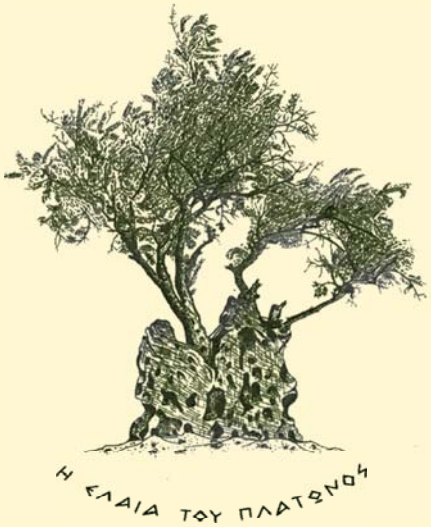
Volume 3, Issue 1, January 2010
ISSN 1791-3691

Hellenic Plant Protection Journal



A semiannual scientific publication of the
BENAKI PHYTOPATHOLOGICAL INSTITUTE

The *Hellenic Plant Protection Journal* (ISSN 1791-3691) is the new scientific publication of the Benaki Phytopathological Institute (BPI) replacing the *Annals of the Benaki Phytopathological Institute* (ISSN 1790-1480) which had been published since 1935.

Starting from January 2008, the Benaki Phytopathological Institute is publishing the *Hellenic Plant Protection Journal* semiannually, in January and July each year, and accepts for publication any work related to plant protection in the Mediterranean region regardless of where it was conducted. All aspects of plant protection referring to plant pathogens, pests, weeds (identification, biology, control), pesticides and relevant environmental issues are topics covered by the journal.

Articles in the form of either a complete research paper or a short communication (including new records) may be submitted. Instructions on how to prepare the manuscript are provided at www.bpi.gr. Manuscripts should be submitted in electronic form either by e-mail at editors@bpi.gr or by post on a disk addressed to the Editorial Board, Benaki Phytopathological Institute, 8 St. Delta str., GR-145 61 Kifissia (Athens), Greece. Only original articles are considered and published after successful completion of a review procedure by two competent referees.

For subscriptions, exchange agreements, back issues and other publications of the Institute contact the Library, Benaki Phytopathological Institute, 8 St. Delta str., GR-145 61 Kifissia (Athens), Greece, e-mail: A.Papanikolopoulou@bpi.gr.

This Journal is indexed by: *CAB Abstracts*-Plant Protection Database, *INIST* (Institute for Scientific and Technical Information) and *SCOPUS*.

The olive tree of Plato in Athens is the emblem of the Benaki Phytopathological Institute

Hellenic Plant Protection Journal **3:** 1-5, 2010

SHORT COMMUNICATION

New threat from an insect borer in urban trees in Greece

F. Karamaouna[1] and D.C. Kontodimas[2]

Summary The insect borer *Phloeosinus bicolor* (Brullé) [=*aubei* (Perris)] det. Thompson (Coleoptera: Curculionidae: Scolytinae) was found on 35-40 year-old Mediterranean Cypress trees in urban areas of Attica, Greece, in June 2008. The scolytid has been previously recorded in Greece with sparse references on its host plant species. The potential risk posed by the dispersal of the borer, which is also a vector of the cypress canker fungus *Seiridium cardinale* (Wag.) Sutt. et Gibs., to urban trees as well as to nurseries and forests located near urban and suburban areas is stressed and control measures are presented.

Additional keywords: *Cupressus sempervirens, Phloeosinus bicolor,* scolytid, *Seiridium cardinale*

Ornamental trees in urban areas in Greece have been threatened by several new or reappearing insect and mite pests during the last 10 years, i.e. *Marchalina hellenica* (Hemiptera: Margarodidae) on pine trees, horse chestnut leafminer *Cameraria ohridella* Descha and Dimic (Lepidoptera: Gracilariidae) on horse chestnut, *Eutetranychus orientalis* (Klein) (Acari: Phytoseiidae) on lemon and orange trees, South American palm borer *Paysandisia archon* Burmeister (Lepidoptera: Castniidae) and red palm weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) on palm trees (2, 21). The scolytid borer *Phloeosinus bicolor* (Brullé) [=*aubei* (Perris)] det. Thompson (Coleoptera: Curculionidae: Scolytinae), which was collected in the urban region of Attica, Greece, in June 2008, should be added to this list. The aforementioned species was identified at the Natural History Museum in London (Richard Thompson, personal communication) in October 2008. A literature review of the biology, damage and control of *P. bicolor* is presented due to the potential risk it imposes to urban trees or by its dispersal to nurseries and forests near urban and suburban areas.

P. bicolor was found on 35-40 year-old Mediterranean Cypress (*Cupressus sempervirens* L.) trees in the suburbs of Glyfada (2 trees) and Kifissia (1 tree). It occurs in central and southern Europe (Austria, Czechoslovakia, Cyprus, France, Greece, Italy, Spain including Canary Islands, Switzerland), Africa (Ethiopia, Kenya, Somaliland, Tunisia), North America (Greenland) and Asia (Caucasus, China, Iran, Israel, Lebanon) (1, 4, 5, 6, 7, 8, 19, 23, 25, 28, 29).

Plant hosts of *P. bicolor* include *Callitris* spp., *Cephalotaxus fortunei, Chamaecyparis lawsoniana, Cupressus funebris, C. glabra, C. lusitanica, C. macrocarpa, C. sempervirens, C. torulosa*, *Juniperus communis, J. excelsa, J. foetidissima, J. macrocarpa, J. phoenicea, J. sabina, Oxycedrus* spp.*, Platycladus orientalis, Sabina chinensis, Sequoia gigantea, Taxodium distichum, Tetraclinis articulata, Thuja occidentalis, T. orientalis, Thujopsis dolobrata* and *Wellingtonia* spp. (8, 9, 17, 25).

P. bicolor shows a very strong specificity for *Cupressus* among ten conifer species of the genera *Abies, Picea*, *Pinus* and *Cupressus*,

Laboratory of Biological Control of Pesticides, Department of Pesticides Control and Phytopharmacy (1) and Laboratory of Agricultural Entomology, Department of Entomology and Agricultural Zoology (2), Benaki Phytopathological Institute, 8 St. Delta str., GR-145-61 Kifissia (Athens), Greece
Corresponding author: f.karamaouna@bpi.gr

mainly resulting from the synergism of the different terpenes contained in these plants (12). Attraction of *P. bicolor* to Cupressaceae (especially *Cupressus, Juniperus, Thuja* and *Callitris* spp.) based on olfactory stimulus exerted by the terpenes and sasquiterpenes of these trees was proved in laboratory trials using the olfactometer (10). Further tests on the olfactive adaptation and allotrophy of *P. bicolor* resulted in no feeding on an inhabitual food-plant unless all the terpene compounds were suppressed, in which case the scolytid fed indiscriminately on wood of any of the test tree species (11). In laboratory bioassays, both sexes of the borer were attracted to essential oils from the bark, xylem and leaves (in descending order) of stressed *Platycladus orientalis* (33).

Thin wind-broken branches or those dying from the cypress canker fungus *Seiridium cardinale* (Wag.) Sutt. et Gibs. are commonly infested during the endemic phase of *P. bicolor* population. Well-developed, vigorous trees after a sudden stress due to drought, fire, root damage or heavy infection by *S. cardinale* are preferred for breeding, whereas suppressed trees are usually not attacked (15, 25, 27). *P. bicolor* has been reported in cypress plantations in Greece, being rather rare (4), but it is known to cause serious damage to nursery trees of Cupressaceae in France and North Africa (18, Richard Thompson, personal communication 2008). The scolytid may also transmit the cypress canker fungus, *S. cardinale*, in *Cupressus, Juniperus* and *Thuja* (7, 16, 27, 32).

P. bicolor overwinters at both larval and adult stage under the bark. In the Mediterranean region adults emerge in late spring or early summer and feed on twigs (6, 25). Mating occurs in a chamber bored by the female. After mating, the pair tunnels under the bark a brood chamber, which is at first a short gallery around the entrance and then it is extended along the grain of the wood. Eggs are laid singly in chambers down both sides of the central gallery. Larvae feed perpendicularly to the central egg gallery and girdle the branch. The species completes 2 and 4 generations/year on Mediterranean Cypress in Tunisia and Israel, respectively, with extensive overlapping of generations and 2-3 generations/year on *Thuja* trees in China (6, 13, 15, 25).

Adults of *P. bicolor* feed on and may girdle small twigs typically several inches back from the tip for additional nourishment and hibernation, causing discolored, dead tips or "flags" hanging on the tree. Under an intensive attack, the number of dead twigs is very high (22). Excavation activity of galleries occurs in early spring and summer and ceases during winter. The gallery system has a central tunnel running parallel to the branch or trunk with numerous side tunnels coming out of it at right angles (centipede-like pattern). Trees subjected to attack weaken physiologically, grow less vigorously and lose their ornamental appearance. Therefore, the beetles contribute to the decline and eventual death of the trees but they are almost always not the primary cause (13, 22).

Natural enemies of *P. bicolor* comprise two predatory beetles, several parasitoid wasps and a parasitic mite primarily recorded on Mediterranean Cypress in Israel (25, 26). The predators *Aulonium ruficorne* (Olivier) (Coleoptera: Colytiidae) and *Laemophloeus* spp. (Coleoptera: Cucujidae) were found on material infested by *P. bicolor* and another *Phloeosinus* species in Israel (25). The parasitic mite *Pyemotes tritici* Lagrèze-Fossat and Montane (Acarina: Pyemotidae) achieved 67.3% parasitism 30 days after its release under natural conditions in China (35). The complex of the hymenopteran parasitoids consists of 6 pteromalids, 2 braconids, 1 eurytomid, 1 eulophid, 1 eupelmid and 1 bethylid: *Theocolax phloeosini* sp.nov. (Hym.: Pteromalidae) on *Juniperus chinensis* in China (34); *Metacolus unifasciatus* Forster (Hym.: Pteromalidae), *Cerocephala eccoptogastri* Masi (Hym.: Pteromalidae), *Rhaphitelus maculatus* Walker (Hym.: Pteromalidae), *Heydenia pretiosa* Forster (Hym.: Pteromalidae), all on Mediterranean Cypress in Israel (25, 26); *Hecabolus sulcatus* Curtis (Hym.: Braconidae), *Dendrosoter protuberans* (Nees) (Hym.: Braconidae) and the facultatively parasitoid *Eurytoma morio* Boheman

(Hym.: Eurytomidae) associated mainly with *M. unifasciatus* on Mediterranean Cypress in Israel (25, 26); *Entedon ergias* Walker (Hym.: Eulophidae), *Calosota aestivalis* Curtis (Hym.: Eupelmidae) and *Cephalonomia hypobori* Kieffer (Hym: Bethylidae) on living Cupressaceae in Europe and the Near East (20). *M. unifasciatus* and *D. protuberans* were commonly found in most samplings throughout the year in Israel (25, 26).

Control of *P. bicolor* is mainly by improving growth conditions of trees to retain vigor and reduce the threat of future attacks. Wind damage, physical injuries and soil compaction make trees attractive to bark beetles in general (e.g. trees along roadways and parking areas). Infested branches or heavy infested trees should be removed in order to eradicate developing larvae. Corrective pruning or tree removal should be performed in the winter when flight activity of *P. bicolor* is minimal and the plant material should be disposed away from healthy trees (13, 31).

Bait wood/trap-logs are recommended in Israel and China for suppression of the scolytid population in cypress and *Thuja,* respectively, by installing them before the first emergence and then removing and destroying them after infestation (15, 24). Cypress logs baring branches (not pruned) should be used for trapping as they have been proved more effective (significantly higher gallery density, total gallery length and cumulative density of penetration holes) compared to the pruned ones, presumably due to a slower rate of water loss (24).

Unless trees are monitored regularly so that borer attack can be detected early, any chemical spray applied after the penetration of the bark by the borer is likely to be too late and ineffective. Recent transplants and high-value trees, stressed for any reason or located near infested trees, could be protected from bark beetles with topical applications of registered insecticides. These treatments should target the adults by spraying the bark so that the beetles are killed when they land on trees and attempt to bore into the bark to lay eggs (30). Applications should be made at least on the trunk and major limbs of the trees prior to the first flights of the bark beetle in early spring and maintain coverage during the period of adult activity (June-July) (13, 31). Trials on sections of logs with pyrethrins (bifenthrin, permethrin) or carbaryl were effective in preventing successful attacks and colonization of Arizona Cypress (*Cupressus arizonica*) and one-seed juniper (*Juniperus monosperma*) by two other species of *Phloeosinus* (14). However, there are no registered insecticides for bark beetles of Cupressaceae in Greece (3).

Literature cited

1. Alonso-Zarazaga, M.A. 2002. Lista preliminar de los Coleoptera Curculionoidea del área Ibero-Balear, con descripción de *Melicius* gen. nov. y nuevas citas. *Boletín S.E.A.,* 31: 9-33.
2. Anagnou-Veroniki, M., Papaioannou-Souliotis, P., Karanastasi, E. and Giannopolitis, C.N. 2008. New records of plant pests and weeds in Greece, 1990-2007. *Hellenic Plant Protection Journal,* 1: 55-78.
3. Anonymous, 2009. Authorized Plant Protection Products Data Base of the Hellenic Ministry of Rural Development and Food. Available at http://www.minagric.gr/syspest/
4. Avtzis, D.N. and Avtzis, N.D. 2001. Control of the most dangerous insects of Greek forests and plantations. In Liebhold, A.M., McManus, M.L., Otvos, I.S., Fosbroke, S.L.C (eds). *Proceedings: Integrated management and dynamics of forest defoliating insects*. 15-19 August, 1999, Victoria, BC, Gen. Tech. Rep. NE-277, Newtown Square, PA, US. Department of Agriculture, Forest Service, Northeastern Research Station, p. 1-5.
5. Balachowsky, A. 1949. *Faune de France no 50. Coléoptères Scolytides*. Librairie de la Faculté des Sciences, Paris, 320 p.
6. Bel Habib, R., Ben Jamâa, M.L. and Nouira, S. 2007. Biological characteristics of the cypress bark beetle *Phloeosinus aubei* in the Kessra forest, center of Tunisia. *Tunisian Journal of Plant Protection*, 2: 99-108.
7. Bovigny, P.Y. 1994. Dieback in Thujas. *Revue Horticole Suisse*, 67: 136-139.
8. Bright, D.E. and Skidmore, R.E. 1997. *A catalogue of Scolytidae and Platypodidae (Coleoptera), Supplement 1 (1990-1994)*. Ottawa, Canada: NRC Research Press, 368 p.
9. Bright, D.E. and Skidmore, R.E. 2002. *A catalogue of Scolytidae and Platypodidae (Coleoptera), Sup-*

plement 2 (1995–1999). Ottawa, Canada: NRC Research Press, 523 p.

