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SHORT COMMUNICATION

Mosquito (Diptera: Culicidae) fauna in natural breeding sites of Attica basin, Greece

I. Kioulos[1,2*], A. Michaelakis[3], N. Kioulos[4], A. Samanidou-Voyadjoglou[5] and G. Koliopoulos[6]

Summary: Mosquito larvae from natural breeding sites within Attica basin were collected in 15-days intervals from March 2007 to December 2008. The two – year study revealed eight different mosquito species which belong to three different genera: *Anopheles maculipennis* complex, *Anopheles claviger, Culex pipiens, Culex hortensis, Culex theileri, Culex territans, Culex impudicus* and *Culiseta longiareolata*. Three additional species were caught as adults (*Ochlerotatus zammitii*, *Aedes cretinus* and *Aedes albopictus*). *Culex pipiens* and *Culiseta longiareolata* were found in almost all the breeding sites sampled. Potential threats for public health from the above mentioned species are *An. maculipennis*, *Cx. pipiens*, and *Ae. albopicuts*.

Additional keywords: Attica, Greece, larvae, mosquito

The first annotated list of Greek mosquito species by Samanidou-Voyadjoglou and Darsie (1993) was based mainly on data from the literature or specimens retrieved from the National School of Public Health Museum (Athens, Greece). The list contains seven genera, 15 subgenera, 53 species and two subspecies. Soon after, three more records (*Aedes berlandi*, *Aedes annulipes* and *Culex pusillus*) were added to the list (Samanidou-Voyadjoglou & Darsie, 1993). Scattered references basically based on newly discovered species and confirmation by others followed. Kaiser *et al*. (2001) presented 4 new country records from northern Greece (*Ochlerotatus sticticus*, *Oc. pullatus*, *Oc. punctor* and *Oc. cataphylla*), whereas the presence of invasive species of medical importance (*Culex tritaeniorhynchus* and *Aedes albopictus*) was confirmed later (Samanidou & Harbach, 2003; Samanidou-Voyadjoglou *et al*., 2005).

The urban area of the Greek capital, Athens, extends beyond the administrative municipal city limits into the basin of Attica with a population of 5 million habitats, which is approximately half of the total population of the country (EL. STAT, 2012). The current study aims at some qualitative data regarding the mosquito fauna of Athens and its surroundings after the Olympic Games of 2004, when substantial changes in the infrastructure resulted in changes at the geographical landscape and the associated micro climates of Attica (Tziralis *et al*., 2005).

Natural breeding sites of mosquito larvae in the basin of Attica were surveyed for the presence of mosquito species, especially those of medical importance, from March 2007 to December 2008. The study area

[1] Directorate of Plant Produce Protection, Ministry of Rural Development and Food, 150 Syggrou Avenue, GR-176 71 Kallithea, Attica, Greece
[2] Laboratory of Pesticide Science, Agricultural University of Athens, 75 Iera Odos Str., GR-118 55 Athens, Greece
[3] Laboratory of Agricultural Entomology, Department of Entomology and Agricultural Zoology, BenakiPhytopathological Institute, Greece, 8 St. Delta Str., GR-145 61 Kifissia, Attica, Greece
[4] Directorate of Public Health, Prefecture of Korinthos, GR-201 00 Korinthos, Greece
[5] Entomology and Tropical Diseases Laboratory, National School of Public Health, Greece
[6] Laboratory of Biological Control of Pesticides, Benaki Phytopathological Institute, 8 St. Delta Str., GR-145 61 Kifissia, Attica, Greece
* Corresponding author: kioulose@yahoo.ca

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is defined by natural borders: four major mounts (mount Imittos in the East, mount Parnitha in the North, mount Penteli in the North-east and mount Oros-Aegaleo in the West) and the Saronikos Gulf in the South (Figure 1).

The potential natural larval habitats were mapped and eight representative sampling sites were selected, also taking into account accessibility standards of each site: Pikrodafni, Podoniftis, Kokkinos Mylos, Profitis Ilias, Ivis, Maroussi, Chelidonou, Kato Kifissia (Figure 1). Sampling for mosquito larvae was conducted with a 350 ml dipper once every two weeks. Mosquito larvae were then transferred to Benaki Phytopathological Institute (Laboratory of Insecticides of Public Health Importance, Athens-Greece) and to the National School of Public Health (Laboratory of Entomology and Tropical Diseases, Athens-Greece), and were reared to adults (T= 25±2°C, PH= 14:10 L:D). A few adult specimens were also caught (using mouth aspirator) during larval sampling visits. All specimens were identified to species in the adult stage according to identification keys (Harbach, 1985; Glick, 1992; Darsie & Samanidou-Voyadjoglou, 1997; Samanidou-Voyadjoglou & Harbach, 2001).

Larval surveys revealed 7,896 mosquito specimens classified to eight different species. During the entire study period, larvae of *Cx. pipiens* and *Cs. longiareolata* were constantly recorded in all sampling sites. Four more *Culex* species (*Cx.hortensis*, *Cx. theileri*, *Cx. territans*, and *Cx.impudicus*) were found sporadically in two different sampling sites (Ivis and Profitis Ilias, Figure 1). *Culex territans* seems to appear late in the summer and in early autumn (September 2007 and August – October 2008, Table 1), whereas *Cx. hortensis*, *Cx. theileri* and *Cx. impudicus* occur during the summer months (June – August, 2007 and 2008, Table 1). *Anopheles*

