>
</table>

Means followed by the same letter, in the same row, are not statistically different at P<0.05 (Duncan’s Test).

References


Field tests on the combination of mass trapping with the release parasite *Opis concolor* (Hymenoptera: Braconidae), for the control of the olive fruit fly *Bactrocera oleae* (Diptera: Tephritidae)

Constantin Liaropoulos\(^1\), Vassilis G. Mavraganis\(^1\), Theodore Broumas\(^2\) and Nikitas Ragoussis\(^3\)

\(^1\) National Agricultural Research Foundation, Vine Institute, Lykovrysi 141-23, 1 Venizelou Str., Athens, Greece
\(^2\) Benaki Phytopathological Institute, 8 Delta Str. 14561, Kifissia, Athens, Greece
\(^3\) Vioryl SA, Research Department, Kato Kifissia, 145 64, Athens, Greece

**Abstract:** In the framework of an ongoing program for the development of alternative methods for management of the olive fruit fly *Bactrocera oleae* (Gmelin), the efficacy of mass trapping combined with the release of the entomoparasite, *Opis concolor* (Szepl.) was tested. The experiment was conducted in the region of Marathon, Attiki, Greece from 2000 to 2002. In the year 2000, toxic traps baited with food and sex attractants (ECO-TRAP, Vioryl, S.A. Greece) were used for mass trapping at a density of one trap per two trees. In addition a network of five sticky ECO-TRAPs was installed (one trap per tree) within the test orchard for recording the attracted insects. Five thousand (5,000) and thirteen thousand (13,000) parasites were released at the pupal stage on the 11\(^{th}\) and 27\(^{th}\) of October, respectively (total 18,000 or 120/tree). In the year 2001, no toxic traps were installed and no parasites were released due to small fruit load. In the year 2002, toxic and sticky traps were installed on September 14 at the same densities as in 2000. In the year 2000, fly population density in the test orchard was high due to late toxic trap installation. Fruit infestation in the test orchard at harvest (end of November) was 19.5% compared to 35% infestation in the control. No parasitism of olive flies by either released or native parasites was observed either in the test or the control orchards. In the year 2001, fly population density was high as expected (no toxic trap installation). Fruit infestation at harvest (end November) was near 100% both in test and control orchards. Fly parasitism in the test orchard by the released parasite was 6.5% and 3.5% by native parasite *Pnigalio mediterraneus* (Ferr. & Del.). This indicates that released parasites were able to over-winter in the olive grove and parasitize the flies the following season. Fly parasitism in the control orchard for the released parasites was 0% and for the native parasite *P. mediterraneus* 3.5%. In the year 2002, fly population density was high (late toxic trap installation). Fruit infestation in test orchard was 68.1% compared to 78.4% of the control. Fly parasitism in the test orchard by the released parasite was 0% and by the native parasite *P. mediterraneus* 3.2%. Fly parasitism of the control orchard for the released parasites was 0% and for the native parasite *P. mediterraneus* 3.1%. The results have shown that mass trapping and parasite release may be combined since parasites, released or native, are not attracted to ECO-TRAPs.

**Key words:** *B. oleae*, *O. concolor*, *P. mediterraneus*, mass trapping, released parasite, native parasite.

**Introduction**

The olive fruit fly is a major olive pest and its control is a major concern for olive growers. Present control methods are based on the use of wide-spectrum insecticides in bait or cover sprays causing strong adverse side effects. These have prompted the search for alternative control methods. A mass trapping method has been developed lately with good results under low to medium pest population densities (Broumas *et al.* 1998, 2002). Under high pest
population densities however complementary control methods are required for adequate crop protection. Parasite, *Opius concolor* (Szepl.) releases has been investigated as possible alternative method of this pest with promising results (Abdel-Magid, 2000; Alexandrakis, 1986.; Jimenez, 1982.; Jimenez *et al.* 1990.; Liaropoulos *et al.* 1977.; Michelakis, 1986.). The purpose of the present work was to test the effects of combining the above two alternative methods on *Bactrocera oleae* (Gmelin). The results of three year investigations are reported here.

**Materials and method**

The experiments were conducted in a small, relatively isolated, olive orchard of 150 olive trees of the oil producing varieties Megaritiki and Lianolia at Marathon, Attiki, Greece from 2000 to 2002. A non protected olive orchard in the vicinity was used as control. For mass trapping the ECO-TRAP (Vioryl, S.A. Greece) was used. This trap is a paper envelope with a plastic lining inside containing 70g of ammonium bicarbonate salt (food attractant). A pheromone dispenser (Vioryl, S.A.) containing 80mg of the major pheromone component (1,7-dioxaspiro[5.5]undecane) was fastened on the outside. The surface of the trap was treated with 20mg (a.i) of a special formulation of the pyrethroid insecticide deltamethrin-Decis-flow (2.5% a.i.). The active-life of this trap is about six months.

In 2000, ECO-TRAPs were installed on October 11 at a density of one trap per two trees. In addition a network of five sticky ECO-TRAPs was installed (one trap per tree) within the test orchard for recording the attracted insects. Five thousand (5,000) and thirteen thousand (13,000) parasites were released at the pupal stage on the 11th and 27th of October, respectively (total 18,000 or 120/tree). The *O. concolor* parasites were reared on *Ceratitis capitata* (Gonzalez *et al.* 1996; Jimenez-Alvarez 1978; Jimenez *et al.* 1994).

In 2001, no parasites were released due to low fruit load. A McPhail trap was placed at the centre of the experimental orchard baited with a 3% water solution of the food attractant “Dacus bait”. The trap was checked once a week, and the attractant replaced at every check.

In 2002, ECO-TRAPs toxic and sticky were installed on September 14 at the same densities as in 2000.

For result evaluation the following parameters were recorded: a) Fly population densities.  
b) Response of the released and native parasites to ECO-TRAPS. c) Fruit infestation level by *B. oleae*. d) Level of fly parasitism.

Fly population densities were recorded for 2000 and 2002 by means of sticky ECO-TRAPs, and 2001 by a McPhail trap.

Response of parasites to ECO-TRAPS was checked by means of sticky ECO-TRAPs.

Fruit infestation level was checked at harvest time (November). For this purpose fruit samples were obtained as follows: Four fruit per tree picked randomly from four directions of the tree were collected from all trees of both test and control orchards. Fruit was checked (dissected and examined under microscope) for fly infestation.

The level of fly parasitism was checked by collecting fruit samples at harvest time, as above. Fly infestation level was determined by careful inspection of fruit under a stereoscope. After inspection, fruit was placed in screen cages in which the emerged olive fruit flies and parasites were recorded regularly. Fruit samples were collected both from test and control orchards.

Meteorological data for the three years were obtained from the Marathon Meteorological Stations, of the National Meteorological Service.
Results and discussion

Fly population density in the test orchard was high due to late toxic trap installation. Figure 1. show the number of olive flies males and females captured per trap per week on sticky Eco-traps in the experimental orchard in 2001. More males are captured than females due to the presence of the pheromone and no parasites native or the released were captured on the sticky Eco-traps. Fruit infestation in the test orchard at harvest (end of November) was 19.5% compared to 35% infestation in the control. No parasitism of olive flies by either released or native parasites was observed either in the test or the control orchards.

Figure 1. Numbers of *Bactrocera oleae* flies (males and females) captured per trap per week on sticky traps (Eco-Traps) from October 11 to December 1. Means of five replicates, Marathon, Greece, 2000.

Figure 2. Numbers of *Bactrocera oleae* flies (males and females) captured in a McPhail trap from June 22 to November 30. Marathon, Greece, 2001.
In 2001, fly population density was high as expected (no toxic trap installation). Figure 2. shows the number of olive flies males and females captured in a McPhail in the experimental orchard.

Fruit infestation at harvest (end November) was near 100% both in test and control orchards and fly parasitism in the test orchard by the released parasite was 6.5% and 3.5% by native parasite *P. mediterraneus* (Table 1.).

Table 1. Number of *B. oleae* flies, *O. concolor* and *P. mediterraneus* emerged from fruit sample inside screen cages* Test orchard, 2001.

<table>
<thead>
<tr>
<th>Dates</th>
<th>B. oleae</th>
<th>O. concolor</th>
<th>P. mediterraneus</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>♀♀</td>
<td>♂♂</td>
<td>Total</td>
<td>♀♀</td>
<td>♂♂</td>
</tr>
<tr>
<td>13/12</td>
<td>56</td>
<td>53</td>
<td>109</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>17/12</td>
<td>12</td>
<td>12</td>
<td>24</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>21/12</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>24/12</td>
<td>17</td>
<td>10</td>
<td>27</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>27/12</td>
<td>4</td>
<td>6</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>86</td>
<td>180</td>
<td>4</td>
<td>9</td>
</tr>
</tbody>
</table>

This indicates that released parasites were able to over-winter in the olive grove and parasitize the flies the following season probably due to the mild winter of 2000. Fly parasitism in the control orchard for the released parasites was 0% and for the native parasite *P. mediterraneus* 3.5%.

Figure 3. Numbers of *Bactrocera oleae* flies (males and females) captured per trap per week on sticky traps (Eco-Traps) from September 21 to November 16. Means of five replicates, Marathon, Greece, 2002.
In 2002, fly population density was high (late toxic trap installation). Figure 3. shows the number of olive flies males and females captured per trap per week on sticky ECO-TRAPs in the experimental orchard.

No native or released parasites were found on the sticky ECO-TRAPs. Fruit infestation in test orchard was 68.1% compared to 78.4% of the control. Fly parasitism in the test orchard by the released parasite was 0% and by the native parasite *P. Mediterraneus* 3.2%. Fly parasitism of the control orchard by the released parasites was 0% and by the native parasite *P. Mediterraneus* 3.1%. The luck of parasitism by *O. concolor* may be attributed to the severe winter of 2001 while the native parasite seem to has survived. It is well known that catches of *B. Oleae* by traps (Figures 1, 2, 3) are influenced by environmental conditions (temperature, relative humidity), which appears to be case here as well).

The above results have shown that mass trapping and parasite release may be combined since parasites, released or native, are not attracted to ECO-TRAPs.

**References**


Mass trapping technique in *Bactrocera oleae* control in Tuscany Region: results obtained at different territorial scale

Italo Rizzi, Ruggero Petacchi, Diego Guidotti
*Landscape Entomology Lab (LELab), Scuola Superiore S.Anna – Polo S.Anna Valdera, Viale Rinaldo Piaggio 34, 56125 Pontedera (PI)*

**Abstract:** The aim of the investigation is a result’s evaluation of the “lure and kill technique” also called Mass Trapping (MT) in olive fruit-fly control. The study is referred to experimental trials, which were located in Tuscany-Italy, a Region with a huge MT knowledge in different agro-ecological areas and managerial conditions. Experimental data have been collected during 1999-2002 period from medium and small size areas, which are distributed in the coastal and interior zones of Tuscany. Field data as olive infestation and adult trapping were collected by technical advisors of the “Olive Oil Quality Improvement project” (EEC Regulation 528/99). In all Mass Trapping areas Ecotrap was used for olive fruit fly control with a final trap/tree ratio of 1:1. Monitoring data was collected in MT areas by weekly monitoring from the third decade of July to the end of October in 5-10 farms and was transferred and stored with Information and Communication Technology, by using a web-based data management system. Results obtained in different trials of MT were compared using infestation indexes and relative effectiveness indexes, previously defined. Results were compared by years and by areas that were distinguished in medium and small trail areas. We suggest a guideline to assess MT suitability, and we propose a preliminary Regional map of MT suitability, using a previously defined rule to assess MT suitability and the likely success of MT technique in relation to agro-ecological factors, managerial factors, influencing MT effectiveness are finally discussed.

**Key words:** *Bactrocera oleae*, "lure and kill" technique, mass trapping technique, effectiveness index, MT suitability.

**Introduction**

Plant protection against the olive fruit-fly, *Bactrocera oleae* (Gmel.) (Diptera: Tephritidae) is the main problem to carry out sustainable oliviculture in the majority of countries of mediterranean basin, with an economic loss that have been estimated to reach up to 15% of the olive crop (Montiel Bueno, 2002). In Organic Agriculture, one of the most promising techniques of olive fruit-fly control is the “Lure and Kill” technique. The technique has been developed initially using baited traps with a solution of protein hydrolyzate (Orphanidis *et al.*, 1958), and then olive fruit-fly pheromone combined with a food attractant (Haniotakis 1986, Broumas & Haniotakis, 1987, Montiel Bueno, 1987) to lure *Bactrocera oleae* into killing devices. The late control devices have the advantage to be more selective against olive fruit-fly and less detrimental against beneficial insect (Paraskakis, 1989). Among the different products Ecotrap which is admitted in organic agriculture (Annex II of EC Regulation 2002/91) has been tested in different agro-ecological and management conditions and in different Italian regions (Delrio, 1990; Silvestri, 1999; Petacchi *et al.*, 2001a) and in other Mediterranean countries (Haniotakis 1986, 1991; Broumas, 1990).

Ecotrap comprise, deltamethrine-baited traps with ammonia releasing salts as food attractant and sex pheromone. The “Lure and Kill” technique has been proved to be effective in wide-area management, where the large scale adult movement over short distance do not have
influence on “Lure and Kill” effectiveness (Montiel Bueno, 2002). For that reason and the small size of olive growing farms the Ecotrap have to be placed in a contiguous group of farms.

To provide technical advices to olive growers and to control and validate this technique which is managed on wide area, biological data (olive infestation and adult trapping) are collected weekly. In Tuscany since year 1999 monitoring data on MT trials are transferred using Information Technology (Petacchi et al., 2001c), which allow comparing data in real time.

In this paper we report the results obtained from the evaluation of Lure and Kill method, using Ecotrap in several areas of Tuscany Region – Italy during three years from 2000 to 2002. The evaluation of Lure and kill effectiveness has been realized in the context of the Olive oil quality improvement (EEC Regulation 528/99) organized by ARSIA-Tuscany Region in collaboration with Olive oil Producer Association (P.A.). Our work consisted initially on defining a working protocol for monitoring data collection, and a methodology of result evaluation. In order to allow inserting and sharing real time monitoring data we used AgroAmbiente.info, a web based data management system.

Material and methods

Test areas were 15 up to 25, with a growing number of Lure and Kill areas from 2000 to 2002. The treated surface was circa 1.250 ha in 2000-01 and 1814 ha in 2002, and Lure and Kill areas are located both in the coastal area and in the interior of Tuscany Region. MT areas cover at least 8 ha of olive grove, and all the smaller MT areas were isolated.

Dynamic of *B. oleae* was investigated by using a standard monitoring methodology (Chesi & Quaglia, 1982), and a weekly monitoring was carried out by P.A. field advisors from the end of July to the end of October. Infestation data were collected in monitoring points located within each MT area and in comparison farms located outside the MT areas but having similar pedoclimatic and olive growing conditions. Monitoring was performed on a total of 28 control farms and 130 MT farms in 2002. The device used is Ecotrap, that consist of a food attractant, 70 g of ammonium bicarbonate, within a green coloured bag coated on the outside with the contact insecticide, deltamethrin, 0.0019% (15 mg/device), and a sex pheromone dispenser containing 80 g of spiroacetal (Vyoril A.E. Kato Kifissia, Greece).

Half of Ecotrap were placed at the beginning of the summer (end of June), before the first generation of olive fruit fly and the remaining devices in autumn, before the second generation, with a final trap/tree ratio of 1:1.

On average there were 1 to 2 chemical treatments in the majority of control plots, using dimethoate (a.i.) an organophosphate larvicide authorized by ECC regulation 2078/92.

To evaluate effectiveness of MT system we use indexes that are calculated on active infestation to compare the level of young pre-imaginal stadia and an index calculated on live III instar larvae, to estimate the production damage.

Moreover to evaluate the relative effectiveness of Lure and Kill technique in comparison with conventional pest management practices we use Relative Effectiveness Indexes, that are the ratio between the yearly average of infestation in control farms and in MT areas (Petacchi et al., 2003). Relative effectiveness indexes are calculated on active infestation and on live III instar larvae.

Results and discussion

The average yearly values of Index of active infestation in MT trials (IaiMT) and in control farms (IaiCo) are reported in Table 1.
Infestation indexes
The values of infestation indexes were analyzed in relation to Active Infestation and Damaging Infestation. These values, which are obtained by calculating the average annual infestation in different monitoring points, are useful to compare the infestation levels in monitored areas in different years, or among different areas of the same year.

- Indexes of average annual values of active infestation \((I_{\text{aiMT}}, I_{\text{aiCo}})\).

  The average value of \(I_{\text{aiMT}}\) in 2001 was lower (2.19%, ±0.43%) compared with 2000 and 2002 \(I_{\text{aiMT}}\) average values, respectively 3.29 ± 0.46% and 3.22% ±0.62%. The differences among \(I_{\text{aiCo}}\) are less conspicuous but the average values of \(I_{\text{aiCo}}\) were higher on 2000 year (5.68%, ±1.12%) compared with \(I_{\text{aiCo}}\) average values in year 2001 (4.75%, ±1.04%) and 2002 (4.23%, ±0.70%).

- Indexes of annual average values of live third instar larvae \((I_{\text{dMT}}, I_{\text{dCo}})\).

  The average value of damaging infestation indexes (table 1) based on live third instar larvae in MT areas \((I_{\text{dMT}})\) is lower in 2001 (0.39 ± 0.12%) than in 2000 (0.65 ± 0.13%) and in 2002 (0.65 ± 0.27%). In comparison farms the index value \((I_{\text{dCo}})\) is slightly less in 2002 (0.87 ± 0.20%) than in 2000 (1.10 ± 0.37%) and in 2001 (1.08 ± 0.39%). In short both infestation indexes in MT areas \((I_{\text{aiMT}}\) and \(I_{\text{dMT}}\) were lower on 2001, compared with 2000 and 2002 year, although infestation indexes in comparison farms \((I_{\text{aiCo}}\) and \(I_{\text{dCo}}\) were not lower on 2001.

Comparing infestation indexes for each MT area in different years, where MT has been adopted for three years, there is not a clear evolution of infestation level and we cannot assume a change in the population level of olive fruit-fly related to the adoption of Lure and Kill technique.

Relative effectiveness indexes
Considering all three years, values of the relative effectiveness index on active infestation \((RE_{\text{ai}})\) show that active infestation in MT trials was lower than in comparison farms in the large majority of case, 33 up to 37 MT areas (Graf n.1).