10. Chararas, C. 1976. Study of the primary and secondary attraction in *Phloeosinus* (Coleoptera: Scolytidae). *Comptes Renus Hebdomadaires des Seances de l'Academie des Sciences, D.,* 282: 1793-1796.
11. Chararas, C., Ducauze, C. and Revolon, C. 1978. Olfactive adaptation and allotrophy in certain scolytids exclusive to conifers. *Comptes Renus Hebdomadaires des Seances de l'Academie des Sciences, D.*, 287: 285-288.
12. Chararas, C., Revolon, C., Feinberg, M. and Ducauze, C. 1982. Preference of certain Scolytidae for different conifers. A statistical approach. *Journal of Chemical Ecology*, 8: 1093-1109.
13. Cranshaw, W., Leatherman, D. and Kondratieff, B. 1994. *Insects that feed on Colorado trees and shrubs*. *Bulletin 506A*. Colorado University Cooperative Extension, 176 p.
14. DeGomez, T., Hayes, C.J., Anhold, J.A. McMillin, J.D. and Clancy, K.M. 2007. Evaluation of insecticides for protecting Arisona cypress (*Cupressus arizonica*) and one-seed juniper (*Juniperus monosperma*) from attack by *Phloeosinus* bark beetles. *Arboriculture and Urban Forestry*, 33: 162-167.
15. Dong CunYu. 1997. Observations of the biological characteristics and control of *Phloeosinus aubei* Perris. *Journal of Jiangsu Forestry Science and Technology*, 24: 34-35.
16. Gimenez Verdu, I. 1991. Notes of cypress canker [*Seiridium cardinale* (Wag.) Sutt. et Gibs.]. *Boletin de Sanidad Vegetal Plagas*, 17: 423-439.
17. Gruene, S. 1979. *Handbuch zur Bestimmung der Europaischen Borkenkaefer Hanover*, 182 p. (in German and English).
18. Hastings, C. 2002. Scolytids in nurseries and green spaces. *PHM Revue Horticole*, 441: 27-31.
19. Hrubik, P. 1974. A summary of present knowledge of the occurrence and injurious activity of animals and exotic tree species. *Lensnictvi,* 20: 939-950.
20. Kenis, M., Wermelinger, B. and Grégoire, J.C. 2004. Research on parasitoids and predators of Scolytidae in living trees in Europe - a review. In Lieutier, F., Day, K.R., Battisti, A., Grégoire, J.C. and Evans, H.F. (eds). *Bark and wood boring insects in living trees in Europe, a Synthesis*. Dordrecht: Kluwer Academic Publishers, p. 237-290.
21. Kontodimas, D. 2006. New pests of palm trees in the Mediterranean area. In *Proceedings of the Meeting "The pest of the palm trees Rhynchophorus ferrugineus (Coleoptera: Curculionidae). Present situation and proposals for its control"*, Crete, Greece, 31 May 2006, p. 8-12.
22. Markovic, C. and Stojanovic, A., 2004. *Phloeosinus thujae* (Perris) and *Ph. aubei* (Perris) (Coleoptera: Scolytidae): cause of shoot death in juniper, arbovitae and cypress. *Biljni Lekar Plant Doctor*, 32: 352-354 (Abstract).
23. Masutti, L. 1995. Italian bark and ambrosia beetles (Coleoptera: Scolytidae). Available at http://www.unipd.it/esterni/wwwentom/checklist.htm
24. Mendel, Z. 1983. Effects of pruned and unpruned trap-logs of cypress on infestation and development of two *Phloeosinus* species. *Phytoparasitica*, 11: 83-88.
25. Mendel, Z. 1984. Life history of *Phloeosinus armatus* Reiter and *P. aubei* (Perris) (Coleoptera: Scolytidae) in Israel. *Phytoparasitica*, 12: 89-97.
26. Mendel, Z. and Halperin, J. 1981. Parasites of bark beetles (Col.: Scolytidae) on pine and cypress in Israel. *Entomophaga,* 26: 375-379.
27. Mendel, Z., Golan, Y., Madar, Z. and Solel, Z. 1983. Insect pests and diseases of cypress in Israel. *La Yaaran*, 33: 37-41.
28. Muñoz, C. and Ruperez, A. 1980 Causas de la desaparición de los cipreses en España. *Boletin Servicio de Defensa contra Plagas e Inspeccion Fytopatologica*, 6: 95-104.
29. Perny, B. 1995. Bark beetles as pests of exotic conifers. *Forstschutz Aktuell*, 16: 18-19 (Abstract).
30. Schalau, J. 2003. *Cypress Bark Beetles*. University of Arizona, College of Agriculture and Life Sciences, Tucson, Arizona, 2 p.
31. Sutherland, C. 2006. Bark Beetles. Cooperative Extension Service, College of Agriculture and Home Economics, New Mexico State University, 5 p.
32. Tiberi, R. and Battisti, A. 1998. Relationships between phytophagous insects and cypress canker. *Annali Accademia Italiana di Scienze Forestali*, 47: 35-44.
33. Yang Xue Yun, Zhao Bo Guang, Zho Zhen Yi and Li Yang. 2006. Determination of active components from stressed *Platycladus orientalis* as potential pheromones of *Phloeosinus aubei* Perris. *Journal of Northeast Forestry University,* 34: 10-12 (Abstract).
34. Yang, Y.Q. 1989. One new species and other pteromalids parasitizing bark-beetles in Shaanxi, China (Hymenoptera, Chalcidoidea, Pteromalidae). *Entomotaxonomia*, 11: 97-103.
35. Zhang Zuo-shuang, Xiong De-ping and Cheng Wei, 2008. Control of stem borers by a parasitoid, *Pyemotes tritici* Lagrèze-Fossat and Montane. *Chinese Journal of Biological Control*, 24: 1-6 (Abstract).

Received: 16 February 2009; Accepted: 23 April 2009

ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Νέα καταγραφή ξυλοφάγου εντόμου στο αστικό πράσινο στην Ελλάδα

Φ. Καραμαούνα και Δ.Χ. Κοντοδήμας

Περίληψη Το αστικό πράσινο στην Ελλάδα έχει δεχθεί τα τελευταία χρόνια πολλές απειλές από νέους ή επανεμφανιζόμενους εχθρούς. Τον Ιούνιο του 2008 καταγράφηκαν προσβολές από το ξυλοφάγο έντομο *Phloeosinus bicolor* (Brullé) [=*aubei* (Perris)] det. Thompson (Coleoptera: Curculionidae: Scolytinae) σε τρία κυπαρίσσια (*Cupressus sempervirens*, L.) ηλικίας 35-40 ετών, σε ιδιωτικούς κήπους σε δύο αστικές περιοχές της Αττικής (Γλυφάδα και Κηφισιά). Η παρουσία των εντόμου αυτού έχει καταγραφεί στο παρελθόν στην Ελλάδα με περιορισμένες αναφορές στους ξενιστές του και στην πιθανή επικινδυνότητά του. Επισημαίνεται ο κίνδυνος από την εξάπλωση του *P. bicolor*, το οποίο είναι φορέας του μύκητα *Seiridium cardinale* (Wag.) Sutt. et Gibs. (καρκίνος του κυπαρισσιού), στο αστικό πράσινο, σε φυτώρια κυπαρισσοειδών (Cupressaceae) ή περιαστικά δάση και προτείνονται μέτρα αντιμετώπισης.

Hellenic Plant Protection Journal **3:** 1-5, 2010

Hellenic Plant Protection Journal **3:** 7-8, 2010

SHORT COMMUNICATION

First record of *Physokermes inopinatus* Danzig & Kozár 1973 (Hemiptera: Coccidae) in Greece

G.J. Stathas[1] and F. Kozár[2]

Summary The scale insect *Physokermes inopinatus* Danzig & Kozár (Hemiptera: Coccidae) (Hungarian spruce scale), collected from *Abies cephalonica* (a new host plant for this species) on Taygetos mountain (Peloponnese, Southern Greece), is reported for the first time in Greece. Some data on its phenology are also provided.

Additional keywords: *Abies cephalonica,* Hungarian spruce scale, soft scales

Scale insects infesting fir (*Abies* spp.) and spruce (*Picea* spp.) trees have previously been reported in Greece and have been studied mainly due to their excreting honeydews on which bees feed (5,6). These species are *Physokermes hemicryphus* (Dalman), *P. piceae* (Schrank) and *Eulecanium sericeum* (Lindinger), found on *Abies cephalonica* Loud. and *A. borisii-regis* Mattf. (Pinaceae). Moreover, the coccid *Nemolecanium graniformis* (Wünn) was recorded on *A. cephalonica* in Athens (7) and the diaspidid *Dynaspidiotus abietis* (Schrank) on the same host plant on Taygetos mountain (Peloponnese, Southern Greece) (8).

The scale insect *P. inopinatus* Danzig & Kozár (Hemiptera: Coccidae) (Hungarian spruce scale) was found by the first author to infest *A. cephalonica* growing in a forest area of Taygetos mountain, north-east of the Dyrachi County (37º12΄18΄΄ N, 22º13΄31΄΄E) in an altitude of 1080 m a.s.l., during the period June 2004-July 2008. Identification of the scale species was made by the second author. Female adults and crawlers of the scale were deposited in the collection of the Plant Protection Institute, Hungarian Academy of Sciences, Budapest.

P. inopinatus has so far been recorded only in Hungary, Austria and Ukraine, where it infests *Picea abies* (L.) Karst., *P. glauca* (Moench) Voss and *P. pungens* (Engelm) (1, 2, 4). On the above host plants, the scale is often present in high populations causing extensive damage. *P. inopinatus* completes one generation per year (2). Post-reproductive female is kidney-shaped with a dorsal carina, shiny-brown in color and 5-8 mm in diameter. Detailed description of the female is presented by Kosztarab and Kozár (2). In Hungary, the parasitoids *Aphycoides clavellatus* (Dalman) (Hymenoptera: Encyrtidae), *Microterys* sp. (Thomson) (Hymenoptera: Encyrtidae), *Coccophagus lycimnia* (Walker) (Hymenoptera: Aphelinidae), *C. scutellaris* (Dalman) (Hymenoptera: Aphelinidae) and the predators *Anthribus nebulosus* (Forster) (Coleoptera: Curculionidae), *Chilocorus bipustulatus* L. (Coleoptera: Coccinellidae), *Scymnus* sp. (Kugelann.) (Coleoptera: Coccinellidae) and *Forficula auricularia* L. (Dermaptera: Forficulidae) are reported as natural enemies of *P. inopinatus* (3).

Observations of *P. inopinatus* phenology on the infested trees (on Taygetos mountain)

[1] Highest Technological Educational Institute of Kalamata, School of Agricultural Technology, Department of Crop Production, Laboratory of Agricultural Entomology and Zoology, Antikalamos Messinias, GR-24 100 Kalamata, Greece
[2] Plant Protection Institute, Hungarian Academy of Sciences, Department of Zoology, P.O. Box 102, Budapest. J-1525 Hungary
Corresponding author: georgestathas@hotmail.com

were carried out between June 2004 and July 2008. Scale settles at the base of young shoots and needles sucking juice of fir trees. It is oviparous and biparental. It completes one generation per year and overwinters as 2nd instar nymph. Pre-ovipositing females appear from the beginning of May until the beginning of July. Egg-laying occurs from mid-June to early August. Eggs hatch from mid-July to mid-August and settled 1st instar nymphs occur from mid-August to mid-September. A similar phenology has been reported in Hungary (2). Small differences in the time of appearance of the developmental stages could be attributed to differences in host plants and climatic conditions.

In Greece, larvae and adults of *Chilocorus bipustulatus* (L.) were observed to prey on the scales.

P. inopinatus infestations observed on *A. cephalonica* growing on Taygetos mountain did not cause damage to the plants. Nevertheless, it would be useful to investigate the presence and development of this scale on *Picea abies*, *P. glauca* and other species of *Abies* and *Picea* growing in Greece, on which the scale could potentially develop high populations (2).

Scale insect studies of Dr. F. Kozár were supported by grants of EU (Synthesis, GB-TAF-1318, FR-TAF-1319, ES-TAF-2590) and OTKA (Hungarian National Science Foundation, No. K 75889).