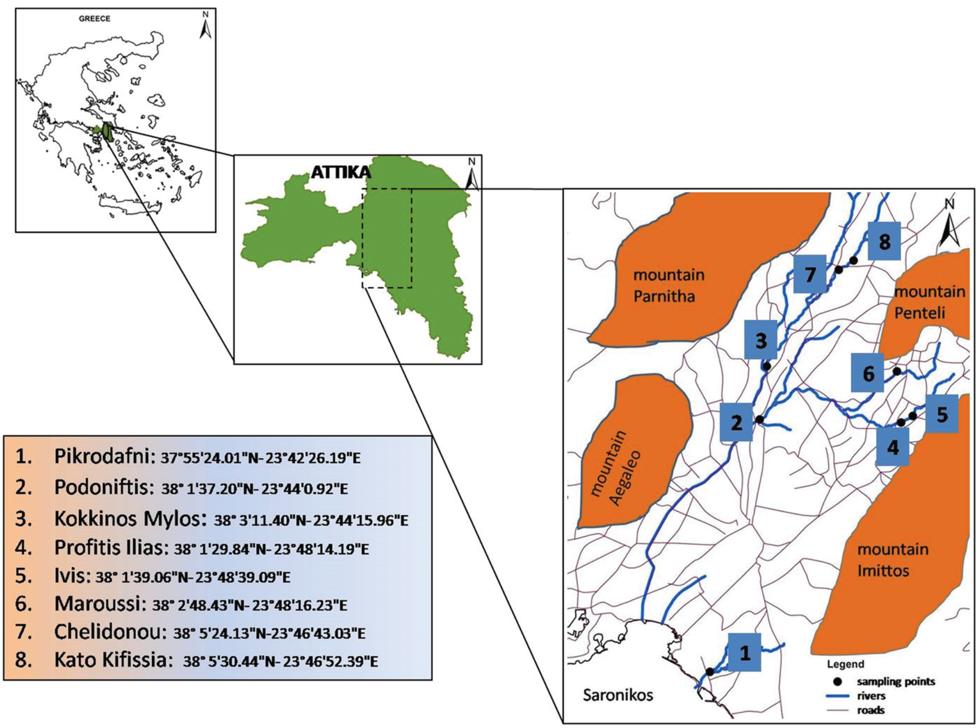

Figure 1. Geographical distribution of collection sites of mosquito species sampled as larvae from natural breeding sites within the Attica basin from March 2007 to December 2008.

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Table 1. Mosquito species sampled as larvae from natural breeding sites within the Attica basin from March 2007 to December 2008.

	2007										2008											
	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
Cx. pipiens	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Cx. territans	-	-	-	-	-	-	√	-	-	-	-	-	-	-	-	-	-	√	√	√	-	-
Cx. impudicus	-	-	-	-	-	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cx. hortensis	-	-	-	√	√	√	-	-	-	-	-	-	-	-	-	√	√	-	-	-	-	-
Cx. theileri	-	-	-	-	√	-	-	-	-	-	-	-	-	-	-	√	-	√	-	-	-	-
An. maculipennis	-	-	-	√	√	√	-	-	-	-	-	-	-	-	√	√	√	-	-	-	-	-
An. claviger	√	√	√	-	√	-	√	-	√	√	-	√	√	√	-	-	√	-	√	√	-	√
Cs. longiareolata	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

(√:denotes presence)

maculipennis complex and *An. claviger* were present in three (Chelidonou, Ivis, Profitis Ilias, Figure 1) and four (Kokkinos Mylos, Ivis, Profitis Ilias, Kato Kifissia, Figure 1) sampling sites, respectively, and their highest larval population densities were recorded also in the summer period (July - August 2007 and 2008). The presence of *An. claviger* larvae during the winter months indicates that this species overwinters at the larval stage in the Attica region, a fact that coincides with findings by Becker *et al.* (2010).

Adult specimens of *Ochlerotatus zammitii* (Pikrodafni, Figure 1), *Aedes cretinus* (Chelidonou, Ivis and Profitis Ilias, Figure 1) and *Aedes albopictus* (Podoniftis, Figure 1) were also caught during the sampling period while females of these three species tried to bite. The two last species (*Ae. cretinus* and *Ae. albopictus*) belong to genus *Stegomyia* and were never sampled as larvae or pupae during the present study. Their larvae develop in tree-holes, phytotelmata and other artificial containers such as tires, barrels, cans etc (Reiter & Sprenger, 1987; Grist, 1993; Simard *et al.*, 2005). *Aedes albopictus* was firstly reported in northwestern Greece in 2003 (Samanidou-Voyadjoglou *et al.*, 2005). It is a species of great medical importance since it can transmit at least 24 diseases, among which dengue and dengue hemorrhagic fever (Mitchell, 1995; Lundstrom, 1999).

Even though Attica is a densely urbanized and populated city, the current study revealed that medical important mosquito species, such as *Cx. pipiens* and *An. maculipennis* may proliferate in the remaining natural breeding sites. These species are active for several months of the year threatening a large amount of the population in many areas of Attica. A mosquito vector surveillance and population monitoring program should be established including the surveillance of invasive species (ECDC, 2012).

Literature cited

Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M., Dahl, C., and Kaiser, A. 2010. Mosquitoes and their control, Verlag Berlin Heidelberg. 2nd edn. 577pp.

Darsie, R.F.J. and Samanidou-Voyadjoglou, A. 1997. Keys for the identification of the mosquitoes of Greece. *Journal of the American Mosquito Control Association,* 13: 247-254.

ECDC. 2012. European Centre for Disease Prevention and Control: Guidelines for the surveillance of invasive mosquitoes in Europe, Stockholm. 95pp.

EL. STAT. 2012. Hellenic Statistical Authority.Statistical information about Greece available on internet (last visit 18/11/2012) www.statistics.gr/portal/page/portal/ESYE/BUCKET/A1602/PressReleases/A1602_SAM01_DT_DC_00_2011_01_F_EN.pdf

Glick, J. 1992. Illustrated key to the female *Anopleles* of Southwestern Asia and Egypt (Diptera: Culicidae). *Mosquito Systematics,* 24(2): 125-153.

Grist, N. 1993. *Aedes albopictus*: the tyre-travelling tiger. *Journal of Infection,* 27(1): 1-4.

Harbach, R.E. 1985. Pictorial keys to the genera of

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mosquitoes, subgenera of *Culex* and the species of *Culex* (*Culex*) occuring in southwestern Asia and Egypt, with a note on the subgeneric placement of *Culex deserticola* (Diptera: Culicidae). *Mosquito Systematics,* 17(2): 83-107.

Kaiser, A., Jerrentrup, H., Samanidou-Voyadjoglou, A. and Becker, N. 2001. Contribution to the distribution of European mosquitoes (Diptera: Culicidae): four new country records from nothern Greece. *European Mosquito Bulletin,* 10(9): 9-12.