In 2001 average \(RE_{\text{ai}}\) is on average higher than 2 (active infestation in MT trials is half that of Control Points) but can reach a value of 6 in the most successful trials ("inside infestation" is 15-20% of "outside infestation"). In 2000 and 2002, the Lure and Kill technique is less effective in reducing active infestation because average \(RE_{\text{ai}}\) in about 10% of Mt areas is lower than 1 (active infestation in MT trials is higher that of Control Points) especially in 2002 year.

The value of relative effectiveness index on damaging infestation \((RE_{\text{d}})\) is less than 1 (\(I_{d}\) is higher in MT trials than in comparison farms) in only 9 trials out of 31: the Mass Trapping technique was able to bring about damage reduction in more than two out of three cases. Average \(RE_{\text{d}}\) was higher in 2001 (2.88 ± 0.62%) when the MT technique was more effective in controlling the olive fruit fly.

We estimate that managerial factors, especially the timing of devices placement, could partially explain the different performance of MT.

On 2002 a fifth of MT areas were smaller than 15 ha (9-13 ha) and the results of Lure and kill technique on reducing active and damaging infestation is similar to bigger MT areas.
Table 1. Indexes of average annual values of active and damaging infestation

<table>
<thead>
<tr>
<th>Area</th>
<th>Year 2000</th>
<th></th>
<th></th>
<th>Year 2001</th>
<th></th>
<th></th>
<th>Year 2002</th>
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<td></td>
<td>Iai(_{Co})</td>
<td>Iai(_{MT})</td>
<td>Id(_{Co})</td>
<td>Iai(_{MT})</td>
<td>Id(_{Co})</td>
<td>Iai(_{MT})</td>
<td>Id(_{Co})</td>
<td>Iai(_{MT})</td>
<td>Id(_{Co})</td>
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<td>Vinci</td>
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<td><strong>Annual average</strong></td>
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<td>3.29</td>
<td>1.1</td>
<td>0.65</td>
<td>4.75</td>
<td>2.19</td>
<td>1.08</td>
<td>0.39</td>
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<td><strong>Std. Deviation</strong></td>
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<td>2.94</td>
<td>1.22</td>
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<td>1.12</td>
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<td>0.43</td>
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</table>

\( Iai_{MT} \) = Index of active infestation on Mass Trapping trial  
\( Iai_{Co} \) = Index of active infestation on Control  
\( Id_{MT} \) = Index of damaging infestation on Mass Trapping trial  
\( Id_{Co} \) = Index of damaging infestation on Control
**Figure 1.** Values of relative effectiveness index of mass trapping on active infestation (REai).

**Figure 2.** Values of relative effectiveness index of mass trapping on damaging infestation (REd).

**Improvement of MT management**
A result of our work in collaboration with field advisors of Olive Producer Associations, the Regional Agency for Development and Innovation in Agriculture (ARSIA) and olive oil producers, was the improvement of Lure and kill management. It consists on the definition of a procedure to carry out the preliminary investigation on olive growing areas, the definition of a proper timing of ecotrap placement and finally the improvement of the technical communication process.
The preliminary assessment of olive growing area is based on agro-ecological features and on management considerations. A rule to assess MT suitability and the likely success of MT technique in relation to agro-ecological factors (Petacchi et al. 2002), can be implemented to define a priori if the olive growing area can be considered suitable for Mass Trapping.

The rule is based on an economic threshold of 15% active infestation (ai) and MT effectiveness in halving active infestation and state that “an area can be considered suitable for MT if its active infestation does not exceed 30% on a majority of farms (80% of farms) within the third week of October of every year”. It follows that the adoption of Lure and Kill technique is linked also to the economic threshold and the harvesting practices that are adopted in the area by the farmers’ majority. In fact where farmers do spraying at very low level of active infestation and practice a late harvesting, starting from December, are not in condition of using the lure and kill technique in a profitable way.

The proper procedure of device placement is a double device positioning, before oviposition of first and second generation of olive fruit fly, with final trap/tree ratio of 1:1, but in some case was proved to be useful a third device placement before third generation, without changing the final trap/tree ratio of 1:1. The lure and kill method could be adopted in a contiguous group of farms, on a olive growing area adequately isolated from other olive groves, of at least 8-10 ha.

**Conclusion**

Mass trapping enable the reduction of active infestation in the large majority of MT areas, allows halving of active infestation in half of MT trials, and allows bringing about damage reduction in more than two out of three cases.

Using a rule to rate the likely success of MT technique and monitoring data of olive fruit-fly infestation at regional level it is possible to create a regional map of MT suitability. Our aim is to integrate next year the MT knowledge achieved and the information on *B. oleae* infestation level that are collected through the regional IPM activities for Olive oil quality improvement.

In our work we discussed main agroecological and management factors influencing the MT effectiveness and we tried to define a short guidelines to be used during the preliminary investigation on olive growing areas. Factors to be considered are the infestation potential of *B. oleae*, the attitude of farmers on using economic threshold and the harvesting practices. The technical assistance remains a central point in order to provide the technical advices for olive fruit fly monitoring and devices placement.

**Acknowledgment**

We are grateful to ARSIA-Regione Toscana, and Olive Producer Associations AIPROL and OTA for providing data and for the fruitful collaboration of the Regional Project of Olive Oil Quality Improvement (Reg. CE 528/99) co-founded by European Union.

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Comparison of different strategies for controlling *Bactrocera oleae* in a coastal area of Abruzzo – Central Italy

Antonio F. Spanedda, Alessandra Terrosi, Claudio Pucci
*Dipartimento di Protezione delle Piante, Università della Tuscia, Via S. Camillo de Lellis, 01100, Viterbo, Italy*

**Abstract:** The experiments were carried out in the two-year period of 2001-2002 in an irrigated orchard near the coast in Abruzzo Region, Italy. The aim of this work was to compare two methods for controlling olive fruit-fly, that is to say the preventive one and the curative one, both guided by a treatment threshold suggested by an infestation forecasting model. Such a model is synthesized by an index (Z) that take into account field data on the females weekly captured and mean temperature of the capture week. Results showed that in a coastal and irrigated area, where infestation usually starts early in the season, treatments carried out in the first half of August are not necessary, as young boring larvae will die because of high maximum temperature of that period. This is well represented by the forecasting model. Both kinds of treatments have demonstrated same effectiveness in controlling olive fruit-fly, either in the first year with medium infestation (2001) or in the second when infestation was very high (2002).

**Key words:** guided control, forecasting model, *Bactrocera oleae*

**Introduction**

According to the guidelines on integrated plant protection, a sound and effective control of pests must deliver its strategy on the knowledge of the elements that make up foundations of pest management. They are, in a broader sense, the study of bionomics of pests, the in-depth cognition of biotic and non-biotic factors of natural control, the practice of a proper monitoring and, last but not least, the definition of accurate intervention thresholds.

On concerning issues related with olive fly control, one could hold that advances have been made in all of the above mentioned topics.

This contribution aims at verifying the effectiveness of a treatment threshold suggested by an infestation forecasting model set up by Plant Protection Department of Tuscia University in Viterbo – Italy (Pucci, 1993). Such a model, even if formerly based on experimental data coming from a particular environment situated in Northern Lazio and referred to the cultivar named “Canino”, seemed to be a valid tool also for others environments and cultivars.

The model is represented by the following linear combination:

\[
Z = 0.039 (F_m - 9.7) - 0.186 (T_m - 22.1)
\]

where:

- **Z:** stands for the index of the linear combination of independent variables (called “canonical”) such as air temperature and females, highly correlated with W, that is the index of dependent variables linked with infestation levels
- **F_m:** is the average number of females weekly captured by means of yellow sticky traps
- **T_m:** is the average of the daily mean temperature recorded in the same capture week

Other values represent constants.
In addition to statistical analysis, that led to the infestation forecast model, \( Z = 0.1 \) was indicated as threshold which, if exceeded, may cause an economic injury.

The application of the forecasting model in the inner hilly areas of Abruzzo Region allowed a quite excellent control of the olive fly by avoiding unreasonable treatments.

On the base of our experience we have noticed that, in the hilly and dry orchards, the main risks appear when the alert for exceeding threshold occurs after first abundant rainfall following a period with high mean temperature. That usually happens not before last decade of August. Such a fact can be explained by considering the high mortality of eggs and larvae inside drupes due to high temperature, with a subsequent reduction of “active infestation” (Pucci et al., 1985). Nevertheless, in particular cases (mainly in coastal areas), we could be in presence of olive-fly attacks early at the end of July or at beginning of August. It may occurs when weather is characterized by abundant and prolonged rainfall that lower daily mean temperatures. The same event takes place in the irrigated orchards as the temperature in the inner layers of the fruit is mitigated by good water availability.

In this experiment the Authors want to verify whether hottest conditions of the summer period can stop infestation or a rainy season or irrigation allows eggs and larvae to survive. In the latter case, treatment performed not later of middle of August would prevent from increasing infestation.

The second aim of the trials was to compare two of the most used methods for the olive-fly control, that is to say the preventive and the curative one. The former is based on proteinaceous baits started with deltamethrin which has an adulticide action (Pucci, 1992); the latter performs as a larvicide by means of cytotropic active ingredients, e.g. dimethoate.

**Materials and methods**

The experiments were carried out in the two-year period of 2001-2002 in an olive grove near the coast in the middle of Abruzzo Region in Central Italy. The predominant cultivar was “Leccino” with plants aged 30 years. The orchard was subdivided into 5 plots, with 50 trees each, corresponding to the following thesis:

- **A)** 3 treatments with adulticide a.i.
- **B)** 2 treatments with adulticide a.i.
- **C)** 3 treatments with larvicide a.i.
- **D)** 2 treatments with larvicide a.i.
- **E)** control not treated

In the olive grove was placed a device for temperature, humidity and rainfall data recording. Daily mean temperature values were used for obtaining the week mean value necessary for the calculation of infestation forecasting index (Z).

The first treatment was executed by observing an incipient infestation, the second was decided when \( Z \) (infestation forecasting index) exceeded the threshold value (Figure 1), the third was performed about one month later (see Table 1).

In every plot at distances of 30 m one from each other, 3 yellow sticky traps, with size of 15 x 20 cm, were placed for monitoring adults of fly. In order to make adults counting the more rapid and simple as possible, the traps were tied at the end of a stake with adjustable height.

In order to have an indication on the trend of infestations, 5 contiguous trees in the inner part of each plot were chosen, from which 40 fruits/tree were taken for a total of 200 fruits/plot. Then, sampled olives were moved to laboratory and their infestation degree was detected, by counting every development stage of the fly.
Infestation was subdivided into three categories, namely:

- eggs + 1st instar alive larvae
- 2nd instar alive larvae + 3rd instar alive, dead and parasitized larvae
- alive pupae + empty cocoons + abandoned galleries

Weekly, also dropped fruits were collected from same sampled trees and their weight was recorded, with the purpose of quantifying production lost.

Table 1. Resume framework of treatments performed in the experiment

<table>
<thead>
<tr>
<th>Mode of action</th>
<th>Adulticide</th>
<th>Larvicide</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode of employ</td>
<td>a.i.: deltametrhin 2.8 % dosis: 100 ml/hl (DECIS) + 1000 g/ml of proteinaceous bait (BUMINAL) quantity: 0.5-1 litres/tree on southern side of canopy</td>
<td>a.i.: dimetato 40% dosis: 150 cc/hl (ROGOR) quantity: 5-7 litres/tree on the whole canopy</td>
<td></td>
</tr>
<tr>
<td>Plot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>3 treatments</td>
<td>B</td>
<td>2 treatments</td>
</tr>
<tr>
<td>Date</td>
<td>2001</td>
<td>9 August</td>
<td>12 September</td>
</tr>
<tr>
<td></td>
<td>2002</td>
<td>14 August</td>
<td>7 September</td>
</tr>
</tbody>
</table>

Results

In the graphs of Figure 2 females population dynamics of *B. oleae* is reported. It can be noticed that, at the end of July-early August, females were present at fairly good density capable to cause injuries due to their egg laying activity. This was the assumption for justifying and testing the “early” treatment. Average percent infestation for each experimental plot is presented in Figure 3.

Resuming the obtained results, it emerges that plot “early” treated, seems to have no improving effects in comparison with plots treated only twice following the alert signal given by the infestation forecasting model. As well as, both kind of control techniques used, the adulticide one and the larvicide one, showed no significant difference between them.

These results are well evidenced in Figure 4, where damages are represented by the percentage of lost product (fallen olives because of fly infestation).
Figure 1. Trend of Z index values calculated for each experimental plot in the two-year period.
Figure 2. Dynamics of captured females for each experimental plot in the two-year period
Figure 3. Percentage of infestation observed on fruits picked up from the canopy for each experimental plot in the two-year period.
Figure 4. Percentage of lost production in the different experimental plots

Acknowledgements

Funding for this research was provided by the Abruzzo Region Agency for Rural Development (ARSSA) in the frame of Programme “Miglioramento della qualità della produzione olivicola” – supported by European Union through Reg. CE n. 528/99 and n. 673/01.

References

Integrated Pest Management
Integrated Protection of Olive Crops
IOBC/wprs Bull. 28(9), 2005
pp. 101-105

Integrated control of olive pests in Morocco

Hilal A., Ouguas Y.
National Institute of Agronomic Research, Marrakech, Morocco

Abstract: In the majority of Moroccan olive groves, the biological balance is established between the pests and their natural enemies. In addition, when the use of pesticides is abusive, the level of the entomophagous populations decreases what involve a great pullulation of the olive pests. The integrated control against the olive pests is thus necessary to ensure a better plant health protection of this culture. The principal components of this technique of control are:

- The microbiological control with *Bacillus thuringiensis*.
- The mass trapping of *Bactrocera oleae*.
- Destruction of the wintering pupes of *Bactrocera oleae* by a farming method based on the covercropage method.
- The generalization of the olive pruning to control the significant stages of certain pests as olive moth and cochineal.

Key words: olive pest, integrated control

Introduction

The olive culture is of a great importance; it occupies the 7th range with a production of over 500,000 tonnes every year. However, the principal damage is caused by phytosanitary problems. The animal pests attack the whole plant (branches, leaves, flowers and fruits). The major danger comes from the following ones:

- The olive moth, *Prays oleae* Bern.
- The olive fly, *Bactrocera oleae* Gmel
- The olive psyllid, *Euphyllura olivina* Costa
- The black cochineal, *Saissetia oleae* Olivier

The olive culture in Morocco was long characteised by an abundance of parasitoides and predators, mainly due to a very limited use of pesticides. However, during the two last decades, the development of agricultural methods and techniques have resulted in an intensification of the olive culture requiring a large use of pesticides. This situation has brought about a quite big destruction of the biological equilibrium in treated olives.

In order to protect this culture, we have tried to develop an integrated control method against the principal pests.

Materials and methods

Ground practices

In the last olive fly generation, L3 larvae move from olives to soil where they became pupae. These pupae, buried under ground on October, become adults after 30 days, whereas those buried during November and December require between 80 and 90 days.

In 1996, we studied pupae spatial distribution inside the ground. We also defined the horizontal pupae localisation vis-à-vis the tree trunk. On the other hand, we tried to precise different depth levels of these pupae.
Mass trapping
In this study, we use alimentary fly traps based on ammonia sulfate and “ammonitrate”. Traps are fixed during fly stage until harvest. Between October and December, we have done two tests on olives infested by *B. oleae*.

Results and discussion

Plant cutting
There are not many olive varieties in Morocco. One variety (*Picholine marocaine*) occupies about 97% of the olive trees. They have an excessive leaf development which makes a good setting for diseases and pests. They also offer good conditions for the pests’ enemies to multiply. This multiplication remains far lower than that of the pests. Hence, the need for other efficient ways, one of which is plant cutting. The latter allows good air circulation and a humidity decrease inside the tree. These conditions lead to a high mortality of sensitive stages (eggs and young larvae) of many pests like *S. oleae*, *P. olea*, *E. olivina*. In 1977, in Ouezzane, we came to the conclusion that the cut olive trees were not covered by *S. oleae*. On the other hand, those which were not cut were covered by fumagine due to a big infestation by *S. oleae* (Hilal, 1984).

Ground practices
Results of vertical distribution of pupae are mentioned in table 1.

Table 1. Vertical distribution of pupae (3,5,10 cm)

<table>
<thead>
<tr>
<th>Dates</th>
<th>Kelâa</th>
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<th>Aït Ourir</th>
<th>Sâada</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>06/2/96</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>12/2/96</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
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<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>15/4/96</td>
<td>–</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>21/5/96</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>4</td>
<td>0</td>
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Table 2. Horizontal distribution of pupae bearing trunk tree distance (0,1,2 m)

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<th>Sâada</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>1</td>
<td>2</td>
<td>0</td>
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<tr>
<td>06/2/96</td>
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<td>–</td>
<td>1</td>
<td>1</td>
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<td>–</td>
<td>1</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>15/4/96</td>
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<td>1</td>
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<td>21/5/96</td>
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<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>2</td>
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<td>2</td>
</tr>
</tbody>
</table>
Most infested ground levels are between 5 and 10 cm (9 pupae) while the less infested are at 3 cm (6 pupae). This result indicates that the practice of a superficial ploughing (cover-cropage) can perturb fly hibernation sites and induce a big mortality. Concerning distribution under the tree, we counted pupae at 0, 1 and 2 m to the trunk. Results are shown in table 2.

The biggest number of pupae was found at 1 m, but there were less pupae near the trunk. These results confirm the importance of ploughing as a beneficial practice to reduce fly proliferation.

**Mass trapping**

Results mentioned in table 3 and 4 show that the two nitrogenous substances have successfully caught flies. In fact, from 30-10-96 and 30-12-96, we captured 587 of adults by using traps baited by “ammonitrate” and 936 adults were captured by 5 % ammonia sulfate baited. This made us conclude that ammonia sulfate traps are far more efficient than “ammonitrate” ones.