Literature Cited

1. Ben-Dov, Y. 2006. Scalenet (available on online). http://www.sel.barc.usda.gov/scalenet/scalenet.htm
2. Kosztarab, M. and Kozár, F. 1988. Scale insects of Central Europe. Akadémiai Kiadó, Budapest, 456 pp.
3. Kozár, F. and Nagy B. 1998. New data to the distribution of some Palaearctic scale insects (Homoptera: Coccoidea). *Folia Entomologica Hungarica*, 59: 53-56.
4. Kozár, F. and Sugonjaev, E.S. 1979. Contribution to the knowledge of parasites of coccids (Homoptera: Coccoidea). *Folia Entomologica Hungarica*, 32: 234-236. (In Hungarian with English Summary).
5. Santas, A.L. 1983. Insects producing honeydew exploited by bees in Greece. *Apidologie*, 14(2): 93-103.
6. Santas, A.L. 1991. New species of honeydew producing insects in Greece. In *Proceedings of the 3rd Panhellenic Entomological Congress*, Thessaloniki, 9-11 October, pp. 174-177. Hellenic Entomological Society.
7. Stathas, G.J. 2001. The scale *Nemolecanium graniformis* (Wünn) (Homoptera: Coccidae) in Greece. *Anzeiger für Schädlingskunde (Journal of Pest Science)*, 74: 57-59.
8. Stathas, G.J. 2008. The scale insect *Dynaspidiotus abietis* (Schrank) on *Abies cephalonica* (Pinaceae). *Entomologia Hellenica*, 17: 28-33.

Received: 11 August 2009; Accepted: 28 August 2009

ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή του *Physokermes inopinatus* Danzig & Kozár (Hemiptera: Coccidae) στην Ελλάδα

Γ.Ι. Σταθάς και F. Kozár

Περίληψη Στην παρούσα εργασία καταγράφεται για πρώτη φορά στην Ελλάδα η παρουσία του κοκκοειδούς εντόμου *Physokermes inopinatus* Danzig & Kozár (Hemiptera: Coccidae) (Hungarian spruce scale) σε έλατα του είδους *Abies cephalonica* (ενός νέου ξενιστή του εντόμου), στο όρος Ταϋγετος (Πελοπόννησος, Νότια Ελλάδα). Δίδονται επίσης ορισμένα στοιχεία του τρόπου προσβολής και της οικολογίας του κοκκοειδούς.

Hellenic Plant Protection Journal **3:** 7-8, 2010

Hellenic Plant Protection Journal **3:** 9-16, 2010

Determination of operator exposure levels to pesticides during greenhouse applications with new type multi-nozzle equipment and the use of two different protective coverall types

A.N. Tsakirakis[1], K.M. Kasiotis[1], P. Anastasiadou[1] and K. Machera[1]

Summary In the present study, the operator exposure levels during fungicide applications in greenhouse-grown pepper with a novel application tool were determined. For the monitoring of dermal exposure, the whole body dosimetry method was applied. The inhalation exposure was measured by means of personal air pump. Ten applications were carried out with Novi-F (4 nozzles) in Tympaki, Heraklion Prefecture, Crete, Greece and the application duration ranged from 39-77 min. A fully validated GC-ECD analytical method was applied for the determination of iprodione in/on the used personal protection equipment and quality control samples. The recovery ranged between 79 and 98% and the corresponding RSDs were <4.2%. Dermal exposure, both potential (PDE) and actual (ADE), was measured with two different types of outer coveralls (Hydrofoil® and Cotton) as dosimeters. From the results of the present work it is worth mentioning that the ADE, which reflects the actual dermal exposure when Hydrofoil® coverall was used, is drastically reduced compared to the respective values for the cotton coveralls. However, operator exposure levels using Novi-F are much higher than the respective levels determined with conventional spray gun application.

Additional Keywords: greenhouse, operator exposure, pesticides, protective coveralls

Introduction

Assessment of operator exposure during field applications of plant protection products is one of the most critical aspects for the operator safety (3, 6 ,7). Greenhouse applications are considered to be high exposure scenarios for the operators. In Greece, greenhouse applications are usually carried out with hand-held application techniques that involve either knapsack sprayers or spray guns connected via a hose to the pump and a spray tank. Operator exposure trials have been performed in the past addressing the two aforementioned application techniques and different personal protection equipment (PPE) types (2, 5). In the present study, a novel application tool, called Novi-F, has been studied as an alternative to the traditional application equipment. The Novi-F tool is a T-shaped spray gun device having four nozzles (i.e. two pairs placed at each end) and allowing the operator to hold it horizontally underarm with the two nozzle pairs oriented towards the crop at the height of the operator's shoulder and waist, respectively. This new type of equipment was pilot-tested as an alternative to the conventional spray guns used normally by the operators in the greenhouses of the Tympaki region (Heraklion Prefecture, Crete, Greece), as it was introduced in the market as more convenient and less time consuming than the traditional knapsack sprayers or the hand-held spray guns.

In a previous greenhouse study, the performance of two different protective coverall types was tested, compared and evaluated. The performance evaluation was related to penetration resistance properties and the overall degree of provided protection for the operator (2, 4). In the present study, the

[1] Laboratory of Pesticides Toxicology, Benaki Phytopathological Institute, 8 St. Delta str., GR-145 61 Kifissia (Athens), Greece
Corresponding author: k.machera@bpi.gr

same two protection coverall types were used in order to collect more data for their performance in high exposure scenarios, where it was anticipated that a significant difference between these two types could be apparent.

With the above considerations, the scope and the objectives of the present study were: a) the determination of the operator exposure levels using the newly introduced Novi-F tool, b) the comparison of the present trial results with the respective results from previous work using conventional spray gun application, and c) the evaluation of coverall performance for the two different coverall types. For the aims of this study the SC formulation of iprodione (Rovral 50 SC) was used, which could provide surrogate data for risk assessments with similar use scenarios.

Materials and Methods

The operator exposure measurements were carried out with the whole body dosimetry method according to the OECD Guidance Document (7). Ten applications were carried out in greenhouse-grown pepper in Crete following good agricultural practices. The application parameters are given in Table 1. The spraying application technique involved the use of a four-nozzle, T-shaped hand-held spraying equipment similar to a lance, called Novi-F. The potential dermal exposure (PDE), the actual dermal exposure (ADE) and the hand-, head- and inhalation exposure of operators were determined. The fungicide used was a SC formulation (Rovral 50 SC) containing 50% w/v iprodione as active substance (a.s.).

Dermal exposure, both potential and actual, was measured with two different types of outer coveralls as dosimeters (five applications per type). The inner coveralls and the rest of the personal protective equipment were of the same type in all ten applications. Both types of the protective coveralls used were made of woven, permeable fabrics that had shown satisfactory results in laboratory permeability tests with the pipette test (ISO 22608:2004). The first coverall tested (Type A), was made of 50/50%, cotton/polyester (Twill, 215 g/m^2 Hydrofoil®) treated with a water repellent finish attached at the nano (sub-micron) level to the fibres. The second coverall (Type B) was made of 100% cotton (twill, 287g/m^2). None of the operators were involved in mixing and loading of the formulation or in any other activities in the field. The operators were asked to follow their normal routine and application practices.

Field Part

Before each application, the operators were dressed in the inner and outer whole body dosimeters, which were worn for the duration of the monitoring period. The inner dosimeters consisted of a long sleeved shirt and a pair of long johns (100% cotton). The active substance (a.s.) deposited in/on the inner clothing represents the actual dermal exposure for the upper body (shirt) and lower body (long pants). The residues of the a.s. retained by each part (jacket and trousers) of the outer coverall were also determined. The actual head exposure was estimated from the residue of the a.s. detected on baseball cap, using an extrapolation factor of 2 to account for the whole head surface. Nitrile gloves were worn over inner cotton gloves by all operators as dosimeters for hand exposure. Actual hand exposure corresponded to the amount of the a.s. found on the inner gloves, while potential exposure for the hands was estimated from the total amount of the a.s. found on both inner and outer gloves. The footwear was assumed to provide complete protection; therefore, exposure was not monitored or estimated for this area.

Personal air sampling pumps with XAD-2 filter tubes were used to monitor inhalation exposure. The XAD-2 filter tubes were placed in the breathing zone of the operators and the airflow was calibrated at 2.0 l/min. Inhalation exposure values were derived from the residues found on XAD-2 tubes multiplied by a factor of 29/2 (assum-

Table 1. Application conditions and parameters for the greenhouse trials using Novi-F sprayer.

Application number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Operator/Trial Code	**A1**	**A2**	**A3**	**A4**	**A5**	**B1**	**B2**	**B3**	**B4**	**B5**
Monitoring Method/Coveralls	**Whole Body Dosimetry / Outer Hydrofoil, inner cotton**					**Whole Body Dosimetry / Outer Cotton, inner cotton**				
Operator height (cm)	185	170	178	175	168	178	168	180	176	188
Operator weight (kg)	75	85	82	82	68	74	68	120	65	115
Age (years)	55	60	43	49	32	29	32	41	32	40
Experience (years)	10	3	2	20	10	1	10	20	2	15
Nominal FST concentration (g/l)	750	750	750	750	750	750	750	750	750	750
Crop height (cm)	160-200	190	100	165	170	140	170	150-200	200	170-205
Row distance (cm)	110-120	120	200	150	150	120	150	110-180	140	110-125
Application Duration (min)	39	65	39	49	53	64	53	68	77	65
Application Area (ha)	0.296	0.315	0.500	0.374	0.357	0.401	0.305	0.309	0.396	0.275
FST volume /time (l/h)	323	256	415	331	442	295	362	333	304	267
FST volume/area (l/ha)	710	880	540	722	1093	785	1051	1221	984	1053
Temperature	19-26	16-22	26-27	28-31	28-30	26-29	24-29	24-29	22-26	25-31
Relative Humidity (%)	52-60	56-75	47-51	46-59	38-40	51-58	28-43	48-58	49-67	42-63
Cross contamination	-	-	cap	-	-	-	-	-	-	-

	Parameters common for all trial codes
Operator	Male, right-handed
Location	Tympaki, Heraklion Prefecture, Crete, Greece
Crop	Greenhouse-grown pepper
Formulation	Rovral 50 SC
Active substance	Iprodione 50% w/v
Formulation Dilution (ml/100 l)	150
Air sampler flow rate (l/min)	2
Application scenario	Full cover spraying with Novi-F (4 nozzles)
Nozzle(s) type	Novi-F
Nozzle distance from ground (cm)	50-180
Mean nozzle flow (l/min)	5.5
Hand exposure monitoring	Inner cotton gloves / outer nitrile gloves
Head exposure monitoring	Baseball cap
Inhalation exposure monitoring	XAD-2

ing an inhalation rate of 29 l/min, divided by the air sampler's pumping rate of 2 l/min).

Following application, the dosimeters were removed, wrapped in aluminum foil, labeled and packed in individual plastic bags. Field samples were placed in a cool box and transferred to a freezer below –18°C within 2 hours. The outer nitrile gloves were extracted directly in the field, since it was known from previous studies that recovery of the a.s. decreases over time in nitrile gloves (1). The aforementioned extraction was done by rinsing the gloves in 400 ml of hexane contained in a polyethylene bag and shak-

ing the bag 50 times.

Quality control samples were prepared with fortification of matrices on each application day. Thus, three samples of different types of dosimeters (outer, inner fabric, inner and outer gloves, caps and XAD-2 tubes) were fortified at two fortification rates. The fortification solution was taken directly from the nozzle of the tank in the field. The field fortified samples were exposed to the environmental conditions for the duration time of the application. During this period the XAD-2 tubes were left attached to air pumps operating at an airflow rate of 2.0 l/min. Additionally, field blank samples for each dosimeter type were treated as the fortified samples.

For the accurate determination of the actual spray solution concentration, three volumetric flasks of 10 ml each were fortified in the field concurrently with the dosimeter fortification. The flask fortification volume was 1 ml taken from the spray solution used for the application.

Analytical Part

A fully validated, in-house analytical method was applied for the determination of iprodione in the different types of dosimeters and in quality control samples. For the analysis of all field and quality control samples used in the study, the principal steps of the method included sample extraction with *n*-hexane, extract concentration, filtration and Gas Chromatography determination using Electron Capture Detector (GC-ECD) and pendimethalin as internal standard.

The *n*-hexane solvent, P-R grade, was purchased from Merck (Darmstadt, Germany). Iprodione (99.3 % pure) and pendimethalin (98.4% pure) analytical standards were purchased from Sigma-Aldrich (Steinheim, Germany). The commercial iprodione SC formulation (Rovral 50 SC, 500 g a.s. iprodione/l) was purchased from Basf Agro Hellas (Sindos, Greece). For the filtration of extracts, PTFE (polytetrafluoroethene) 0.45 μm syringe filters (Acrodisc, p/n 4219T, PALL Dreieich, Germany) were used. Stock solutions of iprodione analytical standard (1000 μg/ml) were prepared in P-R grade *n*-hexane and stored at -18°C. Working solutions of the analytical standard (100, 10 and 1 μg/ml) were prepared by further dilution of stock solution in *n*-hexane and stored at -18°C.

Iprodione residues were extracted from the different types of dosimeters with *n*-hexane in high density polyethylene containers on an overhead shaker for 45 min at 200 rpm. The extraction volumes used were 3.5 l for outer garment parts, 1.5 l for inner garment parts and 600 ml for caps and for inner gloves. The extraction of nitrile gloves was performed in the field using 400 ml *n*-hexane, as described above. The extraction of the XAD-2 tubes (extraction volume 10 ml) was carried out in 30 ml screw cap vials after disassembling of the tube and transferring the absorbent layer material to the extraction vial, and placed on the overhead shaker for 45 min. The actual concentration of iprodione in the field spray solution (FST) was determined from the field fortified flasks after solvent (water) evaporation to dryness and re-dissolving of the dry residue in *n*-hexane.

Depending on the initially measured iprodione concentration in the analyzed sample extracts, the extract was either concentrated in a rotary evaporator or diluted with hexane to obtain a concentration into the range of the calibration curve (0.1-0.9 μg/ml). The internal standard pendimethalin was added at 0.04 μg/ml to the final solution, which was filtered prior to GC injection (injection volume 1 μl).