Lundstrom, J. 1999. Mosquito-borne viruses in Western Europe: a review. *Journal of Vector Ecology,* 24: 1-39.

Mitchell, C.J. 1995. Geographical spread of *Aedes albopictus* and potential for involvement in arbovirus cycles in the Mediterranean basin. *Journal of Vector Ecology,* 20: 44-58.

Reiter, P. and Sprenger, D. 1987. The used tire trade: a mechanism for the worldwide dispersal of container breeding mosquitoes. *Journal of the American Mosquito Control Association,* 3: 494-501.

Samanidou-Voyadjoglou, A. and Darsie, R.F.J. 1993. New country records for mosquito species in Greece. *Journal of the American Mosquito Control Association,* 9(4): 465-466.

Samanidou-Voyadjoglou, A. and Harbach, R.E. 2001. Keys to the adult female mosquitoes (Culicidae) of Greece. *European Mosquito Bulletin,* 10: 13-20.

Samanidou-Voyadjoglou, A., Patsoula, E., Spanakos, G. and Vakalis, N. 2005. Confirmation of *Aedes albopictus* (Skuse) (Diptera: Culicidae) in Greece. *European Mosquito Bulletin,* 19: 10-11.

Samanidou, A. and Harbach, R.E. 2003. *Culex* (*Culex*) *tritaeniorhynchus* Giles, a newly discovered potential vector of arbovirus in Greece. *European Mosquito Bulletin,* 16: 15-17.

Simard, F., Nchoutpouen, E., Toto, J-C. and Fontenille, D. 2005. Geographic distribution and breeding site preference of *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae) in Cameroon, Central Africa. *Journal of Medical Entomology,* 42(5): 726-731.

Tziralis, G., Tolis, A., Tatsiopoulos, I. and Aravossis, K.G. 2005. Economic aspects and the sustainability impact of the Athens 2004 Olympic Games: Environmental Economics and Investment Assessment (ed. by K Aravossis, CA Brebbia, E Kakaras & AG Kungolos) WIT press, pp. 21-33.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Καταγραφή της πανίδας των κουνουπιών (Diptera: Culicidae) σε φυσικές εστίες του λεκανοπεδίου της Αττικής

Η. Κιούλος, Α. Μιχαηλάκης, Ν. Κιούλος, Α. Σαμανίδου-Βογιατζόγλου και Γ. Κολιόπουλος

Περίληψη Πραγματοποιήθηκαν δειγματοληψίες ατελών σταδίων κουνουπιών από φυσικές εστίες στο λεκανοπέδιο της Αττικής από το Μάρτιο του 2007 έως και το Δεκέμβριο του 2008 κάθε 15 μέρες. Οι φυσικές εστίες που επιλέχθηκαν βρίσκονται εντός του λεκανοπεδίου Αττικής όπως αυτό ορίζεται από του ορεινούς όγκους της Πάρνηθας, του Υμηττού, του Όρους Αιγάλεω, της Πεντέλης και της θαλάσσιας ζώνης του Σαρωνικού κόλπου. Καταγράφηκαν συνολικά 8 είδη κουνουπιών που ανήκουν στα γένη *Anopheles*, *Culex*, και *Culiseta*. Αναλυτικότερα, τα είδη που αναγνωρίστηκαν ήταν τα ακόλουθα: *Anopheles maculipennis, Anopheles claviger, Culex pipiens, Culex hortensis, Culex theileri, Culex territans, Culex impudicus, Culiseta longiareolata.* Εκτός των ειδών αυτών, τρία ακόμα είδη συλλέχθηκαν ως ακμαία (*Aedes cretinus, Aedes albopictus* και *Ochlerotatus zammitii*), χωρίς όμως να εντοπιστούν τα ατελή τους στάδια κατά τη διάρκεια αυτής της μελέτης. Από όλα τα παραπάνω είδη, σημαντικό υγειονομικό ενδιαφέρον παρουσιάζουν τα είδη *Anopheles maculipennis*, *Aedes albopictus* και *Culex pipiens* γεγονός που καθιστά αναγκαία τη διαρκή παρακολούθηση των πληθυσμών τους καθώς και τον σχεδιασμό και την εφαρμογή κατάλληλων μέτρων καταπολέμησης.

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Enhancement of tomato growth, yield and resistance to the root-knot nematode (*Meloidogyne javanica*) after the field application of *Saccharomyces cerevisiae*

M.R. Karajeh

Summary Effects of the yeast fungus *Saccharomyces cerevisiae* on tomato cv. Asala and its interaction with the root-knot nematode *Meloidogyne javanica* were evaluated under field conditions. The yeast was equally effective as the reference nematicide (a.i. oxamyl). It was more effective than hydrogen peroxide when applied as a rhizospheric soil drench leading to a significant reduction of root galling and nematode reproduction ability as compared to the untreated control. Furthermore, field application of *S. cerevisiae* improved tomato growth and yield and increased nematode resistance of tomato cv. Asala through increasing its root total phenolic content in a similar way as exogenously applied hydrogen peroxide.

*Additional Keywords***:** pest management, *Solanum lycopersicum*, vegetable crops

Introduction

Tomato (*Solanum lycopersicum* L.) is the most economically important and widely cultivated vegetable crop in Jordan. However, yield is reduced by many fungal, bacterial, viral and nematode diseases especially by the root knot disease caused by the root-knot nematode (RKN), *Meloidogyne* spp., e.g. *M. javanica,* which is the most common species in Jordan (Abu-Gharbieh *et al.*, 2005; Karajeh and Al-Nasir, 2013).