Table 3. *B. oleae* adults caught by “ammonitrate” traps

<table>
<thead>
<tr>
<th></th>
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<td>Sex</td>
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<td>♀</td>
<td>♂</td>
<td>♀</td>
<td>♂</td>
<td>♀</td>
</tr>
<tr>
<td>Number</td>
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<td>75</td>
<td>55</td>
<td>94</td>
<td>31</td>
<td>53</td>
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<td>Total</td>
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<td>149</td>
<td>84</td>
<td>38</td>
<td>115</td>
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</tbody>
</table>

Table 4. *B. oleae* adults caught by “ammonia sulfate” traps

<table>
<thead>
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<th></th>
<th></th>
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<td>♀</td>
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<td>106</td>
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<td>97</td>
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Table 5. Number of attacked olives (sample of 23/10)

<table>
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<th>Treatment</th>
<th>Control</th>
<th>Ammonia Sulfate</th>
<th>“Ammonitrate”</th>
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<tr>
<td>Analysed olives</td>
<td>2350</td>
<td>2350</td>
<td>2350</td>
</tr>
<tr>
<td>Infested olives</td>
<td>125</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Infestation %</td>
<td>5,3</td>
<td>0,04</td>
<td>0,08</td>
</tr>
</tbody>
</table>

Table 6. Number of olives attacked by *B. oleae* (sample of 26/11)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Ammonia sulfate</th>
<th>Ammonitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysed olives</td>
<td>2350</td>
<td>2350</td>
<td>2350</td>
</tr>
<tr>
<td>Infested olives</td>
<td>193</td>
<td>41</td>
<td>60</td>
</tr>
<tr>
<td>Infestation %</td>
<td>8,2</td>
<td>1,7</td>
<td>2,05</td>
</tr>
</tbody>
</table>
Level of olive infestation in 47 studied trees showed that percentage of infested olives in the first sample (table 5) is 5.3 % in control, 0.04 % with ammonia sulfate and 0.08% with “ammonitrate “.

According to Arambourg (1986), the threshold of infested fly olives is 2% for table olives. This level is more superior than to oil olives. So, we can affirm that during harvest period of table olives (end October), the two substances assure a good olive protection from flies.

Concerning November sample, results (table 6) showed that 8.2% is recorded for olives-control, 1.7 % for ammonia sulfat and 2.5 % for ammomnitrate. This makes us say that the rate of infested olive in control is superior to tolerable threshold (2 %). On the other hand, % of infested olives in ammonia sulfate experiments is just 1.7 %, which is inferior to 2 %. In the ammonitrate case, infested olive rate is about 2.5 %.

These results show that the use of traps baited with nitrogen substances can assure B. oleae control. Also, the price of this technique is low because the two substances are usually used as fertilizers.

**Microbiological control against P. oleae**

Up now, the control of P. oleae is still carried out by organophosphorus pesticides in order to reduce larvae of first and second generation. The use of pesticides during flower-time is harmful to the useful fauna. Alexandrakis & Neuenschwander (1980) showed that in Greece olive groves a great infestation by Oleander scale Aspidiotus nerii Bouché is noticed, just by an excessive use of pesticides.

On the other hand, Bacillus thuringiensis is widely used in Greece. All this because this bacteria has proved very efficient in reducing insect attacks. The optimum of the application date is when 5 % of flowers are opened. L1 and L2 larval stages are the most sensitive. In the Moroccan Haouz region, the efficiency of B. thuringiensis is more than 60 %.

**Biological control of olive pests**

Some studies have been done on predators and parasites present in olive groves in Morocco.

**Predators**

From the many predator species, which have been studied, the most important are:

*Chrysoperla carnea* Stephens: It exists in all Moroccan olive groves.

*Anthocoris nemoralis* Fabricius: In Morocco, one generation exists per year. Most of the adults are detected during November. This predator’s last larval stages are 10 to 20 times more efficient in controlling psyllid populations than first larval stages. Besides, one adult predator can eat 425 psyllid larvae.

*Pullus mediterraneus* Iablokoff-Khnzorian: In Talda region (Morocco), this species is abundant with one generation a year (Belhamdounia, 1993). In Haouz region, the predator’s evolution coincide with high Sessetia oleae density. One Pullus can consume 597, 69 in average of *S. oleae*. The 4th predator stage is the most voracious.

Others: In Morocco Haouz region, three species of hoverflies (Syrphidae) prove to be good predators of psyllid larvae and adults at flower-time. One syrphid larvae can eat about 30-40 psyllid larvae a day, at 24.5°C (Tajnari, 1992).

**Parasitoids**

*Chelonus eleaphilus* Silvestri: It’s a Braconidae fly which attacks P. oleae. In the Moroccan Tadla region this species is very abundant. It’s efficient on the second generation mainly in fallen olives (Chemseddine, 1988 and Belhamdounia, 1993 ).
*Opius concolor* Szepligeti: It is a Braconidae fly which attacks *B. oleae*. It’s found in most olive trees.

*Metaphycus lounsburyi* Howard: It belongs to the Encyrtidae family. Its presence in Tadla area is evaluated by holes-percentage caused by *M. lounsburyi* or by their presence in *Sessetia oleae* females. It’s present mainly during spring and summer and can induce 60% of mortality.

**Economic effect of reasonable chemical control on farmers**

Following pest-populations closely has allowed us to determine the optimal date of chemical treatment as well as the number of interventions per year. These factors depend on:

1. Knowledge of biological cycle
2. Population importance of each generation
3. Stage of plant growth

**Conclusion**

We have developed some practices to control olive pests without disturbing the biological equilibrium:

- Microbiological control with *B. thuringiensis* against *P. oleae*
- Mass-trapping technique using ammonia sulfate
- Biological study of *B. oleae* pupae in the soil which makes ploughing an efficient way to reduce pupae frequency in soil.
- Good pruning allows a significant reduction of animal pests. The generalisation of integrated control among Moroccan farmers allows a good production of olive trees. This technique also contributes to the improvement of the olive products quality.

**References**


Lepidopterous Pests of Olives
Unexpected mass collection of the olive moth, *Prays oleae* Bern. by non-traditional black light traps

Esmat M. Hegazi ¹, Wedad E. Khafagi²
¹Department of Entomology, Faculty of Agriculture, Alexandria University, Egypt
²Plant Protection Research Institute, Sabahia, Alexandria, Egypt

Abstract: An olive farm of 240 ha near Cairo was selected to control the olive moth, *Prays oleae* Bern. by an integration of egg parasitoids and pheromones upon a promise of the farm’s owner to keep his farm pesticide-free. During the 2nd flight of the olive moth, its population density in olive groves other than the experimental ones, exceeded our expectations. Therefore, two black light traps namely UV-sticky (BLS) and UV-water (BLW) traps were constructed easily by the first author from materials generally available around the farm. When 3 traps/each type were used, unexpected mass-trapping of the olive moth was recorded. The traps saved the fruit yield of the whole farm by sweeping over most population of *P. oleae* moths from the farm without using any insecticide. The BLS traps proved to be safer for the environment than the BLW. The trap may be integrated with other approaches to control the olive moth, e.g., the mating disruption method. Additional careful testing is required to fully reveal and document the basic trap’s true potential.

Key words: Black light traps, *Prays oleae* moths, mass-trapping.

Introduction

*Prays oleae* Bern. (Lepidoptera; Plutellidae), the olive moth, is one of the most important pests of olive trees throughout the Mediterranean Basin (Arambourg, 1983). In particular areas or conditions, the jasmine moth, *Palpita unionalis* (Lepidoptera; Pyralidae) occurs at population levels that cause serious damage (Katsoyannos, 1992). Both pests are mainly controlled by insecticide applications. In November 2001, an international research project was started to promote biological and biotechnical methods for the control of these pests. The project is funded by the European commission within the specific programme (contract ICA4-CT-2001-10004). The project title is sustainable control of lepidopterous pests in olive groves - Integration of egg-parasitoid and pheromone. One of the main farms where the study was conducted is a private farm “Paradise Park” of ca 240 ha 177 km south Alexandria. The farm consists of 88 uniform olive groves (2.1-3.3 ha, each). Ten olive groves were selected for the project work. The owner agreed not to apply insecticides on the whole area. In late April 2002, the population levels of the olive and jasmine moths on olive groves other than selected ones for mating disruption or inundative releases of *Trichogramma* were heavily infested. To solve this problem, we had to use some of black light traps to reduce adult population of the target pests. There are different types of light traps that can be used for monitoring or controlling pests. The traps range from simple and inexpensive to elaborate and costly (e.g., Barr *et al.*, 1960; Barr *et al.*, 1963; Oloumi *et al.*, 1975; Hines & Heikkenen, 1977; Barnard, 1980; Furniss, 1981; Willson *et al.*, 1981). Thus, our objective was to develop simple and inexpensive traps that will capture a large percentage of target moths. The materials that are generally available around the farm were used to construct two black light traps namely UV-sticky and UV-water traps. Fortunately, the traps proved to be important tools for *P. oleae* control and survey traps for *P. oleae*, *P. unionalis* and other pests.
Materials and methods

Description of the traps

Two traps were constructed easily from materials generally available around the farm. Both traps do not include fans or any motorized method of draft induction. Each trap is equipped with two 15 watt ultraviolet fluorescent tubes (F 15 T 6/BL), which emit a highly visible bluish-white light.

The first trap “UV-sticky trap” is non-directional type (Fig. 1) consists of a roof which is adjusted over the collecting device that serves fixture for UV lamps. Two tilted mirrors were fixed on one of both opposite sites of a wooden frame (50x50x15 cm). A board covered with white sheet of paper “light sheet” is laid down on the bottom of the wooden frame. Then the board is covered with a clear plastic (0.2 cm thick), to which non-poisonous glue for trapping rates (Temo Bi, Italy) was applied. As the insects are attracted to the UV lamp and alight on the sticky surface they are unable to extricate themselves. The plastic sheets are changed daily. The sticky material is later dissolved with a suitable solvent for screening the insect collection. In most cases, the insect catches were counted directly.

Figure 1. The components of an UV-sticky trap.

The collecting device of the second type of traps (UV-water trap, Fig. 2) is empty oil barrel found around the farm. The barrel is longitudinally cut into 2 halves “two troughs”, each is suspended horizontally on a rack. A piece of netting is stretched on the trough’s bottom. To construct bidirectional trap, a central UV lamps were placed above the container and a glass baffles were fixed in the middle to intercept the attracting insects that are hit the baffle and dropped into the container beneath and perish. The trough is filled to ca 2/3 full with water. A few drops of detergent or some other surfactant is added to the water to break the surface tension. The water is daily drained out through a hole in the side view of the container, then the trapped insect on the net was left in the sun to dry out.
Each type of the above traps is easily handled because of their simple construction and light weight but very effective and useful.

**Sites and sampling**
The farm, namely Paradise Park, where the study was conducted is located in an arid area, 177 km south Alexandria. It is strip-shaped private farm of ca 240 ha. It has a main road (8 m width, 6.5 km length). On both sides locate a total of comparable 88 groves (2.1-3.3 ha, each). Each grove is surrounded by wind break trees (*Casuarina stricta*, Casuarinaceae) isolating each grove from the others. The groves have fruitful olive fruits of 6-7 years old. Dolce, Sinnara, Shamy, Manzanilla, Toffahi, Hamedy, Kalamata, Bicual and Aks are the principal cultivars of table olives, constituting approximately 20.3, 20.1, 18.4, 12.8, 11.9, 9.7, 9.7, 6.3 and 4.9%, respectively of the total of 61774 olive trees. Two olive cultivars in each of 90% of olive groves of the farm are planted near each other in the same grove.

In 2002, six light traps (3 of each type) were stationed throughout six olive groves. Another three olive groves were selected to install pheromone traps for comparison. At each grove, 2 delta-wing traps (baited with (Z)-Z-tetradecenol) and 2 funnel traps (baited with (E)-11-hexadecenol and (E)-11-hexadecenyl-acetate) were installed to monitor the flight phenology of the olive and jasmine moths, respectively. The distance between these traps were >60 m from each other. The pheromone dispensers in the traps were changed every month. To minimize competition between trapping systems, each light trap was stationed a minimum of 250 m from a sex pheromone trap. Captured insects were counted daily and weekly for light and pheromone traps, respectively. The harvest of UV-water trap catches was transferred to the laboratory. The contents of each trap were spread out in a white basin to
facilitate processing, which involved separating insect species from each others. Data were analyzed for significance (P=0.01) by Duncan’s multiple range test.

Results and discussion

Table 1 gives results of the initial tests comparing UV-sticky (BLS) and UV-water (BLW) traps. From May 13 to June 24, 3762.4 and 231.2 *P. oleae* and *P. unionalis* moths were captured in BLS traps with average catches of 87.4 and 5.37 moths/trap/night, respectively. With BLW traps, catches increased dramatically to an average of 242.7 and 12.6 moths/trap/night for *P. oleae* and *P. unionalis*, respectively. This may be due in part to some attractancy of sex pheromone emitting from olive female moths floatation on the water surface of the trap, which stay alive for some hours.

Both traps significantly (P<0.01) caught fewer numbers of *P. unionalis* than *P. oleae* moths. However, each type of trap could be baited with special insect pheromone depending on kind of insects the collector hopes to capture, i.e., to increase the efficiency of the trap for special purposes. In fact, the structure of each type of trap is simple, but very effective in mass trapping of *P. oleae* (Table 1).

Table 1. Weekly captures of *P. oleae* and *P. unionalis* moths in two UV-trap types (3 replicates/type), Cairo, May-June, 2002.

<table>
<thead>
<tr>
<th>Date</th>
<th>P. oleae (UV-sticky)</th>
<th>P. unionalis (UV-sticky)</th>
<th>P. oleae (UV-water)</th>
<th>P. unionalis (UV-water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 13</td>
<td>440.5 + 15.3</td>
<td>15.1 + 1.3</td>
<td>1650.2 + 17.3</td>
<td>85.6 + 4.5</td>
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<td>18.2 + 3.1</td>
<td>1000.0 + 13.3</td>
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</tbody>
</table>

It is known that the BL traps indiscriminately kill many species of insects, i.e., harmful to important beneficial species. Insects attracted in large numbers by both traps represented nine orders: Coleoptera (Carabidae, Staphilinidae, Scarabaeidae, Buprestidae, Histeridae, Elateridae, Dermentidae, Anobiidae, Bostrichidae); Lepidoptera (Cossidae, Phycitidae, Pyralidae, Sphingidae, Lasiocampidae, Noctuidae); Diptera (Muscidae, Sarcophagidae, Calliphoridae, Tachinidae, Culicidae, Asilidae, Tabanidae, Syrphidae); and Hemiptera (mainly water bugs and some plant bugs). Some Odonata (damsel flies), Orthoptera (mole crickets, crickets, grasshoppers), Dictyoptera and Neuroptera (Chrysopidae and Myrmeleonidae). No effort was deployed to screen the catches of all above orders for useful insect proportions.

The magnitude of each insect group in trap harvest was type-dependent. The BLW was richer, especially in individuals of histerid species (*Saprinus* spp.), Scarabaeid beetles (*Aphodius* spp.), carrion Diptera (*Calliphora* spp., *Wohlfahrtia* spp., *Lucilia* spp. and *Sarcophaga* spp.) and aquatic insects from Coleoptera (Dytiscidae) and Hemiptera (Belosto-
matidae and Notonectidae). This may be due, in part, to strong attractancy of putrefaction odours emitting from dead animals to the carrion insects.

Although the BLW trap is indestructible; reuse with only simple change of water and caught greatest numbers of *P. oleae* and *P. unionalis*, we did not keep comparative records of insect catches between the 2 trap types to keep of large numbers of non-target insects. Instead, the weekly pattern of BLS catches of *P. oleae* and *P. unionalis* was compared with sex pheromone traps, i.e., with delta-wing and funnel traps, in respect, to optimize the mass-trapping of one or both moths. Preliminary observations of BLS traps with and without tilted mirror indicated that (data not shown) the BLS with mirror was more effective, especially in trapping *P. oleae* moths than without mirror. This may be due to the effect of increasing light intensity by interference of reflected rays and spreading light reflection over a wider area. As shown in Figs. 3 & 4, sex pheromone catches were persistently lower in all collections. The averages for number of moths captured in BLS traps were significantly higher (*P*<0.01) than those in pheromone traps. This may be due, in part, to the exposed of pheromonal sacs of target pests as most insects are stuck head down in BLS trap. However, *P. oleae* moth catches in BLS traps significantly (*P*<0.01) exceeded *P. unionalis* moths. The results indicate that both moth species are not equally attracted to the BL traps. The variation found in the number of trapped moths can be construed, in part, to the relative differences in population abundance and flight activity of both species. The *P. unionalis* was observed during the day time flying in the field. May be eye structure of *P. unionalis* makes it more diurnal than other moths to which it pertain. Thus, the BLS trap may represent an effective tool for mass trapping of *P. oleae* and monitoring tool for both species.

Interestingly, the BLS trap showed the two major periods of the 2nd and 3rd generations of adult activity of *P. oleae* during 2002 season, while the delta trap only showed the 2nd generation (Fig. 3). Also, when moth population was low (Oct.-Nov. for *P. oleae* and August-Sept. for *P. unionalis*), the BLS and pheromone traps showed large difference in their attractancy. The BLS trap was more effective in trapping both moth species even when adult population was low (Figs. 3 & 4) compared with delta and funnel traps. Tingle and Mitchell (1979) reported that captures of sex pheromone traps changed with insect population. Thus, the use of BLS trap may be useful for mass trapping of *P. oleae* and as a mean of monitor for both species of moths to provide field workers with more accurate information for control strategies. By changing trap height, flight behavior of certain pest can be known, e.g., BLS trap at tree top increased its efficiency in trapping *P. unionalis* (data not shown).

The main limitations associated with the use of BLS trap are: availability of electric power, its tendency to become crowded with photopositive insects and some birds and rats which may be also accidentally trapped. Some of non-target fauna may be protected from damage by placing a screen with the proper sized mesh over the entrance or selecting specific lamps of which emission may not attract certain useful insects.