Gas Chromatography

The chromatographic determination was performed with an Agilent GC 6890N instrument (Thermo-Finnigan Italia, Rodano, Italy), equipped with a split/splitless injector (splitless mode), with an ECD and autosampler (Gerstel MPS2-Twister) 2000). Chromatography parameters are presented in Table 2.

Analytical Method Validation

The analytical method validation included study of linearity, accuracy, precision,

Table 2. Chromatography parameters.

Gas Chromatograph	Agilent 6890N
Auto sampler	Gerstel MPS2-twister
Inlet	Split/Splitless injector (splitless mode)
Detector	ECD
Column	HP-5 MS Agilent (PN 19091S-433), length 30m, ID 0.25mm, film thick. 0.25 μm
Temperatures:	
Inlet	210°C
Detector	300°C
Column	70°C, 1 min isothermal 70°C →280°C, 35°C/min, 2 min isothermal
Carrier gas: Helium	1.7 ml/min
Make up gas: Nitrogen	25 ml/min
Retention Times (Rt)	Pendimethaline 7.038 min Iprodione 7.688 min

specificity and limits of detection and quantification. The limit of quantification (LOQ) for the method corresponded to the lowest fortification level giving acceptable recovery (70-120%). Thus the LOQ was 10 μg for the outer dosimeters and caps, 1 μg for inner dosimeters and cotton gloves, 50 μg for nitrile gloves and 0.1 μg for XAD tubes. The method LODs was 1/3 of the respective LOQs. The fortification rates included five replicates at LOQ and five replicates at 10xLOQ levels for each dosimeter type, respectively. The obtained results met the method validation criteria. The accuracy assessment was based on the recovery values obtained from matrices fortified with certified analytical standards of known purity. These recovery values ranged between 79 and 98% and the corresponding RSDs were <4.2%. The above data fulfilled the generally accepted requirements for residue analytical meth-

Table 3. Operator exposure results to active substance (a.s.) of iprodione (mg a.s./kg a.s. applied).

Dosimeters	**Exposure (mg a.s. /kg a.s. applied)**									
	Protective Coverall Type A (Hydrofoil®)					Protective Coverall Type B (Cotton)				
	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5
Inner shirt	1.62	17.29	8.83	3.30	2.01	52.87	19.76	14.86	97.28	13.77
Inner pants	5.93	2.90	2.37	1.41	0.52	5.22	2.01	15.44	1.59	65.66
Actual Dermal Exposure (ADE) (Inner coverall)	**7.56**	**20.19**	**11.20**	**4.71**	**2.53**	**58.09**	**21.77**	**30.30**	**98.86**	**79.43**
Outer jacket	622	983	168	153	126	526	1112	635	957	514
Outer pants	1961	1278	309	83	115	1974	871	896	1301	1368
Outer coverall	2582	2261	477	236	241	2499	1983	1532	2258	1883
Potential Dermal Exposure (PDE) (Inner + Outer coverall)	**2590**	**2281**	**489**	**240**	**243**	**2557**	**2005**	**1562**	**2357**	**1962**
Inner gloves	0.230	2.883	0.808	0.378	0.433	0.196	0.349	0.373	3.803	0.873
Outer gloves	38.764	24.034	16.105	9.555	8.294	3.906	9.332	13.830	5.839	5.844
Head exposure[1]	103.2	212.8	19.4	36.7	20.5	4.26	21.38	12.11	10.58	1.68
PDE + Head + Hand exposure	2732	2521	525	287	273	2566	2036	1588	2377	1971
Inhalation exposure[2]	2.90	2.75	0.75	0.89	0.58	0.90	0.76	0.55	0.48	0.68

[1] The values given for the head exposure derive from the residues (ml spray solution/h) found on operator's cap multiplied by a factor of 2.
[2] The values given for inhalation exposure derive from the residues found on operator's air sampler tube multiplied by a factor of 29/2 (given that the human inhalation rate is 29 l/min, the air sampler's pump flow rate was 2 l/min and the net duration of the operator's pump working was the same with the net duration of the application).

ods. The specificity of the method was verified by the well-resolved peaks obtained for a.s. in combination with the facts that: i) no interferences from other compounds were observed, and ii) no signal peak values exceeding 10% of the respective lowest fortification level were detected in the blank samples of the tested specimens.

Results

As presented in Table 3, the following levels of exposure were obtained. Potential dermal exposure (PDE) corresponding to the total amount of iprodione detected in/on the outer and the inner coverall ranged for the ten applications from 240 to 2590 (mean value 1629) mg/kg a.s. For the operators wearing Type A coveralls, the PDE values ranged from 240 to 2590 (mean value 1169) mg/kg a.s., while for the operators using Type B coverall ranged from 1562 to 2557 mg/kg a.s. (mean value 2089) mg/kg a.s.

The actual dermal exposure (ADE) represented by the amounts of a.s. measured in/on the inner coveralls ranged for the ten applications from 2.5 to 98.9 (mean value 33.5) mg/kg a.s. applied. For the operators using coverall Type A the ADE values ranged from 2.5 to 20.2 (mean value 9.2) mg/kg a.s., while for the operators using coverall Type B ranged from 21.8 to 98.9 (mean value 57.7) mg/kg a.s.

The potential hand exposure (sum of inner and outer glove residues) for the ten applications ranged between 4.1 and 39.0 (mean value 14.6) mg/kg a.s. The respective values for the actual hand exposure (inner glove residues) were between 0.2 and 3.8 (mean value 1.0) mg/kg a.s.

The head exposure values (a.s. residues in caps multiplied by a factor of 2) ranged between 1.7 and 213 (mean value 44.3) mg/kg a.s. and the potential inhalation exposure (a.s. residues in air sampler tubes multiplied by a factor of 29/2) was between 0.48 and 2.90 (mean value 1.13) mg inhaled/kg a.s. for the ten applications.

Discussion and Conclusions

The exposure levels for the trunk and leg parts (outer jacket and outer pants residues) were compared to the respective results of previous work of our team related to operator exposure trials performed in pepper greenhouses in Crete (5). The application in those trials involved the use of spray guns, which is the conventional application method for the greenhouses in the specific region, while the rest of the application conditions were comparable to the present work. The comparison of the respective exposure values in mg/day at the 75th percentile (data not presented) showed that, with the conventional method, the PDE was 6 times lower than that of the present work, where the new application method with Novi-F (75.4 versus 476.8 mg/day) was used. The ADE was 30 times lower in the conventional application method (0.41 versus 12.12 mg/day). For comparison purposes the respective data expressed in mg/kg a.s. are presented in Table 4. From the aforementioned trial results it is apparent that the new application tool does not provide up to now positive ev-

Table 4: Comparison of exposure results (mg/kg a.s.) between conventional[1] (spray guns) and new (Novi-F) application equipment.

Dosimeters	Exposure (mg/kg a.s.)[2]			
	Spray gun Coverall Type A	Novi-F Coverall Type A	Spray gun Coverall Type B	Novi-F Coverall Type B
Inner Coverall	0.41	7.27	1.32	49.6
Outer coverall	123	692	138	2004

[1] The comparison refers to the data of the conventional application with spray guns (4).
[2] The exposure values correspond to the geometric means.

idence for reducing the operator exposure levels during application of plant protection products.

Moreover, in the present study, the comparison of the PDE and ADE values for the A1-A5 and B1-B5 operator groups can be used as a measure of PPE performance in terms of coverall penetration, which can be expressed as:

% penetration = 100 x ADE /PDE

From the aforementioned exposure data, the average penetration for Type A coverall is 0.79%, while for Type B coverall is 2.76% showing that the average Type B coverall penetration is 3.5 times higher than the respective one of Type A. It is noteworthy that this difference in coverall performance is in accordance with the results of previous work addressing spray gun greenhouse applications, where Type B coverall was found to be 3.5 times more permeable than Type A. This difference in coverall performance becomes evident under relatively high exposure conditions, while no significant differences are observed in low exposure scenarios (8). However, both coverall types provided satisfactory protection under the conditions of the specific trials.

Literature Cited

1. Durham, W.F. and Wolfe, H.T. 1962. Measurement of the Exposure of Workers to Pesticides. *Bulletin WHO*, 26: 75-91
2. Goumenou, M. and Machera, K. 2001. Determination of Penconazole on Personal Protection Equipment after Field Applications. *Fresenius Journal of Anaytical Chemistry,* 370: 946-950.
3. Machera, K., Goumenou, M., Kapetanakis, E., Kalamarakis, A. and Glass, C.R. 2003. Determination of Potential Dermal and Inhalation Operator Exposure to Malathion in Greenhouses with the Whole Body Dosimetry Method. *Annals of Occupational Hygiene,* 47: 61-70.
4. Machera, K., Goumenou, M., Kapetanakis, E., Tsatsakis, A. and Glass, C.R. 2001. Determination of Potential Dermal and Inhalation Exposure of Operators following Malathion 50EC Applications on Greenhouse Tomatoes. *Toxicology,* 164: 85.
5. Machera, K, Tsakirakis, A, Charistou, A, Anastasiadou, P. and Glass, C.R. 2009. Dermal Exposure of Pesticide Applicators as a Measure of Coverall Performance under Field Conditions. *Annals of Occupational Hygiene,* 53: 573-584.
6. Maronim, M., Fanetti, A.C. and Metruccio, F. 2006. Risk Assessment and Management of Occupational Exposure to Pesticides in Agriculture. *Medicina del Lavoro, 97*: 430–437.
7. OECD 1997. Environmental Health and Safety Publications Series on Testing and Assessment No. 9: Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides during Agricultural Application. OECD, Paris [OECD/GD (97) 148].
8. Tsakirakis, A., Kasiotis, K.M., Arapakis, N., Charistou, A., Tsatsakis, A., Glass C.R. and Machera, K. Determination of Operator Exposure Levels to Insecticide during Bait Applications in Olive Trees: Study of Coverall Performance and Duration of Application (*submitted for publication*)

Received: 3 December 2009; Accepted: 27 January 2010

Προσδιορισμός επιπέδων έκθεσης ψεκαστών σε φυτοπροστατευτικά προϊόντα κατά τις εφαρμογές σε θερμοκήπια με ψεκαστικό μέσο νέου τύπου πολλαπλών μπεκ και χρήση δύο διαφορετικών τύπων προστατευτικής φόρμας

Α.Ν. Τσακιράκης, Κ.Μ. Κασιώτης, Π. Αναστασιάδου και Κ. Μαχαίρα

Περίληψη Στόχος της συγκεκριμένης μελέτης ήταν α) ο προσδιορισμός των επιπέδων έκθεσης ψεκαστών κατά την εφαρμογή μυκητοκτόνου σε θερμοκηπιακές καλλιέργειες πιπεριάς στο Τυμπάκι Ηρακλείου Κρήτης με ένα νέο ψεκαστικό μέσο εφαρμογής 4 ακροφυσίων (Novi-F), β) η σύγκριση των επιπέδων έκθεσης των ψεκαστών με το νέο αυτό μέσο σε σχέση με συμβατικές τεχνικές ψεκασμού (ψεκαστικό πιστόλι), και γ) η σύγκριση της περατότητας δύο τύπων προστατευτικών φορμών (η μία από

100% βαμβάκι και η δεύτερη από υδροαπωθητικό υλικό επεξεργασμένο με νανοκάψουλες, Hydrofoil®) που χρησιμοποιήθηκαν στα πειράματα. Το σκεύασμα το οποίο χρησιμοποιήθηκε ήταν ένα μυκητοκτόνο τύπου SC, με δραστική ουσία την ιπροδιόνη. Ο προσδιορισμός των επιπέδων έκθεσης βασίστηκε στη μέθοδο ολοκλήρου σώματος. Πραγματοποιήθηκαν δέκα συνολικά εφαρμογές (5 ανά τύπο φόρμας). Ο ποιοτικός και ποσοτικός προσδιορισμός της δραστικής ουσίας πραγματοποιήθηκε με την τεχνική της αέριας χρωματογραφίας σε συνδυασμό με ανιχνευτή σύλληψης ηλεκτρονίων (GC-ECD). Η μέθοδος προσδιορισμού αναπτύχθηκε και επικυρώθηκε πλήρως στο Εργαστήριο (ποσοστά ανάκτησης 79-98% και RSD<4.2%). Η σύγκριση των αποτελεσμάτων της παρούσης εργασίας με αυτά προγενέστερης εργασίας της ερευνητικής μας ομάδας σε θερμοκηπιακές καλλιέργειες, στην οποία είχε χρησιμοποιηθεί το συμβατικό πιστόλι ψεκασμού, δεν παρέχει θετικές ενδείξεις για μείωση των επιπέδων έκθεσης των ψεκαστών με το νέο μέσο. Από τα αποτελέσματα σύγκρισης ως προς την περατότητα των δύο τύπων προστατευτικής ενδυμασίας προκύπτει ότι η φόρμα τύπου Hydrofoil® είναι λιγότερο περατή από την βαμβακερή φόρμα σε συνθήκες υψηλής έκθεσης, ωστόσο και οι δύο τύποι φόρμας παρέχουν ικανοποιητική προστασία για το δεδομένο σενάριο εφαρμογής με βάση τις τιμές της πραγματικής από δέρματος έκθεσης.