The application of nematicides to control plant-parasitic nematodes is expensive and greatly hazardous for the environment and humans (Thomason, 1987). Thus, there is a need to develop alternative control measures to manage plant-parasitic nematode under field conditions. Hydrogen peroxide (H_2O_2) has been reported as relatively environmentally safe and has been used as soil drench for nematode control (Jansen *et al.* 2002; Karajeh, 2008). The application of biological agents may enhance crop production and induce resistance to many diseases. *Saccharomyces cerevisiae* is considered a promising nematode controlling factor that recently became a possible alternative to chemical fertilizers, safe for humans, animals and the environment (Akhtar and Alam, 1990; Omran, 2000). The yeast is a natural growth stimulator due to it's high content in proteins, carbohydrates, nucleic acid, lipids, vitamins and various minerals (Warring and Philips, 1973) and its enhancement of phosphorus and manganese uptake by plant roots (Mekki and Ahmed, 2005). The potential use of yeast fungi as bio-control agents of soil-borne plant pathogens and plant growth promoters has recently been investigated (Ismail *et al.*, 2005; El-Tarabily and Sivasithamparam, 2006; Azzam *et al.*, 2012; Karajeh, 2013). The yeast fungus *S. cerevisiae* reduced *M. incognita* infection of the Egyptian henbane, *Hyoscyamus muticus* and increased its growth (Youssef and Soliman, 1997). *Saccharomyces cerevisiae* was able to suppress the population of *M. javanica* and root gall formation on cucumber through its effect on nematode infectivity and reproduction and through inducing plant resistance and enhancing fruit production of cucumber under field conditions (Karajeh,

Plant Protection and IPM Department, Faculty of Agriculture, Mutah University, P.O. Box 7, Karak, 61710, Jordan
E-mail: muwaffaq@mutah.edu.jo

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2013).

The present study was conducted to investigate and compare the effects of *S. cerevisiae*, H_2O_2 and oxamyl on growth and *M. javanica* management of tomato under field conditions.

Materials and Methods

A root-knot nematode-infested field previously planted with tomato was selected for conducting a field experiment from mid-February to mid-July 2013, which is the main tomato growing season in Karak Valley region (about 200mm annual rainfall, clay loam soil, ca. 200m above sea level) in Karak Province of Jordan, which is a drip-irrigated agricultural area. The field population of RKN was identified as *M. javanica* by observing the perineal patterns of females and measuring the length of second-stage juveniles (Barker *et al*., 1985). Species identification was confirmed by *Meloidogyne* species-specific sequence-characterized amplified region-polymerase chain reaction (SCAR-PCR) test (Karajeh, 2004).

Three-week-old tomato seedlings about 10cm tall of cv. Asala (Eastern Company, Amman, Jordan) were transplanted into black plastic mulch covered rows with 80cm width and 150cm spacing between rows. One week after transplanting, the following treatments were applied into a randomized complete block design and each treatment (7 plants per plot) was replicated five times:

1. *Saccharomyces cerevisiae* (ready-made yeast, Yeast Industries Company, Jordan, $1X10^8$ CFU/g) was added to the rhizospheric soil at the rate of 10g/plant.
2. Hydrogen peroxide (technical grade) was added to the rhizospheric soil at 10mM concentration.
3. Oxamyl (Vydate®L, 24% v/v, Dupont, Delaware, U.S.A.) was added as soil drench into the rhizospheric soil at 3.5 L a.i./ha within its manufacture's recommended application range (2.88-4.80 L a.i./ha) for vegetables.
4. Untreated (control): plants were irrigated with water only.

The treatments were repeated twice after two and four week intervals from the first application. Traditional agricultural practices (drip irrigation, weeding, air-borne pest control and fruit harvesting) were carried out according to technical recommendations in tomato cultivation (Werner, 1981). For the determination of chlorophyll content, fresh leaf samples (about 0.5 g) were immersed after grinding in 5 ml of 100% acetone for 2 min to extract pigments. The absorbance of extracts was measured at 645 and 663 nm using the ultra-violet light–spectrophotometer. The amount of total chlorophyll (chlorophyll a + chlorophyll b) was evaluated using a standard curve determined under the same conditions (Hamid and Jawaid, 2009).

Fruit harvest started eight weeks after transplanting. Total tomato yield per treatment was recorded during the growing season. At the end of the experiment, the plants were up-rooted. Shoot and root dry weights were recorded. Root galling index was evaluated according to the 0–5 scale: 0: no galling, 1: 1–2, 2: 3–10, 3: 11–30, 4: 31–100 and 5 over 100 galls (Taylor and Sasser, 1978). Random root samples (five samples per treatment) were selected for measuring root total phenol content and stored in a deep freezer until use. The amount of total phenolics in roots was determined with the Folin-Ciocalteu reagent procedure (Maurya and Singh, 2010). Gallic acid was used as a standard and total phenolics were expressed as mg/g gallic acid equivalents. Representative rhizospheric soils were collected from each treatment for nematode extraction using the Baermann tray method (Whitehead and Hemming, 1965) to estimate the final nematode population number.

Statistical analysis

Data were analyzed statistically using general linear model (GLM) procedure (SPSS software version 11.5; SPSS Inc., Chicago, USA). Least significance difference (LSD) test was used for mean separation at the 0.05 probability level.

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Results

The results of the study revealed a significant increase in tomato cv. Asala shoot and root dry weights after the application of yeast fungus (*S. cerevisiae*) at about 25.5 and 37.0 % more than the untreated control. In contrast, H_2O_2 or the nematicide did not significantly cause any increase (Figure 1A and B). Total tomato yield was significantly high-