As we know, the water trap (e.g., BLW) and sticky trap (e.g., BLS) are not recommended for adult Lepidoptera or other insects identification that may be ruined if collected in fluid or by sticky substance, in respect, and can not be removed without being destroyed. However, both of BLW and BLS are powerful enough to be useful as control measure of *P. oleae*. They have no residues on crops and they may operate continuously, thereby eliminating the necessity of fixing the time of control application. Another advantages are: they will attract *P. oleae* irrespective of physical condition of the field; their use may be integrated with other approaches to control and the cost of operation is low. Should solar energy is economically harnessed BLS and BLW trap sets in field continuously in all seasons except in winter time, they may gradually uproot all the locally pests.
Figure 3. Weekly catches of *Prays oleae* moths in delta-wing and UV-sticky traps installed in olive groves at Paradise Park, 177 km south of Alexandria, in 2002 season.
Figure 4. Weekly catches of *P. unionalis* moths in delta-wing by Funnel and UV-sticky traps installed in olive groves at Paradise Park, 177 km south Alexandria, in 2002 season.
Acknowledgements

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References

Population dynamics of *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae) in Central and Northern Greece

C. G. Athanassiou¹, N. G. Kavallieratos² and B. E. Mazomenos³

¹Laboratory of Agricultural Zoology and Entomology, Agricultural University of Athens, 75 Iera Odos, 11855, Athens, Attica, Greece
²Laboratory of Agricultural Entomology, Department of Entomology and Agricultural Zoology, Benaki Phytopathological Institute, 8 Stefanou Delta str., Kifissia, Attica, Greece
³Chemical Ecology and Natural Products Laboratory, Institute of Biology, N. C. S. R. “Demokritos”, P.O. Box 60228, 15310 Aghia Paraskevi, Attica, Greece

**Abstract:** The jasmine moth, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae) was monitored in olive orchards by means of pheromone-baited funnel traps, in two regions of Greece, Magnissia (Thessaly, Central Greece) and Chalkidiki (Macedonia, Northern Greece). The pheromone of this species consists of two pheromone components (E)-11-hexadecenal [(E)-11-16: Ald] and (E)-11-hexadecenyl acetate [(E)-11-16: Ac]. The traps were inspected for captured males at weekly intervals from July 2002 until March 2003. During summer and early autumn, adult numbers in traps were extremely low, (usually less than 1 individual per trap). However, later in the trapping period (mid October-early November) captures were increased, for both regions examined. Surprisingly, the highest numbers of males caught were noted during mid November and December, given that more than 70 % of the total number of individuals captured was recorded during this interval. Despite the fact that during January and February captures were notably reduced, the traps continued to contain males even at low temperatures. This is the first quantitative study about the long-term monitoring of *P. unionalalis*, in olive orchards. From the results of the present work it became evident that this species was active during the entire season of the monitoring, in varied densities, at least in the two regions examined.

**Key words:** *Palpita unionalis*, *Olea europaea*, jasmine moth, pheromone, traps, monitoring

**Introduction**

The jasmine moth, *Palpita unionalalis* (Hübner) is an important pest of *Jasminum* sp., *Ligustrum* sp., *Olea europea* L. and *Phillyrea media* L. Larvae of this moth attack tender leaves and particular those of terminal twigs. In nurseries, larvae may devour young leaves and apical buds causing stunted growth of plants. In cases of heavy infestations, larvae attack the olive fruit, by creating irregular holes in the skin (Balachowsky, 1972; Badawi et al., 1976; Triggiani, 1971).

Very little is known about the seasonal occurrence of the jasmine moth in olive trees, despite the fact that heavy infestations are reported very often. According to the available literature, in Italy *P. unionalis* develops 4-5 generations (Martelli, 1916), in Israel 6 (Avidov & Harpaz, 1969), in Spain 5 (Fodale et al., 1988), in France 2 (Balachowsky, 1972) and in Egypt 10 (Badawi et al., 1976) whereas in Greece 3 (Mazomenos et al., 2002). The main reason for this incomplete knowledge is the limitations arising from monitoring *P. unionalalis* populations.

The female sex pheromone of *P. unionalis* consists of two pheromone components (E)-11-hexadecenal [(E)-11-16: Ald] and (E)-11-hexadecenyl acetate [(E)-11-16: Ac] (Mazomenos et al., 1994). Traps baited with the blend of the two components at a ratio 3:7 have
been proved attractive to males and are expected to constitute a valuable tool in the monitoring of this pest (Mazomenos et al., 1994, 2002). Nevertheless, despite the fact that pheromone traps were effective in the field, they have not been yet evaluated for long term monitoring. In this paper we report results on the seasonal occurrence of *P. unionalis* in two olive productive regions of Greece.

**Materials and methods**

**Field experiments**

The experiments were carried out in two regions, where olive trees are among the most common crops: Magnissia (Thessaly, Central Greece) and Chalkidiki (Macedonia, Northern Greece). Apart from easy access, the experimental fields in these regions were selected according to preliminary samplings, which revealed heavy infestations in olive shoots, leaves and fruit, during the entire growing season. Two rectangular olive groves (one in each region) of approx. 9 ha each, were used for experimentation. In Magnissia 5 and in Chalkidiki 9 traps were suspended. The distance between adjacent traps was 100 m. The trap type used was the Funnel trap (Agrisense BCS, UK), on which the moths are captured with the addition of a DDVP strip containing 0.5 g of dichlorvos. Each trap was baited with white rubber septa (Aldrich Ltd No Z-100722-100EA), loaded with 2 mg of the two-pheromone components, (E)-11-16: Ald and (E)-11-16: Ac, blend at a 3:7 ratio. In the Funnels the lure was placed under the roof of the trap. In both regions, the traps were suspended in July 2002, and checked for captured *P. unionalis* males at weekly intervals until January 2003 (Chalkidiki) and April 2003 (Magnissia). On each trap check date, the number of males captured was recorded, traps were cleaned and within the same group were rotated clockwise. Each trap was suspended with its lower part at a height of 2 m on the external part of the canopy. On each trap, the lure was replaced every 4 weeks.

**Results and discussion**

The present study consists the first one concerning the long-term monitoring of *P. unionalis* in Greece. In both regions examined, male captures in pheromone-baited traps indicated similar trends (Figs 1 and 2). At the beginning of the monitoring season (July), captures were rather low with less than 1 male/trap. Captures were continuously low until August and early autumn. A slight increase was noted in Magnissia (with >3 males/trap) during late September. However, the most interesting finding of the present study is the recording of high numbers of adults in traps during November and December. Thus, approx. 80 and 75 % of the total number of individuals caught in Magnissia and Chalkidiki, respectively, were recorded during the aforementioned interval. In Magnissia, the highest densities were observed in late November, with >20 males/trap. An additional peak was recorded two weeks later, in mid December, with >15 males/trap. Similarly, in Chalkidiki, the peak was observed in early December, with more than 15 males/trap. On the other hand, surprisingly, males, though in small numbers, were continued to be found in traps even during January and February, in both regions. Also, in Magnissia, where traps were monitored until the end of the spring, captures were extremely low until the end of the monitoring period.

Pheromone-baited traps can be used successfully for detection and monitoring of the jasmine moth populations in olive groves (Mazomenos et al., 1994). Among the trapping devices which are commercially available, Funnels have proved superior to traps that have a sticky surface, and therefore are recommended for this species (Athanassiou et al., 2004). Funnel traps are traps with high capture potential, and their efficacy is not influenced by
factors that restrict the efficacy of adhesive traps, such as saturation of the sticky surface or contamination by the presence of foreign material (Athanassiou et al., 2002). Apart from the trapping device, male captures of the jasmine moth are determined by several other factors, such as the colour of the trap, the trapping location and the type of the pheromone dispenser (Athanassiou et al., 2004).

Concerning the overall data, the results of our study indicate that adult males of the jasmine moth are highly active during November and December. In contrast, male activity, as reflected by their response to pheromone-baited traps, is low during summer and spring. Nevertheless, *P. unionalis* moths were found in traps during the entire trapping period. Our study stands in agreement with the results reported by Mazomenos et al. (2002), who made the first preliminary observations about *P. unionalis* population fluctuation by means of

Figure 1. Mean number of *P. unionalis* adults/trap, in Chalkidiki (Macedonia, Northern Greece), during the monitoring period

Figure 2. Mean number of *P. unionalis* adults/trap, in Magnesia (Thessaly, Central Greece), during the monitoring period
pheromone-baited traps in Crete. In that study, although the inspection of the traps was terminated in November, an augmentative trend was evident at that period. In light of our findings, the response of males in the pheromonic source may indicate that a) this species exists at the adult stage even at low temperatures and b) the existing males are active and may be capable of mating. Hence, if the response to the pheromone dissemination is indicative of mating or even reproductive development, we can assume that all life stages of the jasmine moth coexist in olive groves, at least in the study areas. This fact stands in antithesis with the phenology of other moth species in olive groves, like the olive moth *Prays oleae* (Bernard) (Lepidoptera: Plutellidae), whose adults do not exist during winter. Although quantitative measurements were not taken in the present study, some observations on the olive trees during trap inspections in November and in winter revealed that larvae of this species were active. The decrease of captures from late December on may indicate that the adult activity is negatively influenced by the decrease in temperatures, or that adult mortality is high at these conditions. Additional experimentation is required to examine the basis of the aforementioned hypotheses for the phenology of the jasmine moth.

In summary, our study provides information about the seasonal trends of *P. unionalis* adults in two regions in Greece. Generally, males are capable of flying and responding to pheromone emission during the entire year, especially during the cold season, and this fact raises a lot of questions about its phenology. Additional experimentation is required to examine the basis of the aforementioned hypotheses.

**References**


Varietal sensitivity of olive trees to the leopard moth, 
*Zeuzera pyrina* L. (Lepidoptera: Cossidae)

**Esmat M. Hegazi**, **Wedad E. Khafagi**

1. **Department of Entomology, Faculty of Agriculture, Alexandria University, Egypt**
2. **Plant Protection Research Institute, Sabahia, Alexandria, Egypt**

**Abstract**: The experimental work was carried out on a large ecological olive area situated near Cairo (177 km, south Alexandria, Egypt). Ten comparable plots which cultivated each with 2 or more of table olive cultivars in lines side by side, were selected to study the varietal sensitivity of olive trees to the leopard moth, *Zeuzera pyrina* L. The obtained data revealed that the olive cultivars could be categorized into three groups, each in descending order of sensitivity as follows: 1) sensitive, Toffahi, Becual, Sennara and Manzanilla; 2) intermediate, Kala mata and Dolce; and 3) resistant, Hamedy, Aks and Shamy. Infestation of olive trees by the leopard moth concentrated in the North-West (N-W) side than in South-East (S-E). The results justify the effect of wind direction on the egg-laying behaviour of *Z. pyrina* females during the peak of their flight activity (Sept.-Oct.). Interestingly, the co-existence of one olive cultivar in lines side by side with another cultivar and the grown cultivars on the neighbouring farm to the farm of interest may decrease or increase the rate of infestation by *Z. pyrina* larvae. Discovery of allomones in resistant varieties which drive away egg laying of *Zeuzera* females may be fruitful in protecting sensitive olive varieties.

**Keywords**: Black light trap, *Zeuzera pyrina*, olive cultivars, infestation rate.

**Introduction**

The olive tree, *Olea europaea* L. is liable to be attacked by several insect pests. The key pests of Mediterranean olive are the olive fruit fly, *Bactrocera (Dacus) oleae* (Gmelin), the olive-kernel borer, *Prays oleae* Bern., and the black scale, *Saissetia oleae* Olivier. Of the less important insect pests, some occur in particular areas or conditions at population levels that cause serious damage (Katsoyannos, 1992). For instance, in Egypt, the jasmine moth, *Palpita unionalis* (Hübn.) affects young nursery plants or young olive groves, while the leopard moth *Zeuzera pyrina* L. seriously attacks both young and old olive trees (Abdel-Kawy et al., 1992). The *Zeuzera* larvae attack living wood by drilling deep tunnels in the main branches and trunks of olive, apple and pear trees. This pest presents continuously threat for olive farming on almost all olive growing areas in Egypt. The damage caused by the *Z. pyrina* larvae has already led to uprooting of many olive groves in Egypt. The moth develops one generation a year (El-Hakim & El-Sayed, 1982).

*Z. pyrina* is a polyphagous pest that attacks more than 70 shrub species belonging to over 30 botanical families, including apple, pear, peach, cherry and olive (Katsoyannos, 1992). It is distributed throughout the western palaearctic region, in all Europe, the Democratic People’s Republic of Korea, the republic of Korea and the United States (Feron et al., 1966). In the eastern Mediterranean, it seriously attack olive: Lebanon, Syria, Jordan (Katlabi, 1989) and Isreal (Navon, 1977).

The control of *Z. pyrina* is necessary and economically justified but chemical control is not successful. In addition, pesticide treatment must eventually affect the consumer directly, since there is growing concern over possible side effects attributable to residual insecticides in the fruits (Arambourg, 1983). Integrated pest management (IPM) is especially important for
the role it can play in reducing the risk of such damage to quality. In IPM, emphasis and priority are given to natural limiting factors, such as plant resistance, antagonists (natural enemies, pathogen) and cultural practices, which prevent the build-up of pest populations (Lopez-Villalta, 1999).

A considerable number of olive cultivars have been identified in different Mediterranean countries, distinguished mainly on the basis of their different morphological characteristics on leaves, flowers and fruits (Katsoyannos, 1992). Thirty-one cultivars are reported in Greece (Katsoyannos, 1992); 22 major and 156 localized cultivars are found in Spain (Barranco & Rallo, 1985). Some are cultivated only for olive oil, others only for table olives and some for both products. The present work discusses the varietal sensitivity of nine common cultivars of table olives to *Z. pyrina* in Egypt.

**Materials and methods**

*Plot selection and description*

The farm where the study was conducted is located in an arid area, 177 km south Alexandria. It is a strip-shaped private farm of ca. 240 ha. It has a main median road (8 m width, 6.5 km length). On both sides, locate a total of uniform 88 plots (2.1-3.3 ha, each). Each plot is surrounded by windbreak trees (*Casuarina stricta*) isolating each plot from the others. The first four plots which are next to the high way (Cairo-Alexandria high way) are cultivated with palm trees. Six of the interior plots are for olives and floriculture nurseries. The remaining plots have fruitful olive trees of 8 years old. Dolce, Sennara, Shamy, Manzanilla, Toffahi, Hamedy, Kalamata, Bicual and Aks are the principal cultivars of table olives, constituting approximately 20.3, 20.1, 18.4, 12.8, 11.9, 9.7, 9.7, 6.3 and 4.9%, respectively, of the total 61774 olive trees. The owner believes that the cultivars bear better yield when two or more cultivars are planted near each other in the same plot as olives are not self-pollinated, thus the majority of olive plots (90%) contain two cultivars, and few (5 plots) are cultivated with 3 cultivars per plot. Some plots (three) at the far end of the farm was stormed by sand and dead olive plants were replaced by different cultivars which were at hand. These plots contain up to five replacement cultivars. The maximum olive trees/plot were 900 trees which were cultivated in lines (30 trees, each). Generally, each cultivar in 3 lines alternates by 3 lines of the other cultivar. For plots that combine 3 cultivars, the first half area is cultivated as mentioned above (i.e., 2 cultivars/plot), while the second half of the plot one of these 2 cultivars was alternated by new one. Each other 8 years, the owner cuts and sells part of windbreak trees during the low fruit year yield.

*Sensitivity of olive cultivars to the leopard moth, Zeuzera pyrina*

To study the sensitivity of olive cultivars to the leopard moth, *Zeuzera pyrina*, ten olive plots “groves” were selected. The number of cultivars/grove was 2, 3 and 5 on 7, 1 and 2 groves, respectively. The groves were comparable to each other in their size (2.1-3.3 ha), number of trees/cultivar, trees density/grove (780-870 trees) and spatial arrangement (5x6 m). All trees were carefully inspected at the peak period of adult emergence of the leopard moth. The moth was monitored in 3 olive groves by Hegazi’s trap (non-traditional black-light trap, Hegazi & Khafagi, 2003). The traps were inspected for captured moths at weekly intervals from June 2002 until mid November. Data were subjected to statistical analysis for determination of differences between means. Where significant differences occurred, Student’s t-test was applied for mean separation.
Results and discussion

Monitoring data of leopard moth, *Z. pyrina* was collected in 3 plots (3 ha, each) by weekly monitoring from third decade of July 2002 to the mid of November of the same year, a year of high yield of olive fruits. Captures of *Z. pyrina* moths by black-light traps are shown in Fig. 1. The traps caught low numbers of moths when inspected on July 1st. The catches were progressively increased on mid August reaching peak period during September. The flight peak of this univoltine pest was reached on September 25th. The results suggest that the active period of this pest, under the conditions of the experimental region, during which insecticide applications will have their maximum effect, is about one month, i.e., September.

Table 1. Infestation rates of trees of nine table olive cultivars by *Z. pyrina* larvae in ten comparable observation plots, with reference to the growth cultivars on the neighboring olive plots at each cardinal direction of interest plot.

<table>
<thead>
<tr>
<th>Observation plots</th>
<th>Cultivars of neighboring plots/cardinal direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Cultivars</td>
</tr>
<tr>
<td>1</td>
<td>Toffahi</td>
</tr>
<tr>
<td></td>
<td>Sennara</td>
</tr>
<tr>
<td>2</td>
<td>Sennara</td>
</tr>
<tr>
<td></td>
<td>Toffahi</td>
</tr>
<tr>
<td>3</td>
<td>Becual</td>
</tr>
<tr>
<td></td>
<td>Manzanilla</td>
</tr>
<tr>
<td>4</td>
<td>Hamedy</td>
</tr>
<tr>
<td></td>
<td>Sennara</td>
</tr>
<tr>
<td>5</td>
<td>Dolce</td>
</tr>
<tr>
<td></td>
<td>Kalamata</td>
</tr>
<tr>
<td>6</td>
<td>Toffahi</td>
</tr>
<tr>
<td></td>
<td>Shamy</td>
</tr>
<tr>
<td>7</td>
<td>Toffahi</td>
</tr>
<tr>
<td></td>
<td>Aks</td>
</tr>
<tr>
<td>8</td>
<td>Sennara</td>
</tr>
<tr>
<td></td>
<td>Kalamata</td>
</tr>
<tr>
<td></td>
<td>Aks</td>
</tr>
<tr>
<td></td>
<td>Kalamata</td>
</tr>
<tr>
<td>9</td>
<td>Manzanilla</td>
</tr>
<tr>
<td></td>
<td>Aks</td>
</tr>
<tr>
<td></td>
<td>Becual</td>
</tr>
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<td></td>
<td>Ziet</td>
</tr>
<tr>
<td></td>
<td>Hamedy</td>
</tr>
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<td>10</td>
<td>Becual</td>
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<td>Hamedy</td>
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<td>Manzanilla</td>
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<td></td>
<td>Sennara</td>
</tr>
</tbody>
</table>

W = West; N = North; E = East; S = South
The field observations on damages caused by *Z. pyrina* larvae were made when the peak of emergence rate of insect moths was reached in September 2002. All trees of 9 table olive cultivars on ten observation plots were carefully inspected. Table 1 summarizes the data of number of trees/cultivar/plot, percentage of infested trees by leopard larvae and cultivar types grown on each neighboring plot from each cardinal direction. According to the rate of infestation of olive trees by leopard moth larvae, the olive cultivars (Table 1) could be categorized into three groups, each is in descending order of sensitivity as follows: 1) sensitive, Toffahi, Becual, Sennara and Manzanilla; 2) intermediate, Kalamata and Dolce; and 3) resistant, Hamedy, Aks and Shamy.