Hellenic Plant Protection Journal **3:** 9-16, 2010

Hellenic Plant Protection Journal **3:** 17-24, 2010

Acute toxicity of Olive Mill Wastewater on rats, *Vibrio fischeri* and *Artemia fransiscana*

A. Charistou[1], K. Kyriakopoulou[1], P. Anastasiadou[1], N. Fokialakis[2], A.L. Skaltsounis[2] and K. Machera[1]

Summary Although the annual quantity of wastewater generated by olive oil mills in the Mediterranean basin is 10-12x10^6 m^3, it is only recently that solutions for proper management of Olive Mill Wastewater (OMW) are under serious consideration. The common practice for handling such liquid by-products has been the deposition in oxidation tanks/ponds. No special attention is paid to the polluting potential of OMW or the intrinsic toxicity of the mixture to aquatic organisms. The acute toxicity of OMW produced in a three-phase olive mill on the island of Crete, Greece, was tested on Wistar rats and on two marine species, the photobacterium *Vibrio fischeri* (Microtox® assay) and the crustacean *Artemia fransiscana*. The acute toxicity to the aquatic organisms was also determined for the treated OMW (following a pilot scale treatment system developed in the frames of the EU LIFE-Environment program MINOS) and the isolated polyphenol mixtures. The untreated OMW exhibited low acute oral toxicity on rats but high toxicity on the photobacterium *V. fischeri* and the crustacean *A. fransiscana*. Treatment of OMW led to the production of wastewater of lower toxicity to the photobacterium *V. fischeri* (eight-fold reduction following one-cycle treatment and about twenty-fold reduction following two-cycle treatment). On the contrary, the above mentioned treatment of OMW had no effect on the exhibited toxicity against the crustacean *A. fransiscana*. The polyphenolic fraction isolated from OMW, on the other hand, exhibited high toxicity on the photobacterium *V. fischeri* but low toxicity on the crustacean *A. fransiscana*. Therefore, further study is needed on the OMW management before its deposition into the environmental system.

Additional Keywords: aquatic organisms, environment, Microtox®, pollution, polyphenols

Introduction

The annual world production of olive oil is more than 2 million tones, and more than 80% of this quantity is produced in Spain, Italy and Greece. In Greece, olive cultivation is placed among the major crops and olive groves cover an area of about 7,371 km^2 (13). In most cases the production of olive oil is made by the centrifugation method, which results in the generation of 5 m^3 wastewater per tone of olive oil produced (4, 8). As a result, the annual yield of Olive Mill Wastewater (OMW) in the Mediterranean basin is about 10-12x10^6 m^3, with more than 2x10^6 m^3 of this quantity being produced in Greece. For the management of OMW the common practice is its deposition in oxidation tanks/ponds, regardless of the fact that OMW contains macromolecules, such as polysaccharides, lipids, proteins as well as a number of aromatic molecules referred to as phenolic compounds. The polluting potential of OMW is 100-fold greater than that associated with urban wastes attributable to its high organic load and its extremely high volume (20). Additionally, the organic content of OMW, mainly due to the presence of polyphenolic compounds, has a great antimicrobial and phytotoxic effect, which suppress the activity of the microorganisms involved in the biodegradation. Therefore,

[1] Laboratory of Pesticides Toxicology, Department of Pesticides Control & Phytopharmacy, Benaki Phytopathological Institute, 7 Ekalis Str., GR-145 61 Kifissia (Athens), Greece
[2] Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis of Zografou, GR-157 71 Zografou (Athens), Greece
Corresponding author: k.machera@bpi.gr

OMW dumping in the environment is an act of pollution of drinking water, and a threat to aquatic organisms and plants (1, 3, 24).

The determination of OMW toxicity to aquatic organisms is of major importance for characterizing its ecotoxicological properties mainly associated with the high polyphenol content. Recently, several methods have been developed for the treatment of OMW aiming at the recovery of high added value-contained polyphenols and the potential reduction of the environmental impact.

In previous studies, the fungi *Phanerochaete* spp. (i.e. *P. flavido-alba* and *P. chrysosporium*) and *Pleurotus ostreatus* had been used for biological treatment of OMW to achieve a reduction in the polyphenol content and decolorization (2, 12, 21). The effect of fungal treatments on OMW toxicity has been studied on the aquatic organisms *Artemia* sp. and *Daphnia magna* (2, 12). Other approaches to the treatment of OMW include the use of microorganisms, such as *Geotrichum* sp., *Aspergillus* sp. and *Candida tropicalis* (7), aiming at reducing its organic potential (Chemical Oxygen Demand, COD), the use of centrifugation–ultrafiltration techniques in order to remove the solid and organic content (22), the use of sodium polyacrylate polymers (6) or the application of electrophysical methods for the reduction of the toxicity potential of OMW (9).

More recently, a pilot scale system for the treatment of OMW has been developed in the frames of the EU LIFE-Environment programme MINOS (1) aiming at the isolation of high added value-contained polyphenols and the minimization of environmental problems.

In the present study, the acute toxicity of OMW, produced in a three-phase olive mill in Rouva municipality in the island of Crete, Greece, was determined on Wistar rats and two marine species, the photobacterium *Vibrio fischeri* (Microtox® assay) and the crustacean *Artemia fransiscana*. These marine organisms have been widely used as indicators for the toxicity evaluation of environmental pollutants or other chemical substances (17, 18, 19, 23). Therefore, in the present study the above mentioned organisms have been used taking into account that the olive mill wastes are a source of pollution for the marine environment. The acute toxicity on the aquatic organisms was also determined for both the treated OMW [as described by Agalias *et al*., 2007 (1)] and the isolated polyphenol mixtures in comparison with the toxicity of the untreated OMW.

Materials and methods

Treatment of OMW

Olive mill wastewater produced in a three-phase olive mill was treated using a recently developed method (1). In brief, fresh OMW (pH=5.15-5.23) was filtrated through two different filters for gradual removal of the wastewater suspended solids of 50 μm in size. Then, filtrate was successively passed through a series of columns packed with XAD-4 and XAD-7HP adsorbent resins (1st cycle extract, pH=5.18). The second step was repeated twice (2nd cycle extract, pH=4.80) for the achievement of maximal deodorization and decolorization of the wastewater and sufficient removal of the polyphenols.

The samples used in the toxicity assays were the following: untreated OMW, treated OMW after one cycle of treatment, treated OMW after two cycles of treatment, polyphenolic mixture recovered from OMW passed through column XAD-4 and polyphenolic mixture produced by OMW passed through column XAD-7HP. The recovered polyphenolic fractions from columns XAD-4 and XAD-7HP were 5.8 and 3 g/l of treated OMW, respectively.

Bioassays for the determination of OMW toxicity

The toxicity tests were performed on Wistar rats and two aquatic organisms, the bioluminescent bacterium *V. fischeri (NRRL B-11177)* and the crustacean *A. fransiscana*. The Wistar rats were obtained from the breeding colony of the Benaki Phytopatho-

logical Institute. All rats were housed in groups of five animals per cage under controlled environmental conditions (according to the national and European legislation) with *ad libitum* consumption of food and water. The bacterium *V. fischeri,* commercially available from Azur Environmental (USA), was kept at -20°C until the time of the experiment and the cysts of the crustacean *A. fransiscana,* obtained from Micro-BioTests Inc (Belgium), were kept at 4°C before hatching.

A limit test assay was conducted to estimate the acute oral toxicity of OMW on rats according to the protocol of OECD 420 (15). The test sample was administrated orally at a limit dose of 2,000 mg OMW per kg body weight (b.w.) by gavage to 5 female Wistar rats (Group A). Another group, served as a negative control (Group B), was also consisted of 5 female Wistar rats to which only the vehicle (tap water) was administrated. All animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hrs and thereafter daily for 14 consecutive days. Observations included the presence of signs of toxicity such as tremor, convulsion, salivation, diarrhea, sleep, changes in skin, fur, eyes, mucous membranes, and also changes in respiratory, autonomic and central nervous system, somatomotor activity and behavioral changes. Additionally, the weight of the animals was recorded weekly during the experimental period. At the end of the observation period all animals were sacrificed by CO_2 and were subjected to necropsy.

The Microtox® Analyzer Model 500 (Strategic Diagnostics Inc., Delaware, USA) was used for the toxicity assay of OMW. The assay is based on the inhibition of the natural luminescence emitted by the bacterium *V. fischeri,* when exposed to toxic compounds. The bacterial suspension was exposed to the test samples and the acute toxicity was expressed as inhibition of bioluminescence. The protocols used for the conduction of the assays were the "81.9% Basic Test" for the untreated and treated OMW and the "90% Basic Test for Pure Compounds" for the polyphenolic mixtures. The end-point for the assessment of toxicity was the median effective concentrations (EC_{50}) at 5 and 15 min. The pH of all sample solutions was adjusted within the range of 6-8. Each sample was tested at least at three replicates. Zinc sulfate ($ZnSO_4.7H_2O$) was used as reference material (positive control) for the toxicity tests performed on *V. fischeri*.

The nauplii of the marine crustacean *A. fransiscana* were hatched from commercially available cysts. The cysts were incubated in artificial seawater (Instant Ocean) at salinity of 35 ppt. The first nauplii usually appeared after 24 hrs of incubation at 25°C under aeration and illumination of 1,000-4,000 lux. They were transferred into new seawater and incubated for 24 hrs under similar conditions and then transferred into a multiwell test plate with the respective concentrations of the test samples. The toxicity of the samples to *A. fransiscana* nauplii was tested after 24 and 48 hrs of exposure at 25°C in the darkness. The end-point for the assessment of toxicity was the mortality incidence after 24 and 48 hrs of exposure. The toxicity test performed on *A. fransiscana* was based on the principles of the OECD 202 (14) test for *Daphnia* spp. adapted for the marine crustacean. Potassium dichromate ($K_2Cr_2O_7$) was used as reference material (positive control) for the toxicity tests on *A. fransiscana*.

The determined EC_{50} values were calculated using linear regression analysis as natural logarithm of sample concentration versus percentage of mortality or percentage of growth inhibition.

Results and Discussion

Acute Oral Toxicity on Wistar rats

Mortality of female Wistar rats was not observed in the acute limit test (2,000 mg/kg b.w.) of OMW. Moreover, no signs of toxicity or abnormal behavior were observed on any of the test animals during the 14-day observation period following the administration. The body weight development was normal for all animals (Table 1). The macro-

scopic examination of all animals during the necropsy at the end of the observation period did not reveal any abnormality. No effect was noted on the negative control group of 5 female Wistar rats either (Group B).

It can be, therefore, concluded that the tested OMW, produced in a three-phase olive mill in Rouva (Crete, Greece), is of low acute oral toxicity to the test animals.

Acute Toxicity on the bacterium *Vibrio fischeri* (Microtox® assay)

In a preliminary test, the acute toxicity of the untreated OMW was tested on the photobacterium *V. fischeri* following 5, 15 and 30 min of exposure and the determined EC_{50} values for the 15 and 30 min exposure did not significantly differ. Therefore, in the tests, in order to follow the acute toxicity of the untreated and treated OMW on the photobacterium *V. fischeri,* the EC_{50} values were determined only after 5 and 15 min of exposure.

Considering the EC_{50} values (Table 2) determined for the OMW sample and its filtrates (1), significant reduction in the toxicity to *V. fischeri* after the 1st and 2nd cycle of treatment was observed. More specifically, the EC_{50} values determined for the untreated OMW, the treated OMW after 1 cycle and 2 cycles of treatment were 0.219, 1.612 and 4.606% v/v, respectively. In addition, the differences between the EC_{50} values determined for the 5 min exposure were almost the same with those after 15 min of exposure.

The determined EC_{50} values for polyphenolic mixtures isolated from the tested OMW, after passing through column XAD-4 or XAD-7HP on the photobacterium *V. fischeri,* were 37.795 and 50.970 mg/l, respectively, following 5 min of exposure. Comparisons of the EC_{50} values obtained at 5 and 15 min of exposure (Table 3) demonstrated that the toxicity of the tested samples on the photobacterium was almost completed

Table 1. Acute oral toxicity of Olive Mill Wastewater (OMW) on female Wistar rats, expressed as body weight data.

	Code no.	Body weight (g)		
		Before oral administration	7 days post administration	14 days post administration
Group A	A-01	180	180	179
	A-02	162	168.5	170
	A-03	193	191.5	195
	A-04	165	170	174
	A-05	167	175	175
Group B	B-01	175	178	182
	B-02	188	192.5	193
	B-03	180	185	186
	B-04	176	167	172
	B-05	172	180	184

Table 3. Acute toxicity of the polyphenolic mixtures recovered from Olive Mill Wastewater (OMW) on the photobacterium *Vibrio fischeri* (Microtox®), expressed as median effective concentrations (EC_{50}).

Duration of exposure (min)	Polyphenolic mixture XAD-4		Polyphenolic mixture XAD-7HP	
	EC_{50} (% v/v) (± SD[1])	R^2	EC_{50} (% v/v) (± SD)	R^2
5	37.795 (± 3.656)	0.978	50.98 (± 3.352)	0.987
15	37.995 (± 1.407)	0.938	52.42 (± 3.224)	0.977

[1] standard deviation

Table 2. Acute toxicity of untreated and treated Olive Mill Wastewater (OMW) on the bacterium *Vibrio fischeri* (Microtox® assay), expressed as median effective concentrations (EC_{50}).

Duration of exposure (min)	Untreated OMW		Treated OMW (1 cycle)		Treated OMW (2 cycles)	
	EC_{50} (% v/v) (± SD[1])	R^2	EC_{50} (% v/v) (± SD)	R^2	EC_{50} (% v/v) (± SD)	R^2
5	0.219 (±0.044)	0.945	1.612 (±0.687)	0.958	4.606 (±1.51)	0.976
15	0.187 (±0.035)	0.931	1.361 (±0.528)	0.944	4.374 (±1.54)	0.967

[1] standard deviation

in the first 5 min of exposure. In addition, it is noted that the polyphenolic mixture recovered from the XAD-4 exhibited slightly higher toxicity compared to the mixture recovered from the XAD-7HP column.