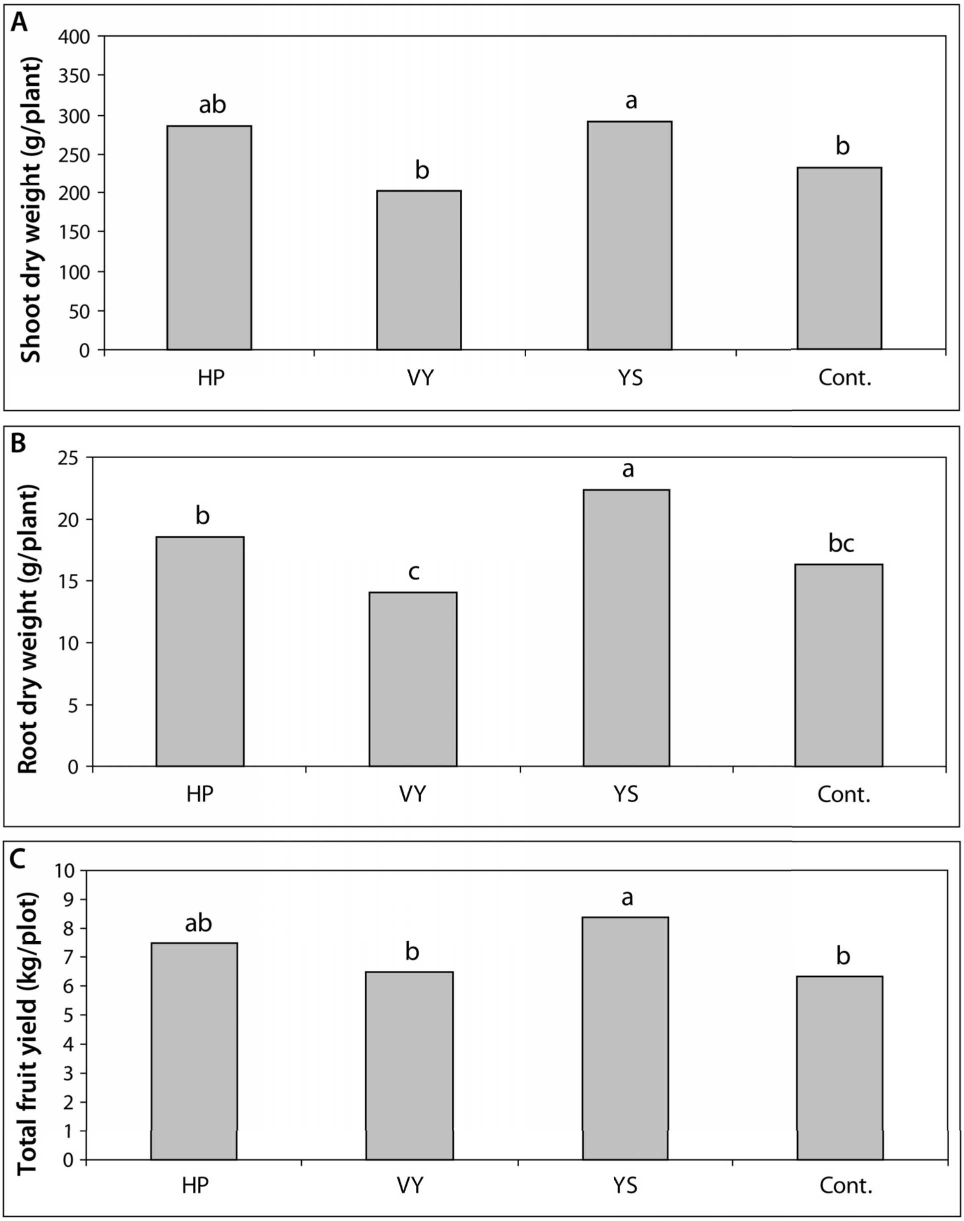

Figure 1. Effects of different treatments [HP: hydrogen peroxide, VY: Vydate (oxamyl), YS: yeast (*Saccharomyces cerevisiae*)] on (A) shoot dry weight, (B) root dry weight and (C) total fruit yield in tomato.

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er for the yeast treatment than that of the control followed by H_2O_2 and the nematicide treatments (Figure 1C). There was no significant variation in chlorophyll content of leaves due to the treatments (Figure 2 A). The highest significant level of total phenolics in roots of the tomato cv. Asala plants treated with H_2O_2 followed by plants treated with the yeast, which caused significant but lower increase than H_2O_2. There was no variation between oxamyl treated plants and non-treated control plants in their phenol content (Figure 2B).

With respect to root-galling reduction, the yeast treatment was similar to oxamyl followed by the H_2O_2 treatment (Figure 3A). Both yeast and oxamyl treatments significantly reduced *M. javanica* final populations in the rhizospheric soil as compared to the untreated controls. Oxamyl was significantly better than the yeast in decreasing the nematode population. However, H_2O_2 treatment did not significantly affect the final populations under field conditions (Figure 3B).

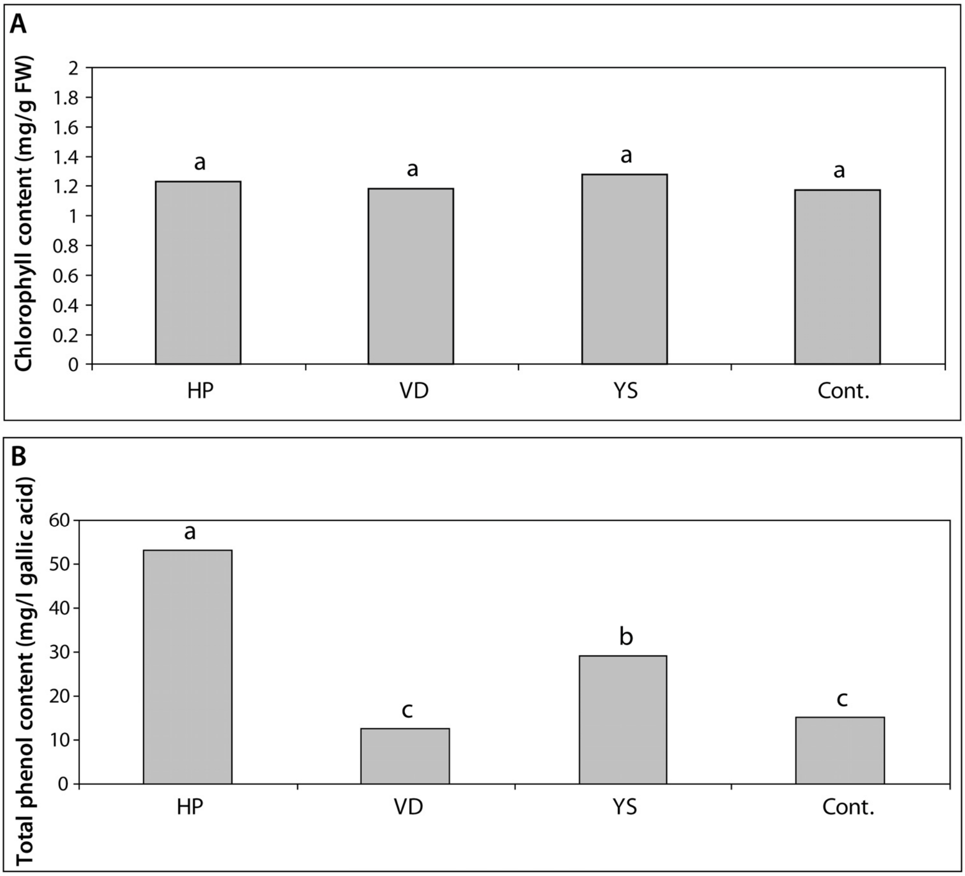

Figure 2. Effects of different treatments [HP: hydrogen peroxide, VD: Vydate (oxamyl), YS: yeast (*Saccharomyces cerevisiae*)] on (A) leaf chlorophyll content and (B) root total phenol content in tomato.