![Figure 1. Weekly catches of leopard moth (*Zeuzera pyrina*) in light traps (trap and kill-method) installed in an olive grove at Paradise Park, Cairo, 177 km south Alexandria, in 2002.](image)
Figure 2. Sensitivity of olive trees of Toffahi and Sennara cultivars to the leopard moth, *Zeuzera pyrina*. 
Figure 3. Sensitivity of olive trees of Becual and Manzanilla cultivars to the leopard moth, *Zeuzera pyrina*.
Figure 4. Sensitivity of olive trees of Dolce and Kalamata cultivars to the leopard moth, *Zeuzera pyrina*.
Figure 5. Sensitivity of olive trees of Hamedy and Sennara cultivars to the leopard moth, *Zeuzera pyrina*. 
Figure 6. Sensitivity of olive trees of Toffahi and Shamy cultivars to the leopard moth, *Zeuzera pyrina*.
Figures 2 and 3 show the infestation rates of four olive cultivars by the leopard moth, *Zeuzera pyrina* at 2 observation plots of olive trees. All of these olive cultivars seem to be susceptible to attack by *Z. pyrina*. The figures show that these sensitive cultivars are in the following order: Toffahi > Becual > Sennara > Manzanilla. Their pattern of infestation by *Z. pyrina* moths seem alike, more concentrated infestation in the north-west (N-W) than south-east (S-E), especially in the shelter of windbreak (Fig. 2) of the above intercardinals. In some cases, some trees were killed by *Z. pyrina* larvae next to the windbreak trees (Fig. 2). This trend of peripheral distribution of infested trees may be due to migration of *Z. pyrina* moths from the neighboring plots, especially when the grown trees of nearby plots are sensitive cultivars. For instance, although both of the same sensitive cultivars (Toffahi & Sennara) coexist in plots 1&2, the percentage of infested trees of Toffahi cultivar was significantly (P<0.01) higher than corresponding figures in plot 2. This can be attributed to the cultivar types grown on the other nearby groves. The same sensitive cultivars are grown in plots from the W-N, N-E and S-W cardinal directions of plot 1. While the nearby groves to plot 2 contained some other cultivars (e.g., Dolce and Kalamata from N-E and Aks and Kalamata from W-N direction, Table 1).

Figure 4 shows that Dolce and Kalamata are intermediate cultivars in their infestation by *Z. pyrina* (9.2 and 10.3%, in respect) and show the same distribution of W-N infestation as the sensitive cultivars (Figs. 2&3).

Assuming that other factors of egg-laying behaviour of *Z. pyrina* moths are the same in all comparable plots (1-10), i.e., irrigation, tree vigour, control measures of other pests, plot size, spatial arrangement of olive trees, ... etc., the intervarietal effects on sensitive cultivars are reflected from Figs. 5&6. The presence of resistant cultivars in lines side by side to the sensitive ones drastically decreased the percentage of infestation in sensitive ones as Sennara. (Fig. 5) and Toffahi (Fig. 6). Interestingly, the effect of co-existence of the most susceptible cultivar (Toffahi) with the resistant one (Shamy) is far more than that of the other sensitive cultivar (Sennara). In combination of Sennara and Hamedy (resistant), the percentage of infestation of Sennara by *Z. pyrina* dropped to 4.6% (Fig. 5). However in Fig. 6, Shamy which looks to be the most resistant (0.9%) of all cultivars, significantly (P<0.001) reduced infestation of Toffahi, the most susceptible cultivar to 1.8%. The same trend could be detected in plot 7 (Table 1). In combination of Toffahi with resistant cultivar (Aks), the percentage of infestation by *Z. pyrina* of the former cultivar dropped to 7.2%.

In combination of 3 cultivars in the same plot (Table 1, plot 8), the percentage of infested Kalamata trees in the first half area of the plot 8 which includes Sennara and Kalamata reached higher rate (7.0%) than that in the second half area of the plot (5.7%), where Kalamata is grown alternately with resistant cultivar (Aks).

Surprisingly, the present study also shows the effect of grown cultivars of the neighboring farms on the infestation levels of the farm of interest. Plot 6 (Fig. 6), which was the least infested plot by *Z. pyrina*, is surrounded by resistant cultivars of Shamy of N-E side and Aks from S-E side. While plot 1 (Fig. 2), which was heavily infested with *Z. pyrina*, was neighboring with the same sensitive cultivars on W-N, N-E and S-W sides. The same trend could be applied on the other heavily infested plot (Table 1, plot 3). The grown cultivars of this plot are also surrounded with the same susceptible cultivars (Becual and Manzanilla) from all sides except W-N one.

Finally, varietal resistance to pests and diseases is one field that is quite unknown in the case of olive crop (Lopez-Villalta, 1999). The present work throws light on the different degrees of susceptibility of olive cultivars to attack of *Z. pyrina*, which could be useful in choosing suitable cultivars for management of new olive farms. Also, discovery of allomones in resistant cultivars which drive away egg laying of *Z. pyrina* females may be fruitful in protecting sensitive cultivars.
Acknowledgements

This study has been carried out with partially financial support from the Commission of the European Communities, contract ICA4-CT-2001-1004 “Sustainable control of lepidopterous pests in olive groves – Integration of egg-parasitoid and pheromones. The authors thank Dr. Ahmed El-Shazli, Professor of Entomology, Faculty of Agriculture, Alexandria University for reviewing the earlier draft of this paper and for his editing of this work.

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Other Olive Pests
Fruit damage by *Rhynchites cribripennis* (Desbr.) (Coleoptera: Attelabidae) and its population in an olive grove

Dionyssios Lykouressis¹, Agelos Kapsaskis¹, Dionyssios Perdikis¹, Anastassios Vatos², Argyro Fantinou³

¹ Laboratory of Agricultural Zoology and Entomology, Agricultural University of Athens, Iera Odos 75, 188 55 Athens, Greece
² Directorate of Rural Development, 29 100, Zakynthos, Greece
³ Laboratory of Ecology and Environmental Sciences, Agricultural University of Athens, Iera Odos 75, 188 55 Athens, Greece

**Abstract**: The damage caused on olive fruits by *Rhynchites cribripennis* adults was estimated by collecting fruit samples from an olive grove in the island of Zakynthos, Greece, during 1995. The distribution of adult weevils on olive trees was studied by beating twigs from the four directions (NW, NE, SE and SW) in 1994 and 1995. Also, the distribution of larvae, pupae and adults in the soil, was investigated by collecting soil samples from two soil depths (0-4 and 4-8cm), from October 1994 to October 1995. The percentage of olive fruits damaged by this weevil was 19% on May 29 and remained at similar levels until middle of July when it peaked (26%). Generally, adult population densities on the tree canopy was not found to be favored in a particular side. According to the results, the number of larvae, pupae and adults were similar in the upper and in the lower soil layer on each sampling date.

**Key words**: *Rhynchites cribripennis*, olive trees, damage, weevil

**Introduction**

*Rhynchites cribripennis* (Desbr.) (=*Rhynchites ruber* Schilsky) may cause significant damages on olive trees in several areas of Greece (Issaakidis, 1936; Pelekassis, 1962) and yield losses have reported ranging from 30 to 80% (Issaakidis, 1936). This species is widely distributed in Mediterranean basin and Arambourg (1985) referred significant losses in olive yield which can range from 40 to 70%. In recent years, the damages by this weevil have been more common than in the past, particularly at the Ionian Islands, Peloponnesus and Crete. Despite its importance as an olive pest, the available information on its bioecology and damage is scarce and it comes mainly from observations (Hoffmann, 1958; Della Beffa, 1962; Arambourg, 1985; Monaco, 1986).

This work aimed at investigating a) the damage levels caused by adult feeding of *R. cribripennis* on olive fruits b) the spatial distribution of *R. cribripennis* adults on olive trees and c) the abundance and distribution of larvae, pupae and adults in the soil.

**Material and methods**

This study was conducted in a 0.2 ha olive grove of cultivar “Koroneiki” at the village Pantokratoras, on the Island of Zakynthos in the Ionian Sea (western Greece).

Damage caused by adult feeding on olive fruits was estimated by collecting samples from 10 randomly selected olive trees, at weekly intervals from 29 May to 7 August in 1995. At each sampling date, 20 olive fruits were randomly collected from each tree. Those olive fruits were examined under a stereomicroscope so that to record feeding holes caused by adults.
The distribution of *R. cribripennis* adults on olive trees was examined by collecting samples from ten randomly selected olive trees, at weekly intervals during 1994 and 1995. Four twigs, one of each the four directions (NW, NE, SE and SW), were randomly selected from each tree. Each twig consisted of an average of 20 shoots. The volume of the twig together with its shoots had a nearly cylindrical shape with a base diameter and height about 40 cm. Each twig with its shoots was shaken above a 50 cm diameter plastic container, for adult collection. The sampling was undertaken between 6:30 and 8:00 a.m., when the adults were still resting on the trees.

The distribution of larvae, pupae and adults of *R. cribripennis* in the soil was studied in soil samples which were taken from October 9 in 1994 to October 2 in 1995, at 20-day to 1-month intervals. In each sampling date 5 olive trees were randomly selected and under the canopy in each of them, one soil sample was examined. In each sample, an area of 25x25 cm was carefully examined by digging the soil at a depth to 8 cm. The number of larvae, pupae or adults was recorded at two depth layers 0-4 cm and 4-8 cm.

Based on the number of adults on the shoots of twigs from the four different directions, their numbers in the northern, southern, eastern and western part of the tree canopy were estimated. These data and the data on abundance of larvae, pupae and adults in the two different depths in the soil, were analyzed using a t-test. Analyses were conducted using the statistical package STATISICA (Statsoft. Inc, 1995).

**Results**

The percentage of olive fruits with feeding holes caused by adult weevils was found as high as 19% on the first sampling on May 29, and remained at similar levels until July 16 when it peaked reaching to 26% (Fig. 1). From the next sampling it started decreasing and fell to 16% on August 7.

![Figure 1. Percentage (%) of olive fruits damaged by *Rhyncites cribripennis* adults in an olive grove on the island of Zakynthos in 1995.](image-url)
Figure 2. Number (mean + SE) of *Rhyncites cribripennis* adults recorded in the different parts of the tree canopy in an olive grove on the island of Zakynthos in 1994. Columns followed by different letters in each sampling date are significantly different (t-test, P < 0.05).

In the soil layer of 0 - 4 cm, larvae were present in all sampling dates but higher numbers were recorded from November to March whereas their population reached to a peak in December. In both depths the numbers of larvae showed a peak in December (Fig. 4a).

Pupae were found with low numbers during October and November (Fig. 4b).

Adults were present in the soil samples from December to May with higher numbers in December (Fig. 4c). In the soil layer of 0 - 4 cm adults were recorded from early December to end of May, with higher numbers in December. In the depth layer of 4-8 cm adults were recorded from December to May but not in all sampling dates (Fig. 4c).

**Discussion**

In this study the percentage of damaged olive fruits reached to lower levels (Fig. 1) than those reported by Issakidis (1936) and Arambourg (1985) (reaching as high as 80%) and by Lykouressis et al. (2001) (44%). However, if we consider that this relatively low damage level occurred in 1995 when the adult population was much lower, almost one third, than that in 1994 (Figs 2 and 3), then it is obvious that in years when high populations of *R. cribripennis*
occur then yield reduction could reach high levels. Damage by this weevil on olives before the stone hardening is the most severe damage caused by this insect as has been stressed by other authors (Arambourg, 1985; Monaco, 1986). Later in the season, the percentage of damaged olive fruits was decreased due to olive drop and to the elimination of adult weevil population.

On the other hand, the percentage of olive fruits damaged by the weevil was highest on July 16, one week after the number of adults peaked (Figs 2 and 3). Therefore, the damage on olive fruits seems to be strongly correlated with the presence of adults on the olive trees as expected. Consequently, to prevent damage on olive fruits, insecticide applications should be conducted soon after their emergence and before their population increase to high levels.

Insecticide sprays should be directed on the entire canopy of the tree because adults did not show a particular tendency to be concentrated on shoots of a specific direction and therefore they are active moving to all directions on olive trees (Figs 2 & 3).

The seasonal abundance of larvae, pupae and adults in the soil showed that they are more abundant in the upper layer (0 - 4cm) (Fig. 4a). Larval population increases in November and December since in that period larvae exit from the olive fruits in which they had been developed. In January and February larval population seem to decrease, due to some mortality, reaching almost to 40%.

![Figure 3](image-url)
Figure 4. Number (mean + SE) of Rhyncites cribripennis larvae (a), pupae (b) and adults (c) recorded in two soil layers in an olive grove on the island of Zakynthos in 1995. Columns followed by different letters in each sampling date are significantly different (t-test, P < 0.05).
In conclusion, *R. cribripennis* can cause significant olive damage with apparent reduction on olive yield, at least in some years when it develops high populations. Adult population increases on olive trees in May indicating that control measures should be taken at that period as mentioned earlier. Studies to investigate the economic threshold of this insect are needed. Also, studies which would elucidate and other aspects of its bioecology could contribute to a more friendly to the environment management of this pest in olive groves and they would be of great importance.

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Reaction of olive cultivars to *Meloidogyne javanica*

Hosseininejad Syed Abbas, Ramezani Malakrodi Mohammad  
Plant Pests and Diseases Research Institute, PO.Box 1454, Tehran – 19395, Iran  
Olive Research Station, Roodbar, Iran

**Abstract:** Nineteen olive cultivars were screened against *Meloidogyne javanica* under glasshouse condition. Seedlings raised in pots containing 1 kg of sterilized soil were artificially inoculated with 2000 second stage juveniles of the nematode and experiment was terminated one year after inoculation. Plant growth, nematode growth and reproduction parameters were recorded. Cultivars Coratina, Manzanillo and Leccino were found to be resistant whereas Amigdalifolia and Frangionato showed moderately susceptible reaction against the nematode. Cultivars Marii, Fishemi, Shengeh, Kalamata, Amphisis, Clonaris, Bldy, Conservallia, Mission and Sevillano were susceptible, Zard-e-Jonoub 1, Zard-e-Jonoub 2, and Roghani showed highly susceptible reaction against the nematode.

**Key words:** Olive, *Meloidogyne javanica*, resistant.

**Introduction**

Several plant parasitic nematodes have been reported to parasitize olive trees (Lamberti and Vovlas, 1993). Almost all olive seedlings produced in different parts of the country were found to be infested with phytonematodes and the root knot nematode, *Meloidogyne javanica*, being the most important endoparasite nematode isolated (Hosseininejad et al., 1997). Cultivars Coratina and Leccino are reported to be resistant to *M. javanica* (Sasanelli et al., 1997) and it was shown that *M. javanica* is pathogenic to the cultivars Ascolano, Sevillano and Manzanillo (Lamberti and Baines, 1969).

Reaction of some olive cultivars, including Roghani, Marri, Shengeh and Fishemi, which are widely grown in Iran was evaluated against *M. javanica* under artificial inoculation condition.

**Material and methods**

Roots of olive seedlings infected with root-knot nematode were collected from nurseries from Mazandaran Provence. Root samples collected in polythene bags and properly marked were brought to the laboratory for the identification of the species and establishing of single egg mass population. The species of root-knot nematode present in the root samples was identified by using perineal pattern characteristics of mature females. Single egg mass culture of *Meloidogyne javanica* was raised on tomato cv. Rutgers seedling in glasshouse at 25 ± 2°C. Sub-culturing was done by inoculating new tomato seedling in order to maintain sufficient inoculum for conducting experiment. Second stage juveniles (J2) of the root-knot nematode obtained by incubating eggmasses in sterilized water in petri plates at 25°C were used as a source of inoculum. The number of juveniles in the suspension was standardized before inoculation. The required amount of suspension was taken by micropipette controller and added near the roots into 4-5 holes made around the seedlings. The holes were covered with the soil after inoculation.
Equal size cutting of olive cultivars were dipped for few seconds in 2000 ppm indolebutyric acid and planted in perlite beds in mist chamber with bottom heating of 21 ± 2°C.

After 60 days, rooted cuttings were transplanted to pots containing 1 kg steam sterilized soil, sand and manure in the ratio of 1:1:1 and were kept in glasshouse at 25 ± 2°C. After two months, olive seedlings were inoculated with 2000 freshly hatched second stage juveniles of the nematode. Each treatment was replicated three times including control and the pots were arranged in randomised block design in glasshouse benches. The experiment was terminated one year after inoculation and following parameters were measured i.e., fresh and dry weights of shoot and root, length of shoot and root, No. of galls and eggs / root, No. of J2 in soil and No. of females / root. The final nematode population density (Pf) in each pot was determined by processing all soil by Jenkins (1964) and the roots by method adopted by Coolen (1979). Root gall index was assessed on a scale of 1-6 (Marull and Pinochet, 1991), where 1 = no gall, 2 = 1-10 galls, 3 = 11-30 galls, 4 = 31-70 galls, 5 = 71-90 galls and 6 = more than 91 galls per root system. Reaction of each cultivar was evaluated on the basis of reproduction factor, \( r = p_f/p_i \) in which 0 = highly resistant, less than 1 = resistant, 1-2 = moderately resistant, 2.1-5 = moderately susceptible, 5.1-10 = susceptible and more than 10 = highly susceptible (Di Vito et al., 1996).

Data were statistically analysed and means were compared by the Students \( t \) test for plant growth parameters and Duncan’s multiple range test for nematode reproduction indices.

**Results and discussion**

The root-knot nematode, *M. javanica*, caused galling in all tested olive cultivars within the gall index range of 2-5 under artificial inoculation. Highest root gall index was recorded in cvs. Zard –e– Jonoub 1, Zard –e– Jonoub 2, Roghani and Sevillano whereas lowest was in Manzanillo and Leccino. Lowest nematode reproduction factor was observed in Leccino and Coratina whereas highest was observed in Zard –e– Jonoub 1 and Zard –e– Jonoub 2 (Table 1).