Acute Toxicity on the crustacean *Artemia fransiscana*

Following the testing of OMW at high dilutions (1.25 and 2.5% v/v) on *A. fransiscana,* a slight toxicity was observed. On the contrary, 100% mortality occurred following a 24-hr exposure to OMW at 1:5 dilution in water (20% v/v). The OMW toxicity on *A. fransiscana* was increased after a 48-hr exposure period to 1:10 dilution (10% v/v) causing 100% mortality (Table 4). Based on the results obtained for the tested OMW samples and its filtrates, it can be concluded that the treatment used did not alter the toxicity of crude OMW to *A. fransiscana*.

The polyphenolic mixtures recovered from OMW with XAD-4 or XAD-7HP exhibited lower acute toxicity to *A. fransiscana* than to photobacterium *V. fischeri*. The EC_{50} values (Table 5 & Table 6) obtained after 48 hrs of exposure were higher than those estimated after 24 hrs of exposure.

All the above results are in agreement with findings mentioned in the literature, where the estimated 48-hr EC_{50} values for OMW were 2.5% v/v and 4.5% v/v for *D.*

Table 4. Acute toxicity of treated and untreated Olive Mill Wastewater (OMW) on the crustacean *Artemia fransiscana*, expressed as mortality (%).

Concentration (% v/v)	Mortality (%) (±SD[1])					
	Untreated OMW		Treated OMW (1 cycle)		Treated OMW (2 cycles)	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
0	0	1.6 (±1.4)	1.7 (±1.7)	3.3 (±1.7)	5 (±2.8)	5 (±4.2)
1.25	12.9 (±6.8)	27.4 (±4.2)	7 (±2.8)	16.5 (±8.9)	5.2 (±0)	5.2 (±0)
2.5	14.1 (±5.8)	28.1 (±13.4)	17.5 (±3.5)	17.5 (±7.0)	7.5 (±3.5)	10.2 (±0.3)
5	25.4 (±15.0)	40.3 (±20.7)	17.5 (±7.7)	55 (±7.0)	48.6 (±26.3)	97.5 (±0.1)
10	80.6 (±12.8)	100 (±0)	77.5 (±17.6)	100 (±0)	75.6 (±7.6)	100 (±0)
20	100 (±0)	100 (±0)	89.7 (±14.1)	100 (±0)	100 (±0)	100 (±0)
40	-	-	-	-	100 (±0)	100 (±0)

[1] standard deviation

Table 5. Acute toxicity of the polyphenolic mixture isolated from OMW on *Artemia fransiscana*, expressed as mortality (%).

Concentration (mg/l)	Mortality (%) (±SD[1])	
	Polyphenolic mixture [XAD-4]	
	24 hrs	48 hrs
0	0	0
204.375	0	0
408.75	0	5 (±2.8)
817.5	0	10 (±2.8)
1635	55 (±9.8)	100 (±0)
3270	100 (±0)	100 (±0)
EC_{50} (mg/l)	1.441	1.079

[1] standard deviation

Table 6. Acute toxicity of the polyphenolic mixture isolated from OMW after passing through column XAD-7HP on *Artemia fransiscana*, expressed as mortality (%).

Concentration (mg/l)	Mortality (%) (±SD[1])	
	Polyphenolic mixture [XAD-7HP]	
	24 hrs	48 hrs
0	0	5 (±4.2)
199.625	0	0
399.25	0	0
798.5	15 (±7.0)	20 (±2.8)
1597	55 (±18.3)	90 (±4.2)
3194	100 (±0)	100 (±0)
EC_{50} (mg/l)	1.317	1.078

[1] standard deviation

magna and *Artemia* sp., respectively (2). Moreover, the results of this study indicate that the treatment of OMW, according to the technology developed within the frames of the EU LIFE-Environment programme MINOS (1), reduces OMW toxicity to the photobacterium *V. fischeri*, which among other marine organisms exhibits the highest sensitivity (16). On the contrary, there are studies showing that the photobacterium *V. fischeri* exhibits higher tolerance compared to other aquatic organisms when exposed to plant protection products (5, 10, 11). Although the treatment of OMW with the aforementioned technology renders a twenty-fold reduction in *V. fischeri* toxicity, the obtained treated OMW still exhibits significant toxicity to *A. fransiscana* indicating the necessity for additional information on OMW bioactive constituents capable of causing adverse effects on aquatic organisms. Therefore, further study is needed on the OMW management in order to suggest a completely satisfactory solution with regard to deposition of such materials into the environmental system.

Literature Cited

1. Agalias, A., Magiatis, P., Skaltsounis, A.-L., Mikros, E., Tsarbopoulos, A., Gikas, E., Spanos, I. and Manios, T. 2007. A new process for the management of olive mill waste water and recovery of natural antioxidants. *Journal of Agricultural Food and Chemistry*, 55: 2671-2676.
2. Aggelis, G., Iconomou, D., Christou, M., Bokas, D., Kotzailias, S., Christou, G., Tsagou, V. and Papanikolaou, S. 2003. Phenolic removal in a model olive mill wastewater using *Pleurotus ostreatus* in bioreactor cultures and biological evaluation of the process. *Water Research*, 37: 3897-3904.
3. Cappasso, R., Evidente, A., Schivo, L., Orru, G., Marcialis, G. and Cristinzio, G. 1995. Antibacterial polyphenols from olive oil mill waste waters. *Journal of Applied Bacteriology*, 79: 393-398.
4. Chartzoulakis, K., Psarras, G. and Moutsopoulou, M. 2004. Application of olive mill Wastewater (OMW) to olive orchards. *International Water and Irrigation*, 24: 34-38.
5. Chrysayi-Tokousbalides, M., Machera, K., Kyriakopoulou, K., Aliferis, K.A., Schrader, K., Tsoutsanis, I. and Anastasiadou P. 2007 Comparative toxicity of the phytotoxins (8R,16R)-(-)-pyrenophorin and (5S,8R,13S,16R)-(-)-pyrenophorol on aquatic organisms. *Bulletin of Environmental Contamination and Toxicology*, 79: 499-503.
6. Davies, L.C., Novais, J.M. and Martins-Dias, S. 2004. Influence of salts and phenolic compounds on olive mill wastewater using superabsorbent polymers. *Environmental Technology*, 25: 89-100.
7. Fadil, K., Chahlaoui, A., Ouahbi, A., Zaid, A. and Borja, R. 2003. Aerobic biodegradation and detoxification of wastewaters from the olive oil industry. *International Biodeterioration and Biodegradation*, 51: 37-41.
8. Kapellakis, I.E., Tsagarakis, K.P., Avramaki, Ch. and Angelakis, A.N. 2006. Olive mill wastewater management in river basins: A case study in Greece. *Agricultural Water Management*, 82: 354-370.
9. Knoufi, S., Feki, F. and Sayadi, S. 2007. Detoxification of olive mill wastewater by electrocoagulation and sedimentation processes. *Journal of Hazardous Materials*, 142: 58-67.
10. Kyriakopoulou, K., Anastasiadou, P. and Machera, K. 2009. Comparative toxicity on different aquatic species of fungicide and herbicide formulations. *Bulletin of Environmental Contamination and Toxicology*, 82:290-295.
11. Machera, K., Cotou, E. and Anastassiadou, P. 1996. Fenbutatin acute toxicity on *Artemia nauplii*: effects of sublethal concentrations on AT-Pase activity. *Bulletin of Environmental Contamination and Toxicology*, 56:159-164.
12. Martinari, L., Giardina, P., Marzullo, L. and Sannia, G. 1996. Reduction of phenolic content and toxicity in olive mill waste waters with the ligninolytic fungus *Pleurotus ostreatus*. *Water Research*, 30: 1914-1918.
13. Migdalas A., Baourakis G., Kalogeras N. and Meriem, H.B. 2004. Sector modeling for the prediction and evaluation of Cretan olive oil. *European Journal of Operational Research*, 152: 454-464.
14. OECD 2004. Guideline for Testing of Chemicals. *Daphnia* sp. Acute Immobilisation Test. No 202.
15. OECD 2001. Guideline for Testing of Chemicals. Acute Oral Toxicity – Fixed Dose Procedure. No 420.
16. Paixão, S.M., Mendonça, E., Picado, A. and Anselmo, A.M. 1999. Acute toxicity evaluation of olive mill wastewaters: A comparative study of three aquatic organisms. *Environmental Toxicology*, 14: 263-269.
17. Ruiz, M.J., Lopez-Jaramillo, M., Redondo, G. and Font, G. 1997. Toxicity assessment of pesticides using the Microtox test: Application to environmental samples. *Bulletin of Environmental Contamination and Toxicology*, 59: 619-625
18. Sanchez-Fortun, S., Sanz-Barrera, F. and Bara-

hona-Gomariz, M.V. 1995. Acute toxicities of selected insecticides to the aquatic arthropod *Artemia salina*. *Bulletin of Environmental Contamination and Toxicology*, 54: 76-82.

19. Somasundaram, L., Coats, J.R., Racke, K.D. and Stahr, H.M. 1990. Application of the Microtox System to assess the toxicity of pesticides and their hydrolysis metabolites. *Bulletin of Environmental Contamination and Toxicology*, 44: 254-259.
20. TDC OLIVE NET. Bremerhaven Technology Transfer Center. Waste management. 2007. Programme TDC-OLIVE, Instituto de la Grasa (CSIC). Available at: http://www.tdcolive.net.
21. Tsiouplas, A., Dimou, D., Ikonomou, D. and Aggelis, G. 2002. Phenolic removal in olive oil mill waste-water by strains of *Pleurotus* spp. in respect to their phenol oxidase (laccase) activity. *Bioresource Technology*, 84: 251-257.
22. Turano, E., Curcio, S., De Paola, M.G., Calabro, V. and Iorio, G. 2002. An integrated centrifugation system in the treatment of olive mill wastewater. *Journal of Membrane Science*, 209: 519-531.
23. Varo, I., Serrano, R., Navarro, J.C., Lopez, F.J. and Amat, F. 1998. Acute lethal toxicity of the organophosphorus pesticide chlorpyrifos to different species and strains of *Artemia*. *Bulletin of Environmental Contamination and Toxicology*, 61: 778-785.
24. Report of MINOS Programme: "Process development for an integrated olive oil mill waste management recovering natural antioxidants and producing organic fertilizer", LIFE Project Number LIFE ENV/GR/000671, April 2004. Available at: http://www.pharm.uoa.gr/minos.

Received: 4 December 2009; Accepted: 14 January 2010

Οξεία τοξικότητα υδατικών αποβλήτων ελαιοτριβείων σε επίμυες, στο φωτοβακτήριο *Vibrio fischeri* και στο καρκινοειδές *Artemia fransiscana*

Α. Χαριστού, Κ. Κυριακοπούλου, Π. Αναστασιάδου, Ν. Φωκιαλάκης, Α.-Λ. Σκαλτσούνης και Κ. Μαχαίρα

Περίληψη Τα ελαιοτριβεία των Μεσογειακών χωρών παράγουν ετησίως περίπου 10-12 εκατομμύρια m^3 υδατικών αποβλήτων, με την Ελλάδα να ξεπερνά τα 2 εκατομμύρια m^3. Η συνηθέστερη πρακτική διαχείρισης των υδατικών αυτών αποβλήτων, που κοινώς αναφέρονται ως κατσίγαρος, είναι η εναπόθεσή τους σε δεξαμενές/λίμνες εξάτμισης, παραβλέποντας το γεγονός ότι ο κατσίγαρος περιέχει μακρομόρια, όπως πολυσακχαρίτες, λιπίδια, πρωτεΐνες και έναν αριθμό αρωματικών μορίων που αναφέρονται γενικά ως φαινολικές ενώσεις. Η ρυπαντική ικανότητα του κατσίγαρου είναι εκατό φορές ισχυρότερη σε σύγκριση με τα αστικά απόβλητα. Αυτό οφείλεται τόσο στο υψηλό οργανικό του φορτίο όσο και στον πολύ μεγάλο όγκο του. Σκοπός της παρούσας εργασίας ήταν ο προσδιορισμός της τοξικότητας των υδατικών αποβλήτων ενός τριφασικού ελαιοτριβείου και η σύγκρισή της με την τοξικότητα των επεξεργασμένων (με την τεχνολογία που αναπτύχθηκε στα πλαίσια του προγράμματος MINOS*) αποβλήτων και των πολυφαινολών που ανακτώνται κατά την εφαρμογή της εν λόγω επεξεργασίας. Για τον σκοπό αυτό μελετήθηκε (α) η οξεία από στόματος τοξικότητα των ακατέργαστων υδατικών αποβλήτων σε θηλαστικά (επίμυες), (β) η οξεία τοξικότητα των ακατέργαστων υδατικών αποβλήτων σε υδρόβιους οργανισμούς [στο φωτοβακτήριο *Vibrio fischeri* (μέθοδος Microtox®) και στο καρκινοειδές *Artemia fransiscana*], (γ) η επίδραση της εν λόγω επεξεργασίας στην τοξικότητα των υδατικών αποβλήτων στους εξεταζόμενους υδρόβιους οργανισμούς, και (δ) η οξεία τοξικότητα των πολυφαινολών που απομονώθηκαν κατά την εν λόγω επεξεργασία του κατσίγαρου στους εξεταζόμενους υδρόβιους οργανισμούς. Το δείγμα κατσίγαρου που εξετάστηκε ήταν χαμηλής οξείας από στόματος τοξικότητας στα θηλαστικά (επίμυες), ενώ εμφάνιζε υψηλή οξεία τοξικότητα στα φωτοβακτήρια *V. fischeri* και στο καρκινοειδές *A. fransiscana*. Η επεξεργασία του κατσίγαρου με την τεχνολογία που έχει αναπτυχθεί στα πλαίσια του προγράμματος MINOS οδηγεί στην τελική παραγωγή υδατικών αποβλήτων που εμφανίζουν

* "Ανάπτυξη Διαδικασίας για την Ολοκληρωμένη Διαχείριση Αποβλήτων των Ελαιοτριβείων με Ανάκτηση Φυσικών Αντιοξειδωτικών και Παραγωγή Οργανικού Λιπάσματος". Συντονιστής του έργου ήταν το Εργαστήριο Φαρμακογνωσίας του Πανεπιστημίου Αθηνών με συγχρηματοδότηση από την Ευρωπαϊκή Ένωση.