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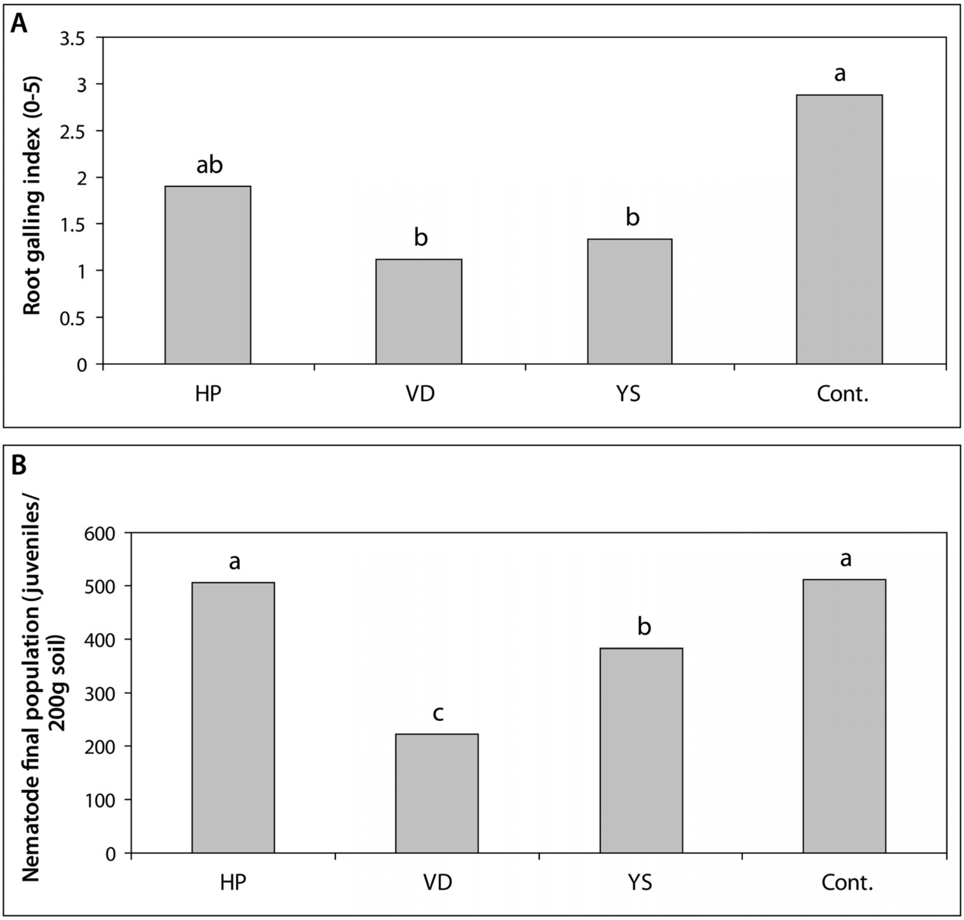

Figure 3. Effects of different treatments [HP: hydrogen peroxide, VD: Vydate (oxamyl), YS: yeast (*Saccharomyces cerevisiae*)] on (A) root galling caused by the root-knot nematode (*Meloidogyne javanica*) and (B) on its reproduction on tomato.

Discussion

Chemical control using nematicides to manage plant-parasitic nematodes is usually an expensive and environmentally hazardous approach and may also negatively affect agricultural soil beneficial microflora. Therefore, searching for nematicide alternatives to manage plant-parasitic nematodes would be crucial. Some microbial agents have been reported as suppressive for nematodes especially RKNs (Youssef and Soliman, 1997; Goswami *et al*., 2008; Jamshidnejad *et al.* 2013).

The yeast fungus *S. cerevisiae* was similarly effective as the systemic nematicide oxamyl when applied as a drench on the rhizospheric soil region, causing a significant decrease in tomato root galling and reducing the reproduction ability of *M. javanica* on tomato roots under the field conditions of Karak Valley region of Jordan. The yeast was also able to reduce infectivity and populations of *M. javanica* on cucumber (Karajeh, 2013). As previously reported, the application of 120g/tree of dry active yeast *S. cerevisiae* FT 700 greatly reduced the populations of *Pratylenchus zeae* and *Helicotylenchus exallus* in both soil and roots of Jasmine, *Jasminum grandiflorum* L. (Ismail *et al*., 2005).

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In addition, as documented by Noweer and Hasabo (2005), a commercial product of *S. cerevisiae* significantly reduced the number of *M. incognita* second-stage juveniles in soil and root galling in squash under field conditions of Egypt, when applied at the rate of 5 g/plant. Furthermore, the yeast was generally more effective at 10 than 5g/l rate in reducing *M. javanica* infection and reproduction on cucumber and promoting its growth (Karajeh, 2013). The suppressing effect of the yeast on *M. javanica* may be due to the ability of the fungus to convert carbohydrates to ethyl alcohol and CO_2 that are toxic to nematodes (Noweer and Hasabo, 2005). Furthermore, *S. cerevisiae* has been effective against soil-borne plant-pathogenic fungi, *i.e. Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina* and *Fusarium solani* (Attyia and Youssry, 2001), and has also effectively controlled *F. oxysporum*, the causal agent of sugar beet damping-off (Shalaby and El-Nady, 2008).