Nematode caused significant reduction in shoot length of Fishemi, Zard –e– Jonoub 1, Zard –e– Jonoub 2, Shengeh and Coratina whereas root length reduction was significant in Fishemi, Roghani and Frangionato. Reduction in fresh and dry weights of shoot was recorded in Zard –e– Jonoub 2 and Roghani whereas in Blady and Leccino the reduction was significant in shoot fresh weight. Root fresh weight reduction was significant in Shengeh, Manzanillo, Sevillano and Leccino whereas the reduction in dry weight was significant in Marii, Zard –e– Jonoub 1, Roghani, Shengeh, Clonaris, Coratina and Frangionato (Table 2).

With reference to the adopted rating cvs. Leccino, Coratina and Manzanillo showed resistant reaction to the tested nematode. Olive cvs. Coratina and Leccino were shown to be resistant to *M. javanica* (Sesanelli et al., 1997) and Manzanillo also was proved to be resistant to *M. javanica* (Lamberti & Baines, 1969). Cultivars Amigdalifolia and Frangionato showed moderately susceptible reaction whereas cvs. Zard –e– Jonoub 1, Zard –e– Jonoub 2 and Roghani showed highly susceptible reaction. Rest of the cvs. showed susceptible reaction to *M. javanica*.

Variation in reaction of olive cultivars against *M. javanica* has been found to be closely correlated with the level of phenols, phenylalanine ammonialyase (PAL) and peroxidase (POD) in plant (Ridolfi et al., 1998). Plants hypersensitive to parasites also elicit metabolic changes activated in the phenylpropanoid pathway. The initial reaction resulting the biosynthesis of a wide variety of phenolic compounds is catalyzed by the enzyme PAL to yield trans – cinnamic acid and NH\(_4\)\. This metabolic pathway is involved in plant resistance mechanisms, i.e. cytotoxic activity exerted by quinones (Ponz & Bruening, 1986) deposition
Table 1. Reproduction of *Meloidogyne javanica* on olive cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>No of galls/root</th>
<th>Gall Index</th>
<th>Eggs &amp; J2 /root</th>
<th>J2 in soil</th>
<th>Femails /root</th>
<th>Total population (soil &amp; root)</th>
<th>R=Pf/Pi</th>
<th>Resistance rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marri</td>
<td>36.3           bc</td>
<td>3.7      b</td>
<td>11640          b</td>
<td>3300       cde</td>
<td>100           b</td>
<td>16040       c</td>
<td>7.5           c</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Fishemi</td>
<td>35              bc</td>
<td>3.7      b</td>
<td>12100          b</td>
<td>3640       c</td>
<td>60            c</td>
<td>15800       c</td>
<td>7.9           c</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Zard-e-Jonoub1</td>
<td>85.3           a</td>
<td>5        a</td>
<td>18427          a</td>
<td>8900       a</td>
<td>100           b</td>
<td>27426       a</td>
<td>13.7          a</td>
<td>Highly Susceptible</td>
</tr>
<tr>
<td>Zard-e-Jonoub2</td>
<td>78.7           a</td>
<td>5        a</td>
<td>18933          a</td>
<td>9000       a</td>
<td>70            c</td>
<td>28003       a</td>
<td>14            a</td>
<td>Highly Susceptible</td>
</tr>
<tr>
<td>Roghani</td>
<td>82.3           a</td>
<td>5        a</td>
<td>17900          a</td>
<td>7000       b</td>
<td>100           b</td>
<td>25000       b</td>
<td>12.5          b</td>
<td>Highly Susceptible</td>
</tr>
<tr>
<td>Shengeh</td>
<td>37.7           bc</td>
<td>3.7      b</td>
<td>10900          b</td>
<td>3200       de</td>
<td>100           b</td>
<td>14200       c</td>
<td>7.1           c</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Manzanillo</td>
<td>6               d</td>
<td>2        c</td>
<td>220            d</td>
<td>1170       f</td>
<td>10            d</td>
<td>1400         e</td>
<td>0.7           e</td>
<td>Resistent</td>
</tr>
<tr>
<td>Amigdalifolia</td>
<td>22              cd</td>
<td>3        bc</td>
<td>4900           c</td>
<td>800        fg</td>
<td>100           b</td>
<td>5800         d</td>
<td>2.9           d</td>
<td>Moderately Susceptible</td>
</tr>
<tr>
<td>Kalamata</td>
<td>37.3           bc</td>
<td>3.7      b</td>
<td>11500          b</td>
<td>3040       e</td>
<td>60            c</td>
<td>14600       c</td>
<td>7.3           c</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Amphisis</td>
<td>39.7           bc</td>
<td>3.7      b</td>
<td>12000          b</td>
<td>3540       cd</td>
<td>60            c</td>
<td>15600       c</td>
<td>7.8           c</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Clonaris</td>
<td>40              b</td>
<td>3.7      b</td>
<td>12620          b</td>
<td>3310       cde</td>
<td>70            c</td>
<td>16000       c</td>
<td>8             c</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Coratina</td>
<td>5.7             d</td>
<td>2.3      c</td>
<td>210            d</td>
<td>1180       f</td>
<td>10            d</td>
<td>1400         e</td>
<td>0.7           e</td>
<td>Resistent</td>
</tr>
<tr>
<td>Blady</td>
<td>36              bc</td>
<td>3.7      b</td>
<td>11500          b</td>
<td>3200       de</td>
<td>100           b</td>
<td>14800       c</td>
<td>7.4           c</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Frangionato</td>
<td>12.3            d</td>
<td>3        c</td>
<td>4850           c</td>
<td>740        g</td>
<td>10            d</td>
<td>5600         d</td>
<td>2.8           d</td>
<td>Moderately Susceptible</td>
</tr>
<tr>
<td>Conservallia</td>
<td>39.3           bc</td>
<td>3.7      b</td>
<td>11800          b</td>
<td>3250       cde</td>
<td>150           a</td>
<td>15200       c</td>
<td>7.6           c</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Mission</td>
<td>37.3           bc</td>
<td>3.7      b</td>
<td>11500          b</td>
<td>3190       de</td>
<td>110           b</td>
<td>14800       c</td>
<td>7.4           c</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Sevillano</td>
<td>77.7           a</td>
<td>5        a</td>
<td>12200          b</td>
<td>3300       cde</td>
<td>100           b</td>
<td>15600       c</td>
<td>7.8           c</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Leccino</td>
<td>9               d</td>
<td>2        c</td>
<td>560            d</td>
<td>430        g</td>
<td>10            d</td>
<td>1000         e</td>
<td>0.5           e</td>
<td>Resistent</td>
</tr>
<tr>
<td>Arbiqueine</td>
<td>39.3           bc</td>
<td>3.7      b</td>
<td>11700          b</td>
<td>3200       de</td>
<td>100           b</td>
<td>15000       c</td>
<td>7.5           c</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

Each value is mean of three replicates. Mean within each column with same letter are not significant (p=0.05) according to Duncan Multiple Range Test.
Table 2. Effect of *Meloidogyne javanica* on growth of olive cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Treatment</th>
<th>Shoot (g)</th>
<th>Root (g)</th>
<th>Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fresh wt.</td>
<td>Dry wt.</td>
<td>Fresh wt.</td>
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<tr>
<td></td>
<td>Control</td>
<td>20</td>
<td>9.3</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>17</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>Fishemi</td>
<td>Control</td>
<td>22</td>
<td>10.3</td>
<td>20.3</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>20</td>
<td>9.7</td>
<td>22</td>
</tr>
<tr>
<td>Zard-e-Jonoub 1</td>
<td>Control</td>
<td>30</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>24</td>
<td>11.3</td>
<td>27.3</td>
</tr>
<tr>
<td>Zard-e-Jonoub 2</td>
<td>Control</td>
<td>28</td>
<td>12.3</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>21**</td>
<td>10*</td>
<td>29.7</td>
</tr>
<tr>
<td>Roghani</td>
<td>Control</td>
<td>32</td>
<td>15.3</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>25*</td>
<td>11**</td>
<td>32</td>
</tr>
<tr>
<td>Shenghe</td>
<td>Control</td>
<td>23</td>
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<td>21</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>19</td>
<td>9</td>
<td>19.6*</td>
</tr>
<tr>
<td>Manzanillo</td>
<td>Control</td>
<td>19</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>18</td>
<td>8.3</td>
<td>23**</td>
</tr>
<tr>
<td>Amigdalifolia</td>
<td>Control</td>
<td>26</td>
<td>12</td>
<td>24**</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>25</td>
<td>11.3</td>
<td>23.3</td>
</tr>
<tr>
<td>Kalamata</td>
<td>Control</td>
<td>25</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>21</td>
<td>10</td>
<td>21.3</td>
</tr>
<tr>
<td>Amphisis</td>
<td>Control</td>
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<td>14</td>
<td>27.3</td>
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<tr>
<td></td>
<td>Inoculated</td>
<td>25</td>
<td>12.3</td>
<td>28</td>
</tr>
<tr>
<td>Clonaris</td>
<td>Control</td>
<td>25</td>
<td>12</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>23</td>
<td>10.3</td>
<td>24.7</td>
</tr>
<tr>
<td>Coratina</td>
<td>Control</td>
<td>27</td>
<td>12.7</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>25</td>
<td>11.3</td>
<td>24</td>
</tr>
<tr>
<td>Blady</td>
<td>Control</td>
<td>27</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>25*</td>
<td>11</td>
<td>26.3</td>
</tr>
<tr>
<td>Frangionato</td>
<td>Control</td>
<td>26</td>
<td>12.7</td>
<td>24.7</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>25</td>
<td>10.7**</td>
<td>24</td>
</tr>
<tr>
<td>Conservallia</td>
<td>Control</td>
<td>31</td>
<td>15</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>28</td>
<td>14</td>
<td>29.3</td>
</tr>
<tr>
<td>Mission</td>
<td>Control</td>
<td>24</td>
<td>11.3</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>21</td>
<td>10</td>
<td>23.7</td>
</tr>
<tr>
<td>Sevillano</td>
<td>Control</td>
<td>30</td>
<td>14.3</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>27</td>
<td>13</td>
<td>28**</td>
</tr>
<tr>
<td>Leccino</td>
<td>Control</td>
<td>33</td>
<td>15.7</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>28*</td>
<td>14.7</td>
<td>14*</td>
</tr>
<tr>
<td>Arbiqueine</td>
<td>Control</td>
<td>26</td>
<td>12.7</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Inoculated</td>
<td>24</td>
<td>11.3</td>
<td>26.3</td>
</tr>
</tbody>
</table>

Statically different from control according to Student’s t-test

*for P=0.05 ; ** for P=0.01.

of phenolics in the cell wall (Kimmis & Wuddah, 1977) and constitution of a non-degradable mechanical barrier resulting from cell wall deposition of lignins (Vance *et al*, 1980).
References


Life table parameters of *Rhyzobius lophanthae* Blaisdell (Coleoptera: Coccinellidae)

**G.J. Stathas, D.C. Kontodimas, S.L. Bouras and L.P. Economou**
Benaki Phytopathological Institute, St. Delta 8, 145 65, Kifissia, Greece
E-mail: georgestathas@hotmail.com

**Abstract:** The fecundity of the diaspidids’ predator *Rhyzobius lophanthae* feeding on *Aspidiotus nerii* Bouché (Homoptera: Diaspididae) was measured under controlled laboratory conditions at 25°C. The average total fecundity was 633.7 eggs/female and the average longevity were 63.4. Using additional data on development of the immature stages, a life-fecundity table and a development matrix (Leslie matrix) were constructed and population growth parameters were calculated. The net reproductive rate $R_n$ was estimated 346.2 females/female and the intrinsic rates of increase $(r_m)$ were 0.122 females/female/day. In addition two mathematical models, by Enkegaard $F= (a + b \cdot x) \cdot e^{-(c+d \cdot x)}$ and Analytis $F= q \cdot (\kappa - x_{ma} - x_{fa} \cdot x^n)$ were used to compare data on fecundity. Both models described satisfactorily the experimental data.

**Key-words:** Coccinellidae, *Rhyzobius lophanthae*, fecundity, life table, Leslie matrix

**Introduction**

The Australian native coccoidophagous predator, *Rhyzobius lophanthae*, has been reported as an important natural enemy of most armored scale species of the family Diaspididae (Homoptera: Coccoidea) (Hodek, 1973; Rosen, 1990). It has been successfully introduced to many countries including U.S.A. (California) (Yu, 1973), Italy (Bouvier, 1913; Smirnoff, 1950), Argentina, (Salvadores, 1913), Bermudas (Bennet & Hughes, 1959), Algeria, Tunisia and Morocco (Rungs, 1950; Smirnoff, 1950), and Georgia (Rubstov, 1952) for the control of armored scales. Although there are no reports on its introduction in Greece, *R. lophanthae* was first recorded in 1960 in Peloponnese and in Central and Northern Greece on olive trees harboring armored scales (Argyriou & Katsoyannos, 1977).

The present study deals with the fecundity of the predator in laboratory conditions. The life-fecundity table and the development matrix (Leslie matrix) were constructed and two mathematical models were used to compare data on fecundity. Another mathematical model was adopted in order to study adult survival.

**Material and methods**

In order to study the fecundity of *Rhyzobius lophanthae*, 25 newly emerged pairs of adults of the predator were reared in climatic incubators at 25±1°C, RH 65±2%, a photoperiod of LD 16:8 and abundant food, *Aspidiotus nerii* mounted on potato sprouts. Potato sprouts were chosen because their shape enabled their examination under the stereo-microscope so as to determine the number of *R. lophanthae* eggs. Life table parameters of other coccinellid species have been studied in similar conditions in east Mediterranean. (Uygun & Elekcioglu, 1998; Uygun & Atlinan, 2000). Each pair was reared separately in plastic cylindrical cages (9 cm diameter & 1.6 cm height) and *A. nerii* were added as food source every day.

Survival of the immature stages was observed and sex ratio of the progeny was measured under the same conditions.
Longevity and fecundity of the 25 females was also recorded daily. The following life table parameters were calculated according to Birch (1948):

- the intrinsic rate of increase \( r_m \), which results by iteratively solving the Euler equation,
\[
\sum (e^{-r_m x} \cdot l_x \cdot m_x) = 1
\]
where \( x \) is the mid-point of age intervals, \( m_x \) is the age specific fecundity (mean number of female progeny during age interval \( x \) per female aged \( x \)) and \( l_x \) is the age specific survival (probability of a female surviving to age \( x \));

- the age specific survival \( l_x \) of the 25 females;

- the age specific fecundity \( m_x \) (measured in females/female), estimated by multiplying the mean number of eggs by 0.55, the sex ratio \( (♀/♀ + ♂) \) (Liu et al, 1997);

- the net reproductive rate (the number of times a population will multiply per generation, measured in females/female/generation):
\[
R_0 = \sum (l_x \cdot m_x)
\]

- the mean generation time (measured in days) was estimated with two ways:
1. \( T_c = \frac{\sum (x \cdot l_x \cdot m_x)}{R_0} \) (Izhevsky & Orlinsky, 1988; Birch, 1948) and
2. \( T = \frac{\ln R_0}{r_m} \) (Chazeau et al, 1991; Kairo & Murphy, 1995)

- the finite rate of increase (the number of times the population will multiply itself per unit of time, measured in female/female/day):
\[
\lambda = e^{r_m}
\]

- the doubling time (the time required for a given population to double in numbers, measured in days):
\[
DT = \frac{\ln 2}{r_m}
\]

- the reproductive value of the females (expected number of female progeny during the remaining life time of a female aged \( x \), measured in females/female):
\[
V_x = \sum_{y=x}^{\infty} e^{r_m y} \cdot l_y \cdot m_y
\]

- the expected remaining life time of the females (measured in days):
\[
E_x = \sum_{y=x}^{\infty} \frac{l_y + l_{y+1}}{2 l_x}
\]

- the stable age distribution:
\[
C_x = \frac{l_x \cdot e^{-r_m x}}{\sum_{x=0} l_x \cdot e^{-r_m x}}
\]
The development matrices (Leslie matrices) of the *Rhyzobius lophanthae* were constructed by the Izhevsky & Orlinsky method. Subsequently, they were summarized by classifying the longevity of the females in 8 age categories (A1-A8) in each temperature (Izhevsky & Orlinsky, 1988). Fecundity is presented as females/female.

Two mathematical models have been used to compare the data on fecundity:

1. the Enkegaard model: \( F = (a + b \cdot x) \cdot e^{-c \cdot d \cdot x} \)  
(Enkegaard, 1993; Perdikis and Lykouressis, 2002)

2. and the Analytis model: \( F = q \cdot x_{\text{min}} \cdot x^{m} \cdot \left( x_{\text{max}} - x \right)^{n} \)  

where \( F \) is the fecundity, \( x \) are the days after emergence, \( a, b, c, d, q, x_{\text{min}}, x_{\text{max}}, n, m \): parameters and \( e=2.178 \).

The Weibull frequency distribution was used in order to describe the age specific survival of female adults. The probability that an individual lives at least to time \( t \) is given by:

\[
S(t) = e^{-\left(\frac{t}{b}\right)^c}, \text{ for } t > 0
\]

where \( b \) is a scale parameter that is inversely related to the mortality rate (ie, larger \( b \) value indicates slower decline of the population) and \( c \) is a shape parameter that allows the model to represent survival distributions of different forms, from the exponential to an extreme inverted S shape. Values of the shape parameter \( c > 1, = 1 \) or \( <1 \) correspond to Deevey’s (1947) type I, II or III survivorship curves, respectively (Pinder et al., 1978). Estimates of both values were obtained with the statistical package JMP v.4.0.2 (SAS, 1989). Additionally, coefficient of non-linear regression (\( R^2 \)) for the Weibull curve derived from using SPSS v.9.0.0 statistical program (SPSS, 1999). The LT\(_{50} \) value was calculated by the fitted Weibull curve.

Results and discussion

The females of *Rhyzobius lophanthae* started ovipositing 4-5 days after emergence (Figure 1). The average fecundity of the females was 633.7 (SD: 199.7) eggs per female and the average longevity of the females was 63.4 (SD: 19.8) days. Female fecundity ranged from 222 eggs laid by a female that lived 17 days, to 1152 eggs, laid by a female that lived 110 days. The median was 629 eggs, which corresponds to an individual that lived 63 days.

The proportion of the eggs laid in clutches of 1-5 eggs was also counted. Most of the eggs were found in clutches of 3 eggs (Figure 2) (3699 eggs were measured overall).