μειωμένη οξεία τοξικότητα στο φωτοβακτήριο *V. fischeri* κατά περίπου 8 φορές μετά από έναν κύκλο επεξεργασίας και περισσότερο από 20 φορές μετά από δύο κύκλους επεξεργασίας. Αντίθετα, η εν λόγω επεξεργασία δεν επηρεάζει την τοξικότητα των εξεταζόμενων υδατικών αποβλήτων στο καρκινοειδές *A. fransiscana*. Τέλος, τα μίγματα πολυφαινολών, που παραλαμβάνονται από τον κατσίγαρο κατά την εφαρμογή της εν λόγω τεχνολογίας, εμφανίζουν υψηλότερη οξεία τοξικότητα στο φωτοβακτήριο *V. fischeri* σε σχέση με το καρκινοειδές *A. fransiscana*. Συμπερασματικά, τα ανωτέρω αποτελέσματα φανερώνουν ότι η τοξικότητα των υδατικών αποβλήτων ενός ελαιοτριβείου τριών φάσεων στο βακτήριο *V. fischeri* οφείλεται εν μέρει στην παρουσία μιγμάτων πολυφαινολών. Αντιθέτως, η τοξικότητα των εν λόγω αποβλήτων στο καρκινοειδές *A. fransiscana* δεν μπορεί να αποδοθεί στις περιεχόμενες πολυφαινόλες εφόσον η απομάκρυνσή τους δεν επηρέασε την τοξικότητα των αποβλήτων στο καρκινοειδές. Από τα παραπάνω αποτελέσματα γίνεται σαφές ότι χρειάζεται περαιτέρω μελέτη των μεθόδων διαχείρισης των υδατικών αποβλήτων των ελαιοτριβείων πριν την εναπόθεσή τους στο περιβάλλον.

Hellenic Plant Protection Journal **3:** 17-24, 2010

Hellenic Plant Protection Journal **3:** 25-27, 2010

SHORT COMMUNICATION

First record of *Acizzia jamatonica* (Kuwayama) (Hemiptera: Psyllidae) in Greece

B. Pásztor[1], D. Rédei[2] and G. Vétek[1]

Summary In August 2009, *Albizia julibrissin* trees were found to be in a remarkably bad condition in the centre of Thessaloniki, Greece. After observing the foliage thoroughly, specimens of a psyllid species feeding on the lower surface of the leaves were found in high numbers. The pest has been identified as *Acizzia jamatonica* (Kuwayama). The psyllid is native to East Asia, but has recently been introduced to Europe. The species is new to the fauna of Greece. The morphology, biology and distribution of *A. jamatonica* together with the damage caused and the major aspects of control are briefly discussed.

Additional keywords: *Acizzia jamatonica, Albizia julibrissin,* Psyllidae

Silk tree, *Albizia julibrissin* Durazzini, is a woody ornamental plant introduced from Asia to Europe and then from Europe to North America in the mid-eighteenth century (4). The species is popular with gardeners and landscape designers especially in the southern and southeastern parts of Europe due to its decorative leaf texture, flowers, and broad crown, which provides dappled shape. *A. julibrissin* is often used in private and public gardens, parks or other public places because of its unique appearance. For a long time, the virtual lack of pests on silk trees was regarded as a considerable advantage when choosing this plant for decorating urban environments.

On 29 August 2009, *A. julibrissin* trees were found to be in a remarkably bad condition in the centre of Thessaloniki, Greece. The foliage seemed to be withering and as if it had lost its bright, green colour. Leaves were rather yellow and deformed. After observing the foliage thoroughly, specimens of a psyllid species feeding on the lower surface of the leaves were found in high numbers. The foliage was covered with honeydew and the white, waxy secretion of the insects. Specimens collected by B. Pásztor were identified as *Acizzia jamatonica* (Kuwayama, 1908) (Hemiptera, Sternorrhyncha, Psyllidae) by D. Rédei. This is the first record of this species in Greece. In the review article of pests and weeds reported from the country between 1990 and 2007, *A. jamatonica* was still not indicated (2). Voucher specimens (1 male, 1 female) are deposited in the Hemiptera Collection of the Hungarian Natural History Museum.

Acizzia jamatonica is readily recognized among the other species of the genus *Acizzia* by its nearly uniformly pale fore wings with only small and faint dark spots at the apical margins of cells r_2, m_1, m_2, and cu_1, and by the highly characteristic male terminalia: proctiger elongate with a narrow apical part and large, broadly triangular posterior lobe at its base; distal segment of phallus reniformly widened apically. Diagnostic characters were described and illustrated in detail in different studies (9, 14).

[1] Corvinus University of Budapest, Department of Entomology, Villányi út. 29–43, H-1118 Budapest, Hungary
[2] Hungarian Natural History Museum, Department of Zoology, Baross u. 13, H-1088 Budapest, Hungary
Corresponding author: gabor.vetek@uni-corvinus.hu

This psyllid has several overlapping generations per year and overwinters in the adult stage (6). However, there is still no detailed data available on its life cycle in the European countries except Italy (12).

Acizzia jamatonica is native to East Asia, where it is widely distributed. It was recorded from Japan, Korea and China (8, 9, 10). In Europe, the pest was first found in the northern part of Italy in 2001 (1). Soon afterwards, it was also observed in Switzerland, France and Spain (3, 6, 7, 21). At the same time, the psyllid started invading the eastern Mediterranean region from Italy, and it was recorded in Slovenia and Croatia as well (15, 16, 17, 18). In 2005, the pest was also identified in Hungary (13, 14). Its occurrence has recently been reported from Bulgaria (20). The first report of *A. jamatonica* in North America was given in 2007 (19). According to the authors, the potential biological, economic and aesthetic impacts of the psyllid in the USA needs detailed investigation, as its host plant, *A. julibrissin*, is also considered a weed in the Southeast by many, hence the insect might be a biocontrol agent against this plant species.

The primary damage of *A. jamatonica* is the weakening of different parts of silk tree caused by sucking, or, in the case of heavy infestations, the discoloration, desiccation and falling of leaves (1, 19). The secondary damage, namely the excreted, sticky honeydew, may cause further problem and inconvenience by dropping onto and covering the surface of any objects (e.g. parked cars, deckchairs, tables of open-air restaurants) under the infested silk trees. Being a decorative ornamental plant, numerous *A. julibrissin* trees are planted in the streets and parks of Thessaloniki, so the occurrence of the pest in the town is obviously undesirable. Although *A. jamatonica* was not found either in Karditsa or in Athens during the summer of 2009, it can not be excluded that the pest will be reported soon from the central or southern regions of Greece, too. It is not easy to prove the origin of infestation in the case of silk trees in Thessaloniki, but it may be assumed that the specimens have probably been transported passively by the wind from long distances. Nevertheless, in order to prevent further spread of the pest, the use of healthy planting material has to be emphasised. The trade of *Albizia* trees can ensure the dissemination of the pest as it was demonstrated once in the UK (5). Insecticides can be used, but several applications may be necessary to control the overlapping generations. In addition, treatments are difficult to perform on amenity trees in urban environments, where only a limited number of active substances are registered (6), and, during summer months, the continuous flowering might increase the risk of poisoning insect pollinators. Control trials against this pest were performed in Italy during 2003 and 2004. Different active substances and application methods (endotherapy and aerial applications) were used. Endotherapy with imidacloprid or abamectin gave satisfactory results with only one application. Among the active substances tested with aerial applications during 2003 and 2004, only lambda-cyhalothrin and thiamethoxam gave good control results with at least two applications (11). Several species of natural enemies (e.g. coccinellid and anthocorid predators) have been identified, and studies are being done on their possible release (3, 6).

Literature Cited

1. Alma, A., Tedeschi, R. and Rossi, J. 2002. *Acizzia jamatonica* (Kuwayama) nuova psilla per l'Europa (Homoptera Psyllidae). *Informatore Fitopatologico*, 52: 64-65.
2. Anagnou-Veroniki, M., Papaioannou-Souliotis, P., Karanastasi, E. and Giannopolitis, C.N. 2008. New records of plant pests and weeds in Greece, 1990-2007. *Hellenic Plant Protection Journal*, 1: 55-78.
3. Chapin, É. and Cocquempot, C. 2005. Psylle de l'*Albizia*: 1ers signalements en France. *PHM Revue Horticole*, 467: 49-52.
4. Cothran, J.R. 2004. Treasured ornamentals of southern gardens—Michaux's lasting legacy. In *The Proceedings of the André Michaux International Symposium. Castanea Occasional Papers* No. 2: 149-157.
5. EPPO 2003. EPPO report on notifications of non-

compliance (detection of regulated pests). *EPPO Reporting Service* 2003/175.

6. EPPO 2004. First report of *Acizzia jamatonica* in France: addition to the EPPO Alert List. *EPPO Reporting Service* 2004/153.
7. Grimau, R. 2006. *Acizzia jamatonica* (Kuwayama 1908). *Paisajismo*, 5: 44-47.
8. Kuwayama, Sh. 1908. Die Psylliden Japans. I. *Transactions of the Sapporo Natural History Society*, 2: 149-189.
9. Kwon, Y.J. 1983. Psylloidea of Korea (Homoptera: Sternorrhyncha). *Insecta Koreana*, 2: 1-181.
10. Miyatake, Y. 1963. A revision of the subfamily Psyllinae from Japan. I. (Hemiptera: Psyllidae). *Journal of the Faculty of Agriculture, Kyushu University*, 12: 323-357.
11. Pellizzari, G., Mori, N., Galbero, G. and Antonucci, C. 2005. Risultati di due anni di sperimentazione contro la Psilla dell'Albizzia, *Acizzia jamatonica* (Kuwayama). *Informatore Fitopatologico*, 55: 33-39.
12. Pellizzari, G., Fiorot, L. and Zampini, M. 2008. La psilla dell'Albizzia, *Acizzia jamatonica* (Kuwayama). Veneto Agricoltura Azienda Regionale per i settori Agricolo, Forestale e Agroalimentare. 4 p.
13. Pénzes, B., Rédei, D. and Vétek, G. 2005. The psyllid *Acizzia jamatonica* in Hungary (A selyemakác-levélbolha Magyarországon) *Kertészet és Szőlészet*, 54: 19 (in Hungarian).
14. Rédei, D. and Pénzes, B. 2006. First occurrence of *Acizzia jamatonica* (Kuwayama, 1908) (Sternorrhyncha: Psyllidae: Acizzinae) in Hungary (A selyemakác-levélbolha, *Acizzia jamatonica* (Kuwayama, 1908) (Sternorrhyncha: Psyllidae: Acizziinae) megjelenése Magyarországon) *Növényvédelem*, 42: 153-157 (in Hungarian).
15. Seljak, G. 2003. Asian psyllid threatening albizia (Azijska bolšica ogroža albicije) *Moj mali svet*, 35: 20-21 (in Slovenian).
16. Seljak, G. 2006. An overview of the current knowledge of jumping plant-lice of Slovenia (Hemiptera: Psylloidea). *Acta Entomologica Slovenica*, 14: 11-34.
17. Seljak, G., Imala, M. and Stigter, H. 2004. Three new non-European psyllids (Hemiptera, Psyllidae) in Slovenia and Croatia. Abstracts of the *3rd European Hemiptera Congress*, St. Petersburg, Russia, 8-11 June 2004, p. 66-67.
18. Imala, M., Seljak, G., Poje, I. and Masten, T. 2006. Newly recorded psyllids (Hemiptera, Psyllidae) on woody ornamental plants in Croatia. [Novo zabilježene vrste lisnih buha (Hemiptera, Psyllidae) na drvenastom ukrasnom bilju u Hrvatskoj] *Glasilo biljne zaštite*, 6 (1) – dodatak: 16-17 (in Croatian).
19. Ulyshen, M.D. and Miller, D.R. 2007. First record of *Acizzia jamatonica* (Hemiptera: Psyllidae) in North America: friend or foe? *Florida Entomologist*, 90: 573.
20. Vétek, G. and Rédei, D. 2009. First record of *Acizzia jamatonica* (Kuwayama) (Hemiptera: Psyllidae) in Bulgaria. *Acta Zoologica Bulgarica*, 61: 323-325.
21. Wittenberg, R. (Ed.) 2005. An inventory of alien species and their threat to biodiversity and economy in Switzerland. CABI Bioscience Switzerland Centre report to the Swiss Agency for Environment, Forests and Landscape, 416 p.

Received: 7 December 2009; Accepted: 15 December 2009

ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή του εντόμου *Acizzia jamatonica* (Kuwayama) (Hemiptera: Psyllidae) στην Ελλάδα

B. Pásztor, D. Rédei and G. Vétek

Περίληψη Στην παρούσα εργασία καταγράφεται για πρώτη φορά στην Ελλάδα η παρουσία του εντόμου *Acizzia jamatonica* (Kuwayama) (Hemiptera: Psyllidae) σε ακακίες του είδους *Albizia julibrissin* στην περιοχή της Θεσσαλονίκης. Δίδονται επίσης ορισμένα στοιχεία σχετικά με τη μορφολογία, τη βιολογία, τη διασπορά του εντόμου και τη ζημιά που προκαλεί και συζητούνται οι μέθοδοι για την καταπολέμησή του.