On the other hand, the yeast treatment was able to improve growth and increase fruit yield of tomato cv. Asala when applied at the same rate (10g/l). Furthermore, *S. cerevisiae* was able to promote growth of sugar beet (Shalaby and El-Nady, 2008), squash yield (Noweer and Hasabo, 2005), cucumber growth and yield (Karajeh, 2012) and growth and yield of Egyptian henbane, *Hyoscyamus muticus,* infected with *M. incognita* (Youssef and Soliman, 1997).

The yeast was able to increase plant resistance of tomato cv. Asala to *M. javanica* infection through increasing its root total phenolic content similarly to exogenous H_2O_2 application. The mechanism behind the biocontrol activity of *S. cerevisiae* may also include nutrient and site competition and induced resistance and/or make physical and chemical soil properties unfavorable for plant pathogens (Ahmed *et al.*, 1972; Alam *et al.*, 1977; Sitaramaiah and Singh, 1978; Noweer and Hasabo, 2005).

In conclusion, the application of the yeast *S. cerevisiae* could suppress the infection and population of *M. javanica* on tomato cv. Asala and increase its resistance and improve tomato root growth and yield under field conditions.

Literature cited

Abu-Gharbieh, W.I., Karajeh, M.R. and Masoud, S.A. 2005. Current distribution of the root-knot nematodes (*Meloidogyne* species and races) in Jordan. *Jordan Journal of Agricultural Sciences,* 1(1): 43-48.

Ahmed, R., Khan, A.M. and Saxena, S.K. 1972. Changes resulting from amending the soil with oilcakes and analysis of oilcakes. Proc. 59^{th} Ses. Indian Sci. Cong., Calcutta, Part3: 164 (abstract).

Akhtar, M. and Alam, M.M. 1990. Control of plant parasitic nematodes with agrowastes soil amendment. *Pakistan Journal of Nematology* 8: 25-28.

Alam, M.M., Siddiqui, S.A. and Khan, A. 1977. Mechanism of control of plant parasitic nematodes as a result of the application of organic amendments to the soil. 3. Role of phenols and amino acids in host root. *Indian Journal of Nematology,* 7: 27-31.

Attyia, S.H. and Youssry, A.A. 2001. Application of *Saccharomyces cerevisiae* as a biocontrol agent against some diseases of Solanaceae caused by *Macrophomina phaseolina* and *Fusarium solani*. *Egyptian Journal of Biology,* 3: 79-87.

Azzam, S.A., Karam, N.S., Hameed, K.M., Goussous, S.J., Maraqa, A.D. and Makhadmeh, I.M. 2012. Investigation of Indigenous Plant Root Associated Bacteria and Yeast Isolates for Plant Growth Promoting Ability. *Jordan Journal of Agricultural Sciences,* 8: 1-14.

Barker, K.R., Sasser, J.N., and Carter, C.C. 1985. An Advanced Treatise on *Meloidogyne*. Vol. II: Methodology. North Carolina State Univ. Graphics, Raleigh, NC, USA, 223 pp.

El-Tarabily, K.A. and Sivasithamparam K. 2006. Potential of yeasts as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. *Mycoscience,* 47: 25-35.

Goswami, J., Pandey, R.K., Tewari, J.P. and Goswami B.K. 2008. Management of root knot nematode on tomato through application of fungal antagonists, *Acremonium strictum* and *Trichoderma harzianum*. *Journal of Environmental Science Health,* 43(3): 237-40.

Hamid, N. and F. Jawaid. 2009. Effect of short-term exposure of two different concentrations of sulphur dioxide and nitrogen dioxide mixture on some biochemical parameter of soybean (*Glycine max* (l.) Merr.). *Pakistan Journal of Biology,* 41(5): 2223-2228.

Ismail, A.E., El-Nagdi, W. and Hasabo, S.A. 2005. Efficacy of some Local Isolates of *Saccharomyces cerevisiae*, *Trichoderma harzianum* and *T. ressei* as Bioagents for Controlling *Helicotylenchus ex-*

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allus and *Pratylenchus zeae* Infecting Jasmine in Egypt. *Egyptian Journal of Biology,* 33: 27-40.

Jamshidnejad, V., Sahebani, N. and Etebarian, H. 2013. Potential biocontrol activity of *Arthrobotrys oligospora* and *Trichoderma harzianum* BI against *Meloidogyne javanica* on tomato in the greenhouse and laboratory studies. *Archives of Phytopathology and Plant Protection,* 46: 1477-2906.

Jansen, W.T., Bolm, M., Balling, R., Chhatwal, G.S. and Schnabel, R. 2002. Hydrogen peroxide-mediated killing of *Caenorhabditis elegans* by *Streptococcus pyogenes*. *Infect Immun.,* 70: 5202-5207.

Karajeh, M.R. 2004. Identification, Distribution, and Genetic Variability of the Root-knot Nematodes (*Meloidogyne* spp.) in Jordan. Ph.D. Thesis, University of Jordan, Amman, Jordan, 152 pp.

Karajeh, M.R. 2008. Interaction of root-knot nematodes (*Meloidogyne javanica*) and tomato as affected by hydrogen peroxide. *Journal of Plant Protection Research,* 48(2): 181-188.

Karajeh, M.R. 2013. Effcacy of *Saccharomyces cerevisiae* on controlling the root-knot nematode (*Meloidogyne javanica*) infection and promoting cucumber growth and yield under laboratory and field conditions. *Archives of Phytopathology and Plant Protection,* 46: 2492-2500.

Karajeh, M.R. and Al-Nasir, F.M. 2013. Ability of nitrogen containing salts to control the root-knot nematode (*Meloidogyne javanica*) on tomato. *Hellenic Plant Protection Journal,* 6: 19-27.

Maurya, S. and Singh, D. 2010. Quantitative analysis of total phenolic content in *Adhatoda vasica* Nees extracts. *International Journal of Pharmaceutical Technology Research,* 2(4): 2403-2406.

Mekki, B.B. and Ahmed, A.G. 2005. Growth, yield and seed quality of soybean (*Glycine max* L.) as affected by organic, biofertilizer and yeast application. *Research Journal of Agricultural and Biological Sciences,* 1(4): 320-324.

Noweer, E.M. and Hasabo, S.A. 2005. Effect of different management practices for controlling root-knot nematode *Meloidogyne incognita* on squash. *Egyptian Journal of Phytopathology,* 33: 73-81.