In addition, summaries of the life table and of the development matrix (Leslie matrix) were constructed and the life table parameters were calculated (Tables 1, 2 and 3).

The comparison of the fecundity data with two mathematical models is presented in Figure 3. The parameters of the Enkegaard equation were estimated: \( a=-14.993997, b=3.638430, c=-0.056794, d=0.062795, \) determination coefficient \( R^2=0.9455, \) Residual Sum of Squares RSS=222.7799. The parameters of Analytis equation were: \( q=5.2941 \times 10^{-7}, x_{\text{min}}=4.026661, x_{\text{max}}=91.999999, n=0.947242, m=3.432867, R^2=0.9697, \) RSS=102.9390.

The Weibull model produced excellent fits to the survival data, with a value of the coefficient of non-linear regression equal to 0.980. The shape parameter \( c \) obtained value >1, classifying the survival curve of *R. lophanthae* into Type I, in which the risk of death increases with age. The LT\(_{50} \) value estimated by the Weibull distribution (LT\(_{50w} \)) lied within the range obtained by the experimental data (LT\(_{50o} \)) (Table 4, Figure 4).
The above biological parameters in combination with data of the development under constant temperatures and of the predatory activity of this insect (Stathas, 2000), prove that \textit{Rhyzobius lophanthae} would be an effective biological control agent against Diaspidids.

Table 1. Summary of the life table of the laboratory population of \textit{Rhyzobius lophanthae}.

<table>
<thead>
<tr>
<th>Observation (day)</th>
<th>Age specific survival ((l_x))</th>
<th>Age specific fecundity females/female ((m_x))</th>
<th>Reproductive value females/female ((V_x))</th>
<th>Expected remaining lifetime ((E_x))</th>
</tr>
</thead>
<tbody>
<tr>
<td>immature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.00</td>
<td>0.0</td>
<td>1.1</td>
<td>86.6</td>
</tr>
<tr>
<td>14</td>
<td>1.00</td>
<td>0.0</td>
<td>5.5</td>
<td>73.6</td>
</tr>
<tr>
<td>mature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>1.00</td>
<td>0.0</td>
<td>30.7</td>
<td>59.6</td>
</tr>
<tr>
<td>42</td>
<td>1.00</td>
<td>60.0</td>
<td>74.4</td>
<td>45.6</td>
</tr>
<tr>
<td>56</td>
<td>0.92</td>
<td>132.4</td>
<td>66.3</td>
<td>35.2</td>
</tr>
<tr>
<td>70</td>
<td>0.88</td>
<td>111.8</td>
<td>31.1</td>
<td>22.3</td>
</tr>
<tr>
<td>84</td>
<td>0.76</td>
<td>46.0</td>
<td>11.2</td>
<td>9.8</td>
</tr>
<tr>
<td>98</td>
<td>0.32</td>
<td>23.1</td>
<td>6.9</td>
<td>15.8</td>
</tr>
<tr>
<td>112</td>
<td>0.16</td>
<td>12.7</td>
<td>2.6</td>
<td>10.8</td>
</tr>
<tr>
<td>126</td>
<td>0.04</td>
<td>10.7</td>
<td>4.6</td>
<td>11.0</td>
</tr>
<tr>
<td>136</td>
<td>0.04</td>
<td>7.7</td>
<td>7.2</td>
<td>1.0</td>
</tr>
<tr>
<td>adult</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>136</td>
<td>0.04</td>
<td>7.7</td>
<td>7.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 2. Life table parameters and stable age distribution of the laboratory population of \textit{Rhyzobius lophanthae}.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Stable age distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R_0)</td>
<td>346.2</td>
<td>Egg</td>
</tr>
<tr>
<td>(r_m)</td>
<td>0.122</td>
<td>Larva</td>
</tr>
<tr>
<td>(T_c)</td>
<td>56.7</td>
<td>Pupa</td>
</tr>
<tr>
<td>(T)</td>
<td>47.8</td>
<td>Adult (1-30 days)</td>
</tr>
<tr>
<td>(DT)</td>
<td>5.7</td>
<td>Adult (&gt;30 days)</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>1.13</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Summary of the development matrix (Leslie matrix) of the laboratory population of *Rhyzobius lophanthae*.

<table>
<thead>
<tr>
<th>Immature stages</th>
<th>Adult</th>
<th>Immature stages</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>egg</td>
<td>larva</td>
<td>pupa</td>
<td>A1</td>
</tr>
<tr>
<td>(female /female)</td>
<td></td>
<td></td>
<td>(1-14) (15-28)</td>
</tr>
<tr>
<td>Fecundity</td>
<td></td>
<td>within 14 days</td>
<td>A2</td>
</tr>
<tr>
<td>(female /female)</td>
<td></td>
<td></td>
<td>(15-28)</td>
</tr>
<tr>
<td>egg to larva</td>
<td>1.00</td>
<td>0 0 0 0 0 0 0 0</td>
<td>A3</td>
</tr>
<tr>
<td>larva to pupa</td>
<td>0</td>
<td>1.00 0 0 0 0 0 0</td>
<td>A4</td>
</tr>
<tr>
<td>pupa to adult</td>
<td>0 0 1.00 0 0 0 0 0</td>
<td>A5</td>
<td></td>
</tr>
<tr>
<td>A1 to A2</td>
<td>0 0 0 1.00 0 0 0 0</td>
<td>A6</td>
<td></td>
</tr>
<tr>
<td>A2 to A3</td>
<td>0 0 0 0 0.96 0 0 0</td>
<td>A7</td>
<td></td>
</tr>
<tr>
<td>A3 to A4</td>
<td>0 0 0 0 0 0.96 0 0 0</td>
<td>A8</td>
<td></td>
</tr>
<tr>
<td>A4 to A5</td>
<td>0 0 0 0 0 0 0.95 0 0 0</td>
<td>Fecundity (female /female)</td>
<td></td>
</tr>
<tr>
<td>A5 to A6</td>
<td>0 0 0 0 0 0 0 0.50 0 0 0</td>
<td>within 14 days</td>
<td></td>
</tr>
<tr>
<td>A6 to A7</td>
<td>0 0 0 0 0 0 0 0 0.40 0 0</td>
<td>A7 to A8</td>
<td></td>
</tr>
<tr>
<td>A7 to A8</td>
<td>0 0 0 0 0 0 0 0 0 0.25 0</td>
<td>0 0 0 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Weibull parameter values for the survival curve of the laboratory population of *Rhyzobius lophanthae*.

<table>
<thead>
<tr>
<th>number of replications</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weibull scale parameter</td>
<td>b</td>
</tr>
<tr>
<td>Weibull shape parameter</td>
<td>c</td>
</tr>
<tr>
<td>LT_{50} value calculated from the fitted Weibull equation</td>
<td>LT_{50w}</td>
</tr>
<tr>
<td>LT_{50} value obtained by the observed experimental data</td>
<td>LT_{50o}</td>
</tr>
<tr>
<td>coefficient of non linear regression.</td>
<td>R^2</td>
</tr>
<tr>
<td>type</td>
<td>I</td>
</tr>
</tbody>
</table>

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Figure 1. Daily fecundity (A) and longevity (B) of the 25 females of *Rhyzobius lophanthae*

Figure 2. Proportion of eggs in clutches of 1, 2, 3, 4, and 5 eggs
Figure 3. Comparison of two mathematical models with the fecundity data

Figure 4. Survival of the laboratory population of *Rhyzobius lophanthae*
References


Control of diaspidid scales on olive trees by releasing coccinellid predators

George J. Stathas¹, Stelios L. Bouras¹, Panagiotis A. Eliopoulos¹ & Nicolas G. Emmanouel²
¹Benaki Phytopathological Institute - Department of Entomology and Agr. Zoology, Laboratory of Biological Control, 8 St. Delta Street, GR 145 61 Kifissia, Athens, Greece
²Agricultural University of Athens – Faculty of Plant Production, Laboratory of Agricultural Zoology and Entomology, 75 Iera Odos, 118 55 Athens, Greece

Abstract: The present paper deals with data on the phenology of the scale insects Aspidiotus nerii, Lepidosaphes ulmi and Parlatoria oleae of olive trees as well as on their potential control with releases of the predatory coccinellid Rhyzobius lophanthae and Chilocorus bipustulatus reared in insectaries. Parlatoria oleae was studied in Southern Greece, while A. nerii and L. ulmi were studied in Central Greece during November 2001 – October 2002. The scales L. ulmi, P. oleae and A. nerii completed one, two and three generations during this period respectively. The hymenopterous parasitoid Aphytis mytilaspides and the parasitic mite Hemisarcoptes nr. malus were recorded as natural enemies of L. ulmi. Four natural enemies of A. nerii were found, namely the parasitic wasps Aphytis melinus and Aphytis chilensis and the coccinellid predators R. lophanthae and Ch. bipustulatus, while Aphytis maculicornis, Ch. bipustulatus and R. lophanthae acted against P. oleae. Releases of R. lophanthae and Ch. bipustulatus, were not responsible for the reduction of the population of L. ulmi which was due to the activity of the local populations of H. nr. malus. As for A. nerii and P. oleae, the reduction of infestation level was credited to the activity of R. lophanthae and Ch. bipustulatus respectively.

Key words: Aphytis, Aspidiotus nerii, Chilocorus bipustulatus, Hemisarcoptes, Lepidosaphes ulmi, Parlatoria oleae, phenology, predator, Rhyzobius lophanthae.

Introduction

Among scale insects that infest olive trees, the most common species are the soft scales Saissetia oleae Olivier, Lichtensia viburni Signoret and Philippia folicularis Targioni-Tozzeti, the diaspids Aspidiotus nerii, Parlatoria oleae, Lepidosaphes ulmi and Leucaspis ricae Targioni and the scale Pollinia pollini Costa belonging to the family Asterolecaniidae. Scarcely, olive trees are damaged by various other species of the families Diaspididae (belonging to genus Aonidiella, Epidiaspis, Lepidosaphes, Getulaspis, and Quadraspidiotus), and Pseudococcidae (Katsoyannos, 1992). From the abovementioned species, S. oleae is the most important pest and has often induced great losses of major economic importance in several countries. Following in order of importance, are the species of family Diaspididae, that damage trees by sucking sap and when they infest fruits by causing a decrease in their commercial value due to greenish-yellow marks (A. nerii), or darkly pigmented spots and deformations (P. oleae) (Katsoyannos, 1992).

Several biological control methods have been put into practice in order to control scale insects in olive trees in Greece and other countries as well. Saissetia oleae has been successfully controlled in Greece with inundative releases of the coccinellid predator Exochomus quadripustulatus L. (Katsoyannos, 1976). Additionally, releases of parasitoids have
been made against this scale in Greece (Argyriou & Michelakis, 1975; Argyriou & Katsoyannos, 1976), while the efficacy of its natural enemies has also been recorded in other countries, such as Cyprus (Orphanides, 1988), France (Lenfant & Marro, 1997) and USA (Daane & Caltagirone, 1989). As far as the diaspidids are concerned, parasitoids have been used against *P. oleae* in USA (Huffaker *et al.*, 1994).

The use of massive releases of predators against diaspidids hasn’t been cited in the past. Thus, it was tempting to examine the possibility of implementing releases of the predatory coccinellids *Ch. bipustulatus* and *R. lophanthae* in order to control the scales *A. nerii, P. oleae* and *L. ulmi*. During the study, recording of data on development of the scales also took place, so as to detect possible variations in their phenology as it has been previously recorded in other regions of Greece (Argyriou & Kourmadas, 1980a; Argyriou & Kourmadas, 1980b; Katsoyannos & Statthas, 1995).

**Materials and methods**

Fieldwork was carried out from November 2001 till October 2002 in three olive groves. Phenology and natural enemies of *P. oleae* were studied in an olive grove located in Southern Greece (Peloponnesse, Co. Akrata), while studies on *A. nerii* and *L. ulmi* were conducted in Central Greece (Attica, Co. Laurion and Co. Marathon, respectively). All plots were left untreated during the course of the study.

In order to study phenology, 15 infested trees were sampled on each grove. Two offshoots 20 cm long were cut off each tree and subsequently examined at the laboratory, where recording of the number of scale individuals and their developmental stage took place. Samplings during April-September were done every 15 days, while a 30-day period was inserted between sampling dates from October to March.

Parasitized individuals of scale insects were counted in order to calculate the parasitization level. Moreover, they were maintained in incubators at 25±1°C, RH 65±5% and a photoperiod of LD 16:8 until adult parasitoids emerged.

Predator activity was studied by counting the number of predated individuals found in the samples that were examined at the laboratory and through field assessments. Those assessments comprised beatings of 12 randomly chosen branches, with a rubber-covered stick, over a 1m X 1m cloth screen and they were conducted on each sampling date. The number and species of the predators that fell on the cloth screen were recorded. Consecutively, predators were released to the olive grove. Coccinellid predators were released twice on all three olive groves. The first release occurred on 7/5/2002, with 450 adults of *Ch. bipustulatus* and with 450 adults of *R. lophanthae*. The second one occurred on 4/8/2002, when 280 adults and 100 mature larvae (L3, L4) of each of the abovementioned species were released.

*Rhyzobius lophanthae* and *Ch. bipustulatus* were reared in *A. nerii* infested plants of *Cucurbita maxima*, placed inside cylindrical plexiglass cages (60 cm in length and 30 cm in diameter) in insectaries under controlled conditions (25±1°C, RH 65±5% and a photoperiod of LD 16:8).

**Results and discussion**

As shown in Figures 1, 3 and 5, peaks of crawlers indicate that *A. nerii, P. oleae* and *L. ulmi*, completed 3, 2 and 1 generation in one year, respectively. *Aspidiotus nerii* and *P. oleae* overwintered as previpositing females, while *L. ulmi* overwintered at the egg stage.
Figure 1. Seasonal variation in percentage composition of various developmental stages of Aspidiotus nerii on olive trees and monthly average temperatures; from November 2001 to October 2002, in Co. Lavrion.
Figures 2A and 4A reveal that during the course of the study, living individuals of *A. nerii* and *P. oleae* reduced in numbers, whereas the number of predators counted after the beatings of the branches increased (Figures 2B and 4B). On *L. ulmi*’s case, living individuals decreased, individuals parasitized by the mite *Hemisarcoptes nr. malus* increased (Figure 6A) and the number of predatory coccinellids remained stable (Figure 6B).

As far as hymenopterous parasitoids are concerned, *Aphytis melinus* DeBach and *Aphytis chilensis* Howard were found to act against *A. nerii*. Parasitization level ranged from 4.4% (on 6/12/2001) to 27% (on 19/10/2002) (Figure 2A). *Aphytis maculicornis* Masi parasitized *P. oleae*, with the parasitization level ranging between 3% (on 7/5/2002) and 28% (on 17/7/2002) (Figure 4A). *Lepidosaphes ulmi* was parasitized by *Aphytis mytilaspidis* (Le Baron) and parasitization level varied from 6.1% (on 15/1/2002) to 22.1% (on 21/8/2002). Finally, the parasitic mite *H. nr. malus* was found to infest *L. ulmi*. Level of parasitism by the mite fluctuated from 6.1% (on 19/1/2001) to 73.7% (on 19/10/2002) (Figure 6A).

Previous studies carried out in Greece present some data on the phenology of the scales *A. nerii, P. oleae* and *L. ulmi*. *Aspidiotus nerii* has been reported to complete 3-4 generations per year in central Greece. Predators such as *Ch. bipustulatus, R. lophanthae, Scymnus* sp. and *Chrysoperla carnea* (Stephens), as well as the parasitoid *A. chilensis* have been cited as its natural enemies in the aforementioned region (Argyriou & Kourmadas, 1980a). During the present study, *A. nerii* was found to complete 3 generations in one year. Despite the fact that safe conclusions cannot be elicited from one-year studies on phenology, small differences in the number of generations completed can be attributed to differences in pedoclimatic conditions and host variety. These also apply to differences in the composition of the population of its natural enemies that occur in different regions or periods of time. *Parlatoria oleae* was found to complete 2 generations per year in Central Greece, having several natural enemies, such as the hymenopterous parasitoid *A. maculicornis* and an endoparasitoid belonging to the genus *Aspidiophagus*, as well as the predators *Ch. bipustulatus, R. lophanthae* and an cecidomyiid *Leptodiplosis* sp. (Argyriou & Kourmadas, 1980b). Katsoyannos & Stathas (1995) report one generation per year for *L. ulmi*. In comparison to the present study, small differences occur, namely little variation in the season where different developmental stages occur and the presence of a second natural enemy of the scale, that of the nitidulid predator *Cybocephalus fodori* Endrödy-Younga. Again, these differences can be can be attributed to differences in pedoclimatic conditions and host variety.

Figures 2A, 4A and 6A show the gradual decrease of the infestation of the olive trees by the scale insects, Figures 2A and 4A show the increase of predated scale individuals, while Figure 6A shows the parasitized individuals. Figures 2B, 4B and 6B, show the respective gradual increase of the natural enemies. The decrease of the infestation by *A. nerii* and *P. oleae* (number of living individuals), can be credited to predator activity, especially to that of *R. lophanthae*, due to the simultaneous increase of predator and predated scale populations. The fact that in both cases, *R. lophanthae* increased without a corresponding increase of *Ch. bipustulatus*, can be possibly explained by the significant decrease of *Ch. bipustulatus* populations during summer months in Greece and other countries as well, due to larvae parasitization which can reach up to 90% (Stathas, 2001). As for *L. ulmi*, it seems that coccinellid predators did not highly contribute to its decrease, whereas the parasitic mite *Hemisarcoptes nr. malus* proved to be the major biological control agent (Figures 6A and 6B). The relatively limited activity of *R. lophanthae* against *L. ulmi* can be due to the scale being an unsuitable food source for the predator because of its hardened scale cover. Honda and Luck (1995), report that anatomic characteristics of the mandibles of *R. lophanthae*, restrain it from being able to feed and reproduce sufficiently on *Aonidiella aurantii* (Maskell) because of the latter having a hard scale cover.
The present paper shows that the natural enemies of the scale insects studied, acted efficiently towards their control. After predator releases, *R. lophanthae* built up higher populations compared to *Ch. bipustulatus*. A resultant conclusion of greater efficacy against *A. nerii* and *P. oleae* cannot be derived by this, because predator efficacy is strongly affected by other factors as well, such as prey consumption (Hodek & Honěk, 1996). Handy mass rearing of *R. lophanthae* and *Ch. bipustulatus* on an alternative host (e.g. *A. nerii*) in insectaries, can be combined with a preliminary examination of the potential use of an olive infesting diaspidid as food source, so as to assist on the evaluation of the predators’ effectiveness prior to them being released in the field.