Hellenic Plant Protection Journal **3:** 25-27, 2010

Hellenic Plant Protection Journal **3:** 29-32, 2010

SHORT COMMUNICATION

First data on the occurrence of *Diabrotica virgifera virgifera* Le Conte (Coleoptera: Chrysomelidae) in Greece

A.N. Michaelakis[1], N.T. Papadopoulos[2], S.A. Antonatos[1], K. Zarpas[2] and D.P. Papachristos[1]

Summary The western corn rootworm, *Diabrotica virgifera virgifera* Le Conte (Coleoptera: Chrysomelidae), is a major pest of cultivated corn in North America. It invaded Europe (Belgrade, Serbia) in the early 90s and since then it has rapidly dispersed to several European countries. In 2009, a survey, supported by the Hellenic Ministry of Rural Development and Food in several corn-producing prefectures of Greece, revealed the presence of *D. virgifera virgifera* for the first time in the country. The survey included sampling of young plants, visual inspection of corn fields and deployment of pheromone traps. The western corn rootworm, which, during the survey, was recorded only in pheromone traps, was first detected in the area of Thessaloniki (northern Greece) in July 2009, and subsequently in Serres, Florina and Pieria prefectures.

Additional keywords: pheromone traps, survey, western corn rootworm (WCR), *Zea mays*

The western corn rootworm (WCR), *Diabrotica virgifera virgifera* Le Conte (Coleoptera: Chrysomelidae), is a major pest of cultivated corn, *Zea mays* (maize), in North America (6). Larvae, mainly those of the 3rd instar, destroy the root system of corn plants causing extensive crop losses (7). Adults feed on leaves and stalks but they seldom cause economic losses. In 1992, WCR was detected, for the first time in Europe, in a small maize field near the Belgrade Airport (Serbia) (4). However, it remains unclear how WCR entered Europe. Gray *et al*. (6) assumed that an accidental transport of WCR adults by commercial aircraft was most likely the cause, as the major airports for intercontinental flights in the United States are located near large maize production areas and the first detection of WCR in Europe was in an area close to Belgrade airport (Serbia). WCR adults may also be carried as contaminants on other means of transport (e.g. boats, trains, trucks, cars, etc). By 2007, WCR was reported in 20 European countries (5, 8). The most significant dispersions of WCR in terms of infested corn-producing areas were reported in Hungary (93,000 km^2), Serbia and Montenegro (73,000 km^2) and Romania (65,000 km^2) (8).

Due to the economic importance of the pest to the maize crop, the European Commission (EC) has implemented measures aiming at preventing the spread of WCR within the Community. In 2003, surveys and eradication measures were established by the Decision 2003/766/EC (1). In 2006, those measures was supplemented by the Decision 2006/564/EC, which introduced additional requirements for the containment of *D. virgifera virgifera* in the infested zones to limit the further spread of the pest (2). The EC Recommendation 2006/565/EC (3) made it possible to switch from an eradication policy to a containment policy.

In 2009, an official and extensive survey, supported by the Hellenic Ministry of Rural

[1] Department of Entomology and Agricultural Zoology, Benaki Phytopathological Institute, 8 St. Delta str., GR-145 61 Kifissia (Athens), Greece
[2] Laboratory of Entomology and Agricultural Zoology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Phytokou str., GR-384 46 N. Ionia, Volos, Magnisia, Greece
Corresponding author: d.papachristos@bpi.gr

Development and Food, was conducted in order to determine the presence and distribution of the pest in Greece. The present study reports on the major findings of this survey.

In order to determine the occurrence of WCR, 174 pheromone traps (CsalMTA NKI ♀m♂ N®, KLPfero+ registered trademark of the Plant Protection Institute, Hungarian Academy of Science) were placed in most of the maize-producing areas in Greece (total of 31 prefectures). Traps were set up into maize fields in mid-June 2009 and were inspected every 10 to 15 days until the end of September 2009. Pheromone lures were replaced every 5 weeks. Captured beetles were identified following the EPPO diagnostic protocol (9). Confirmation of the original identification was done by Dr Sharon Shute in Natural History Museum of London, UK, where specimens of adults have been deposited. Specimens of the captured WCR adult beetles have also been deposited at the Laboratory of Entomology of the Benaki Phytopathological Institute and at the Laboratory of Entomology and Agricultural Zool-

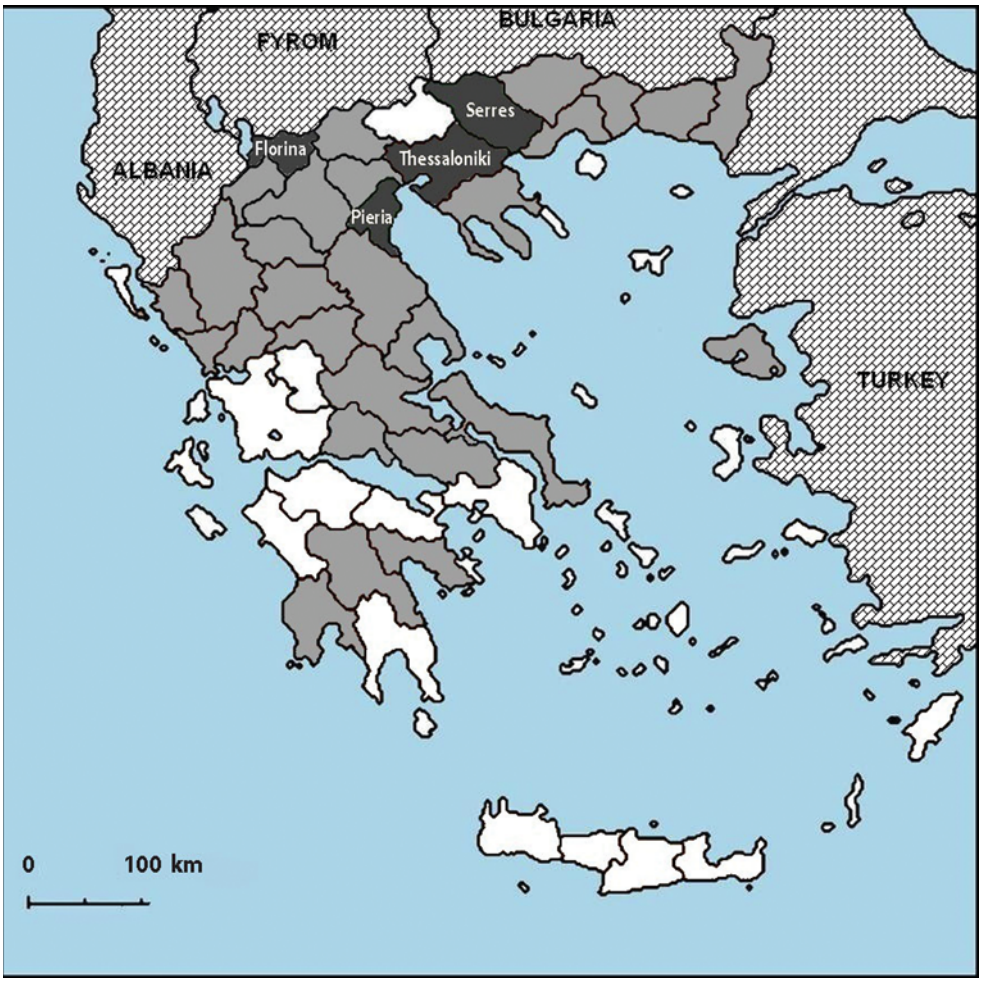


Figure 1. Prefectures of Greece where surveys were conducted (gray and black color) and prefectures where the presence of *Diabrotica virgifera virgifera* Le Conte (western corn rootworm, WCR) (Coleoptera: Chrysomelidae) was recorded (black color).

ogy at the University of Thessaly.

WCR adults were captured in four out of the 31 prefectures, where pheromone traps had been placed i.e. Thessaloniki, Serres, Florina and Pieria (Fig. 1). The first capture was noticed on 13 July 2009 and the last on 30 August 2009. All four prefectures, where WCR was detected, are in the Region of Macedonia (northern Greece) bordering FYROM and Bulgaria. Detections were reported in corn fields neighboring main roads connecting northern Greece with other Balkan countries. Based on the captures of WCR adults in the pheromone traps, the population of WCR adults was considered to be rather low in all four areas. The highest captures were detected in Florina (201 adults captured in 4 sites) and the lowest in Serres (7 adults captured in 6 sites) (Table 1).

Based on the results of the survey, the local authorities of the four prefectures, where WCR was detected, are in the process of identifying zones, a focus zone and a surrounding safety zone, where rotation of maize with other crops will be followed. Moreover, to better define the spread and distribution of WCR in Greece, a more extensive and intensive trapping network will be established in 2010 in both the demarcated zones and neighbouring regions.

Table 1. Captures of *Diabrotica virgifera virgifera* Le Conte (western corn rootworm, WCR) (Coleoptera: Chrysomelidae) in pheromone traps, placed in maize fields in Greece during the period June-September 2009.

Area (Prefecture)	Number of monitoring sites (number of traps)	Number of positive traps	Total number of WCR adults trapped
Thessaloniki	4 (4)	3	51
Pieria	2 (2)	1	15
Serres	13 (13)	6	7
Florina	12 (12)	4	201
Others	143 (143)	0	0

The current study was supported by the Hellenic Ministry of Rural Development and Food. We are grateful to the phytosanitary inspectors who carried out much of the field work.

Literature Cited

1. Anonymous 2003. Commission Decision 2003/766/EC of 24 October 2003 on emergency measures to prevent the spread within the Community of *Diabrotica virgifera* Le Conte. *Official Journal of the European Union* L 275: 49-50.
2. Anonymous 2006a. Commission Decision 2006/564/EC amending Decision 2003/766/EC of 11 August 2006 on emergency measures to prevent the spread within the Community of *Diabrotica virgifera* Le Conte. *Official Journal of the European Union* L 225: 28-29.
3. Anonymous 2006b. Commission Recommendation 2006/565/EC of 11 August 2006 on containment programmes to limit the further spread of *Diabrotica virgifera* Le Conte in Community areas where its presence is confirmed. *Official Journal of the European Union* L 225: 30-31.
4. Bača, F. 1994. New member of the harmful entomofauna of Yugoslavia *Diabrotica virgifera virgifera* Le Conte (Coleoptera: Chrysomelidae). *Zaštita bilja*, 45: 125-131.
5. Edwards, C.R. and Kiss, J. 2007. New WCR 2006 general spread map for Europe. *IWGO Newsletter* 28: 3.
6. Gray, M.E., Sappington, T.W., Miller, N.J., Moeser, J. and Bohn, M.O. 2009. Adaptation and invasiveness of Western Corn Rootworm: Intensifying research on a worsening pest. *Annual Review of Entomology,* 54: 303- 321.
7. Kahler, A.L., Olness, A.E., Sutter, G.R., Dybing, C.D. and Devine, O.J. 1985. Root damage by western corn rootworm and nutrient content in maize. *Agronomy Journal,* 77: 769–774.
8. Kiss, J., Edwards, C.R., Berger, H.K., Cate, P., Cean, M., Cheek, S., Derron, J., Festic, H., Furlan, L., Igrc-Barcic, J., Ivanova, I., Lammers, W., Omelyuta, V., Princzinger, G., Reynaud, P., Sivcev, I., Sivicek, P., Urek, G. and Vahala, O. 2005. Monitoring of western corn rootworm (*Diabrotica virgifera virgifera* Le Conte) in Europe 1992–2003. In: Vidal, S., Kuhlmann, U. and Edwards, C.R. (eds). *Western Corn Rootworm: Ecology and Management*. Cambridge, MA, USA: CABI Publishing, p. 29–39.
9. OEPP/EPPO. 2004. Diagnostic protocols for regulated pests. *Diabrotica virgifera. Bulletin OEPP/EPPO Bulletin* 34: p. 289-293.

Received: 30 December 2009; Accepted: 22 January 2010

ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτα δεδομένα για την παρουσία του εντόμου *Diabrotica virgifera virgifera* Le Conte (Coleoptera: Chrysomelidae) στην Ελλάδα

Α.Ν. Μιχαηλάκης, Ν.Θ. Παπαδόπουλος, Σ.Α. Αντωνάτος, Κ. Ζάρπας και Δ.Π. Παπαχρήστος

Περίληψη Το έντομο *Diabrotica virgifera virgifera* Le Conte (Coleoptera: Chrysomelidae) αποτελεί έναν από τους σημαντικότερους εχθρούς για την καλλιέργεια του αραβόσιτου στη Βόρεια Αμερική. Από την πρώτη καταγραφή της παρουσίας του στην Ευρώπη το 1992 στην περιοχή του Βελιγραδίου της Σερβίας το *D. virgifera virgifera* εξαπλώθηκε μέσα σε σύντομο χρονικό διάστημα σε πολλές Ευρωπαϊκές χώρες. Εκτενείς και εντατικές προσπάθειες που πραγματοποιήθηκαν το 2009 με την υποστήριξη του Υπουργείου Αγροτικής Ανάπτυξης και Τροφίμων οδήγησαν στην καταγραφή της παρουσίας του *D. virgifera virgifera* για πρώτη φορά στην Ελλάδα στην περιοχή της Θεσσαλονίκης τον Ιούλιο του 2009 και στη συνέχεια στους νομούς Σερρών, Φλώρινας και Πιερίας. Το έντομο εντοπίσθηκε με τη χρήση φερομονικών παγίδων που εγκαταστάθηκαν στις περιοχές όπου καλλιεργείται αραβόσιτος σε συνεργασία με το Υπουργείο Αγροτικής Ανάπτυξης και Τροφίμων στα πλαίσια του προγράμματος των επίσημων επισκοπήσεων για οργανισμούς καραντίνας.

Hellenic Plant Protection Journal **3:** 29-32, 2010

Τόμος 3, Τεύχος 1, Ιανουάριος 2010
ISSN 1791-3691

Περιεχόμενα

Volume 3, Issue 1, January 2010
ISSN 1791-3691

Contents

Hellenic Plant Protection Journal

www.bpi.gr