**Figure 2.** Total number of living, predated and parasitized individuals of *Aspidiotus nerii* found on 15 offshoots (A) and predators found after beatings of 12 branches (B), in Co. Lavrion (November 2001 – October 2002)
Figure 3. Seasonal variation in percentage composition of various developmental stages of *Parlatoria oleae* on olive trees and monthly average temperatures, of 12 branches (B), from November 2001 to October 2002, in Co. Akrata.
Figure 4. Total number of living, predated and parasitized individuals of *Parlatoria oleae* found on 15 offshoots (A) and predators found after beatings of 12 branches (B), in Co. Akrata (November 2001 – October 2002)
Figure 5. Seasonal variation in percentage composition of various developmental stages of *Lepidosaphes ulmi* on olive trees and monthly average temperature, from November 2001 to October 2002, in Co. Marathon.
Figure 6. Total number of living and parasitized (by Hymenoptera and Hemisarcoptes nr. malus) individuals of Lepidosaphes ulmi found on 15 offshoots (A) and predators found after beating of 12 branches of olive trees (B), in Co. Marathon (November 2001 – October 2002).

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References


Diseases
Viscum cruciatum: A threat to the olive production in the Moroccan Rif Mountains

Mohamed Besri
Institut Agronomique et Vétérinaire Hassan II, B.P. 6202 Rabat Instituts, m.besri@iav.ac.ma

Abstract: The red leafy Mistletoes, Viscum cruciatum (Viscaceae) is the most important olive parasite in the Rif Mountains at heights between 200 and 2,000 m. This parasitic higher plant is also very common on fruits trees such as almond (Amygdalus communis), prune (Prunus domestica), fig (Ficus carica), walnut (Juglans regia), pears (Pirus malus and P. communis), peach (Prunus persica), grapes (Vitis vinifera), mulberry (Morus alba and Morus nigra), quince (Cydonia vulgaris) and on various wild trees such as Nerium oleander, Populus alba, P. vulgaris.

Infected areas become swollen and produce witches brooms. Infected trees may survive for many years, but they show reduced growth and yield and the portions of the tree beyond the mistletoe often become deformed and die. Viscum cruciatum has a well developed leaves and stems less than 1 or 2 cm in diameter. The high of plants varies from a few cm to a meter or more. Viscum cruciatum produce typical green leaves that can carry on photosynthesis, dioecious flowers and sessile berrylike fruits containing a single seed. They produce haustorial sinkers, rather than roots, however, which grow in branches and stems of trees and absorb water and mineral nutrients. Viscum cruciatum is spread by birds that eat the seed-containing berries and excrete the sticky seeds in the tops of taller on which they like to perch. When mistletoe seed lands on and becomes attached to the bark of a twig or a young olive branch, it germinates and produces a germ tube or a radicle. This grows along the bark until it meets the plant surface, at which point the radicle becomes broad and flattened on the side of the bark. A rootlike haustorium is then produced from the centre of the flattened area of the radicle, which penetrates the bark directly and reaches the phloem and the cambium. From this haustorium develops the system of longitudinal strands and radial sinkers, all of which absorb from the host the nutrients needed for the development of the parasite. The sinkers that reach the cambium of the host become permanently embedded in the wood as the later is laid down each year, but they always retain their connections with the strands in the phloem. After the endophytic system is well established and developed in the host, it produces buds from which shoots develop the following year or several years later. The shoots first appear near the original point of infection, but later more shoots emerge. The parasite removes water, minerals and photosynthates from the host and so starves and kills the portion of the branch lying beyond the point of infection. It also saps the vitality of the branch and, when sufficiently abundant, of the whole tree. Furthermore, it upsets the balance of hormonal substances of the host in the infected area and causes hypertrophy and hyperplasia of the cells with resulting swelling and deformities of various shapes on the branches. This hormonal imbalance also stimulates the normally dormant lateral buds to excessive formation of shoots, forming a dense growth of abnormal appearance.

Key words: Viscum cruciatum

Introduction

More than 2500 species of higher plants are known to live parasitically on other plants. These parasitic plants produce flowers and seeds similar to those produced by the plants they parasitize. They belong to several families (Agrios 1988, Delabraze et Lanier, 1972). No estimate is available of total economic losses in olive from Viscum. However, the disease incidence and severity could be very high in some olive plantations.
Host Range

The red leafy Mistletoes, *Viscum cruciatum* is the most important olive parasite in the Rif Mountains at heights between 200 and 2,000 m. This parasitic higher plant is also very common on fruits trees such as almond (*Amygdalus communis*), prune (*Prunus domestica*), fig (*Ficus carica*), walnut (*Juglans regia*), pears (*Pirus malus* and *P. communis*), peach (*Prunus persica*), grapes (*Vitis vinefera*), mulberry (*Morus alba* and *Morus nigra*), quince (*Cydonia vulgaris*) and on various wild trees such as *Nerium oleander* and *Populus alba*. However, it is unknown if this parasite, as for *V. album*, could be divided in sub species. For *V. album*, at least 3 sub species have been described: *V. album abietis* exclusively on *Abies*, *V. album pini* on *Pinus nigra*, *P. silvestris*, *P. uncinata* and rarely on *Picea excelsa*, *V. album mali* on various leafy fruit (almond, cherry, apple, pear…) and forest (poplar, maple, ash, elm…) trees (Frochot & Lanier, 1980, Frochot & Salle, 1980). Sub species can not be identified on the basis of morphological characters. Therefore, research on olive *V. cruciatum* host specialisation is needed.

The parasite: *Viscum cruciatum*

*V. cruciatum* belongs to the botanical family Viscaceae. This parasitic higher plant has well developed leaves and stems less than 1 or 2 cm in diameter. The height of plants varies from a few cm to a meter or more. *Viscum cruciatum* produces typical green leaves that can carry on photosynthesis, dioecious flowers and sessile berrylike fruits. They produce haustorial sinkers, rather than roots, however, which grow in branches and stems of trees and absorb water and mineral nutrients. The mistletoe fruit is red, smooth and is characterised as a one seeded berry (Kuijit, 1969).

Symptoms

*Viscum cruciatum* is seen in crowns of olive plantation trees. Infected areas become swollen and produce witches brooms. The mistletoe plants sometimes are so numerous that they make up almost half of the green foliage of the tree. Infected trees may survive for many years, but they show reduced growth and the portions of the tree beyond the mistletoe often become deformed and die.

The parasite removes water, minerals and photosynthates from the host and so starves and kills the portion of the branch lying beyond the point of infection. It also saps the vitality of the branch and, when sufficiently abundant, of the whole tree. Furthermore, it upsets the balance of hormonal substances of the host in the infected area and causes hypertrophy and hyperplasia of the cells with resulting swelling and deformities of various shapes on the branches. This hormonal imbalance also stimulates the normally dormant lateral buds to excessive formation of shoots, forming a dense growth of abnormal appearance.

Parasite dispersal

In nature, an agent or mechanism of dispersal is required to take the seed from its fruit and place it in a position where it will germinate (Kuijit, 1969). The dispersal of mistletoe seeds has unexpected facets of great biological interest. It has been known for centuries that mistletoes fruits are eaten by birds, which, by voiding them, disperse the seeds. The association of seeds with bird's excrements may have produced the Germanic name of mistletoe. The correspondence of the Germanic words for manure and mistletoe (“der Mist” “die Mistal” respectively) is too close to be accidental. *Viscum cruciatum* is spread by birds.
that eat the seed-containing berries and excrete the sticky seeds in the tops of taller trees on which they like to perch.

The rapid passage through the digestive tracts is of a great advantage to the mistletoe. It ensures, in return, future crops of berries for the birds off spring. Seeds of *V. cruciatum* spend about 30 min within the bird’s body. A short digestive period prohibits necessarily dispersal over considerable distances.

**Seed germination and parasite penetration**

When mistletoe seed lands on and becomes attached to the bark of a twig or a young olive branch, it germinates and produces a germ tube or a radicle (stage +, Fig 1). This grows along the bark until it meets the plant surface, at which point the radicle becomes broad and flattened on the side of the bark. A rootlike haustorium is then produced from the centre of the flattened area of the radicle (stage a), which penetrates the bark directly and reaches the phloem and the cambium. From this haustorium develops the system of longitudinal strands and radial sinkers, all of which absorb from the host the nutrients needed for the development of the parasite. The sinkers that reach the cambium of the host become permanently embedded in the wood as the later is laid down each year, but they always retain their connections with the strands in the phloem. After the entophytic system is well established and developed in the host, the external parts of the seedling dies (stage b) and it produces buds (stage c) from which shoots develop the following year or several years later. The shoots first appear near the original point of infection, but later more shoots emerge (Agrios, 1988, Fochot & Lanier, 1980, Frochot & Salle, 1980, Kuijit, 1969).

Under what conditions the mistletoe seed does germinate and how it manages to grow toward its host? Host exudates seems not to be required for germination. Many field observations have been made of mistletoe germinating successfully on stones, wires and other inanimate objects (Kuijit, 1969). They do not need any stimulus produced by the host. During the germination phase, *Viscum* seedlings are totally autotrophic. However, the substrate quality is very important for the mistletoe implementation. *Viscum* seeds slide on the smooth periderm of the young branches or on the wet surfaces (Kuijit 1969). Hard branches or trunks will not allow the parasite to penetrate into the host tissues.

In December January, *Viscum* seeds are mature. However, they start germinating only in March or April, after a 3-4 months period of dormancy. Lack of cold will not allow the seed germination. The two first leaves appear only 10-12 months later. It was observed on some olive branches that seedlings could stay at the stage b (death of external part of the seedling) for more than two years, without dying and before the production of the two leaves. Every year, *Viscum* develops an article. The plant age can be calculated by counting the number of articles and then adding one year (Frochot & Salle, 1980).

Light is also very important for germination. *Viscum* did not germinate in dark.

Of the known germination requirements, water is perhaps the most important. *V. cruciatum* do not germinate in darkness. The radicle ability to find the host appears to be due to a negative phototropism and geotropism. The former is dominant under conditions of illumination, forcing the mistletoe to grow to the darkest area of its substrate (Kuijit, 1969).

**Possible role of *Viscum* in virus transmission**

Many Virus like diseases were reported on olives in the Mediterranean area (Kyriakopoulou, 1993,1994, Martelli & Gallitelli 1985, Leitao et al, 1997). Some of them have a wide range of cultivated species (Strawberry Latent Ring Spot Virus = SLRV, Cherry Leaf Roll Virus = CLR, Arabic Mosaic Virus = AMV, Cucumber Mosaic Virus = CMV), others have only
olive as a natural host (Olive Latent Virus= OLV-1, OLV-2, Olive Vein Yellow Associated Virus = OVYaV). The natural spread of these viruses is still unknown. Nematological surveys in the rhizosphere of olive trees hosting nepoviruses have failed to indicate the presence of nematodes vectors (Vanessa, 1995). No animal vector has been yet- found to be involved in the epidemiology of the other olive viruses. The most probable way by which these viruses are spread is the use of infected propagating material, since olive cultivation is not regulated by any form of certification. Olive viruses could also be transmitted from one olive tree to another by *Viscum cruciatum*. Viruses could be introduced in the plant by the parasite haustoria. No report on this mode of transmission is found in the literature.

![Figure 1. Phenological stages of *Viscum* spp (Frochot and Salle, 1980)](image)

**Stage ±:** Germination of the sticky berry, production of a germ tube or radicle  
**Stage a:** Haustorium production  
**Stage b:** Death of the external part of the seed  
**Stage c:** Production of the two first leaves  
**Stage d and next:** Mistletoe growth and leaves production

**Control methods**

The only means of controlling *V. cruciatum* is by physical removal of the parasite. This is done either by pruning infected branches or by cutting and removing the entire infected trees. Many herbicides of the Arloxyacides family (phytohormones) (Delabraze & Lanier, 1972) and others (Dichlorprop (1,5l/hl), glyphosate (10 l/hl), clorame (240 g/hl), Pichlorame+2,4D (1l/hl), Pichlorame+(Dichloprop (1l/hl), Trichloropir (360 g/hl)) were tested. All the herbicides tested were phytotoxic to olive trees.

**Conclusions and prospects**

*V. cruciatum* has been relatively unstudied by plant pathologists. There is a great scientific need for this work. Until now, no study was conducted on the taxonomy, ecology, morphology, histopathology, physiology, general biology and cytology of this parasitic higher plant and on its role on the virus dissemination.

It appears in the surveyed area that no tree is immune from infection by *V. cruciatum*. However, the host specificity is unknown because no attempt was made to inoculate various hosts by the olive parasite. Such investigations are essential to understand the *Viscum* biology and to propose adequate control methods.
References

Change of the copper concentration in olives and leaves of olive trees

S.G. Vleioras, S.N. Pozani, A.C. Traikou, V.K. Papastamou
Department of Quality Control Laboratory, Regional Center of Plant Protection and Quality Control of Magnesia, Ministry of Agriculture, Torouzia-Nikolaidi, Pedion Areos, 38334 Volos, Greece

Abstract: The copper concentration in leaves of olive trees and olives was detected by Atomic Absorption Spectroscopy with flame. Samples of olives and leaves were taken from twenty different fields from the area of responsibility of the Regional Center of Plant Protection and Quality Control of Volos (specifically from the areas of Magnesia and Phthiotida). In those fields copper was sprayed in different forms in order to confront fungal diseases such as olive scab. It has been studied the evolution of copper concentration during the productive period of 2002-2003 taking under consideration the dates of spraying and the frequency of rainfall. It has also been studied the comparison of copper concentration of olives and leaves taken from the same field and the same date. The results showed the fluctuation of copper concentration caused by the sprayings. In all cases copper concentration in leaves was multiple of that in olives.

Key words: olive, olive’s leaves, copper, Atomic Absorption Spectroscopy

Introduction

In Greece olive trees cover the 59% of biologically and integrate cultivated area. In Thessaly olive trees cover an area of 313 hectares. Olive is the first cultivation of trees in Thessaly with a percentage of 23,23% (second after wheat). Copper application is legal. There are different kinds of fungicides based on copper. Samples were taken from fields, where Bouillie Bordelaise, copper oxychloride etc., were added. Copper acts as a fungicide, changing cellular membrane permeability. That causes abnormalities in basic functions, such as formation of proteins. Diseases caused by Cycloconium oleaginum, Pseudomonas syringae and Gloeosporium olivarum can be controlled by copper application. Excessive concentrations can cause reduction of respiration, scalds, growth inhibition and other abnormalities. The above mentioned phenomenon could be aggravated by the environmental conditions such as humidity and low temperatures.

During the period of our experiment (July, August, September of 2002) the height of rain was greater in comparison to previous years (80mm in the region of Lehonia in September). As a result the growth of the above mentioned microorganisms was favored. The Regional Center of Magnesia suggested spraying with copper in late September.

As far as it concerns the final product (olive) the factors that influence it’s quality are:

a) subjective (sensory factors: odor, color, flavor, taste etc),

b) physical (color, etc.)

c) chemical, which influence the safety of the product: i) presence of residues, ii) presence of heavy metals (Cu, As, Cd, Pb), iii) presence of toxins, iv) nutritional factors (e.g. oil concentration), and

d) microbiological.
Copper could have a toxic action for human when excessive quantities are consumed. Consumption of 3-5gr causes gastroenteritis when consumption of 8-12gr causes immediate death.

In view of the general trend and suggestions about the reduction in applied copper’s quantity, our laboratory was occupied with the determination of copper (Cu) in olives and olive tree’s leaves. That project was the succession of a previous one, concerning the presence of copper in samples from the previous cultivating period (2001-2002).

**Materials and methods**

Two kilos of olives was taken from each field, taking care to have some olives from every position of the field and from different heights of the tree. Half a kilo of the sample was taken and was treated in order to remove the seeds. Drying at 105°C to unchangeable weight was following. 1gr of the dried olive was digested with addition of 10ml nitric acid and heating at 45°C overnight. Filtration of the solution was following and collection of the filtrated solution in a volumetric flask of 50ml which was fulfilled with distilled water. The concentration of copper was measured by Atomic Absorption Spectroscopy (AAS, Perkin Elmer 3300) with air-acetylene flame. For the leaves it was followed the same procedure.

**Results and discussion**

The concentrations of copper found in our samples from the areas of Lehonia, Pteleos, and Aghialos are shown in tables 1, 2 and 3 respectively. From the areas of Pteleos and Aghialos we have the concentrations of copper from the leaves of the same trees, same fields.

![Figure 1. Copper concentration in olives from the region of Lehonia](image)

In figure 1 we can see that the concentration of copper is gradually reduced taking its highest price on August the 8th (26.29ppm) and its lowest on November the 26th (6.26ppm). We notice a peak on September the 25th which is probably a natural consequence of the application of copper after the suggestion of the Regional Center of Plant Protection and Quality Control of Magnesia. A period of continuous rainfall forgoing the growth of fungus was favored. In order to avoid that the application of copper was suggested for areas like Lehonia.

In figures 2 and 3 we can compare the copper concentration in olives and leaves. The concentration in leaves is multiple to that in olives. In the sample of Pteleos (figure 2) the concentration of copper was almost the same to that which is naturally found in the plant...
because the application of copper was done a long time before the sampling. In samples from Aghialos (figure 3) copper concentration in olives is much lower to that in leaves where, in some cases reaches the 664,2ppm.

Figure 2: Copper concentration in olives and olive leaves from the region of Pteleos

Figure 3: Copper concentration in olives and olive leaves from the region of Aghialos

In figure 4 we have samples from the area of Magnesia (samples 1-6) and from the area of Phthiotida (samples 7-10). It is obvious that we have a fluctuation in copper concentration independently to the area. We can assume that the concentration depends mainly on the frequency of the fungicide applications and not on the area of sampling.

In figure 5 we have the concentration for samples from the area of Magnesia. As we can see the prices vary from 4.32ppm to 20.51ppm.

Conclusions

Copper concentration was higher in leaves than in olives. In fields where there was no copper application, copper concentration was lower than in fields where copper was added. Height of rainfall during the last cultivating period favored diseases and copper application was suggested. However copper concentration was not in level that could be considered as a potential hazard of human health.
Figure 4. Copper concentration in olives and olive leaves.

Figure 5. Copper concentration in olives from the area of Magnesia.

References