Omran, Y.A. 2000. Studies on histophysiological effect of hydrogen cyanamide (Dormex) and yeast application on bud fertility, vegetative growth and yield of Roumi Red grape cultivar. Ph.D. Thesis, Fac of Agric Assiut Univ Egypt.

Shalaby, M.E. and El-Nady, M.F. 2008. Application of *Saccharomyces cerevisiae* as a biocontrol agent against *Fusarium* infection of sugar beet plants. *Acta Biologica Szegediensis,* 52: 275-271.

Sitaramaiah, R and Singh, RS. 1978. Effect of organic amendments on phenolic content of soil and plant and response of *Meloidogyne javanica* and its host to related compounds. *Plant and Soil,* 50: 671-679.

Taylor, A.L. and Sasser. J.N. 1978. Biology, identification and control of root-knot nematodes *(Meloidogyne* spp.). Coop. Pub. Dep. Plant Pathol., North Carolina State Univ., and U. S. Agency Int. Dev. Raleigh, N. C. 111 pp.

Thomason, I.J. 1987. Challenges facing nematology: Environmental risks with nematicides and the need for new approaches. Pp. 469–476 *in* Vistas on nematology. J. A. Veech and D. W. Dickson, eds. Hyattsville, MD: Society of Nematologists, Inc., USA.

Warring, P.E. and Phillips, I.D. 1973. The Control of Growth and Differentiation in Plants. E.L.B.J. ed., Published by Pergamon Press. Ltd. UK.

Werner, P. 1981. Manual for field trials in plant protection. Agricultural Division, Ciba-Geigy Limited, Basle, Switzerland, 205pp.

Whitehead, A.G. and Hemming, J.R. 1965. A comparison of some quantitative methods for extracting small vermiform nematodes from soil. *Annals of Applied Biology,* 55: 25-38.

Youssef, M.M.A. and Soliman, M.M. 1997. Effect of integrated management on *Meloidogyne incognita* infecting Egyptian henbane, *Hyoscyamus muticus* and on subsequent cowpea plant. 1st Scientific Conference of Agricultural Sciences, Fac. Agric., Assiut Univ., Assiut, pp. 585-594.

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Βελτίωση της ανάπτυξης, της παραγωγής και της αντοχής στο νηματώδη *Meloidogyne javanica* σε καλλιέργεια τομάτας μετά την εφαρμογή σκευάσματος του μύκητα *Saccharomyces cerevisiae*

M.R. Karajeh

Περίληψη Μελετήθηκε η επίδραση σκευάσματος του μύκητα *Saccharomyces cerevisiae* σε τομάτα της καλλιεργούμενης ποικιλίας cv. Asala και η αλληλεπίδρασή της με τον κομβονηματώδη *Meloidogyne*

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javanica σε συνθήκες υπαίθρου. Το υπό δοκιμή σκεύασμα ήταν εφάμιλλης αποτελεσματικότητας με το νηματοδωκτόνο αναφοράς (δ.ο. oxamyl). Η αποτελεσματικότητα του σκευάσματος ήταν καλύτερη από αυτή του υπεροξειδίου του υδρογόνου, όταν εφαρμόστηκε με ριζοπότισμα, επιτυγχάνοντας σημαντική μείωση στο σχηματισμό κόμβων και της αναπαραγωγικής ικανότητας του νηματώδη σε σχέση με τον μάρτυρα (χωρίς επέμβαση). Επιπλέον, η εφαρμογή του *S. cerevisiae* βελτίωσε την ανάπτυξη και την απόδοση της καλλιέργειας και αύξησε την ανθεκτικότητα της καλλιεργούμενης ποικιλίας τομάτας cv. Asala στο νηματώδη μέσω της αύξησης του συνολικού ποσού των φαινολών στις ρίζες κατά τον ίδιο τρόπο όπως η εξωγενής εφαρμογή του υπεροξειδίου του υδρογόνου.

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Toxicity assessment of insecticides to nymphs and adults of *Calliptamus barbarus barbarus* Costa (Orthoptera: Acrididae)

S.A. Antonatos[1,2*] and N.G. Emmanouel[2]

Summary The toxicity of various insecticides to nymphs and adults of *Calliptamus barbarus barbarus* (Orthoptera: Acrididae) was studied in laboratory bioassays. The insecticides used were imidacloprid, spinosad, alpha cypermethrin, lambda cyhalothrin, diflubenzuron and azadirachtin. Lambda cyhalothrin was used only in bioassays with adult grasshoppers whereas diflubenzuron and azadirachtin were used only in bioassays with grasshopper nymphs. The insecticide with the most toxic effect on nymphs and adults of *C. barbarus barbarus* was spinosad followed by imidacloprid and alpha cypermethrin. Ten days after treatment the mortalities obtained from those three insecticides were 98.5%, 89.1% and 81.3% in nymphs and 98.4%, 71.9% and 67.2% in adults, respectively. The toxicity of lambda cyhalothrin to the grasshopper adults was moderate, reaching 43.8% mortality ten days after their exposure. Diflubenzuron and azadirachtin provided moderate and reduced level of mortality, respectively, to the grasshopper nymphs. The mortalities obtained from these insecticides ten days after treatment were 53.1% and 29.7%, respectively. The same pattern was observed for the lethal time with spinosad having the most rapid action.

Additional keywords: grasshoppers, insecticides, lethal time, toxic effect

Introduction

Several grasshopper species of *Calliptamus* are widely distributed throughout the countries of the Mediterranean basin (Larrosa *et al.*, 2008; Wilps *et al.*, 2002; Merton, 1959) and damages on crops have often been reported (Aragón *et al.*, 2013). *Calliptamus barbarus barbarus* (Costa, 1836) along with the closely related species *Calliptamus italicus* (Linnaeus, 1758) are very common in Greece (Willemse, 1984). They are highly polyphagous and they can feed on a broad spectrum of plants belonging to the families Asteraceae, Papilionaceae, Malvaceae, Poaceae, Fabaceae, Solanaceae, Brassicaceae as well as on many fruit trees (Bei-Bienco and Mishchenko, 1963). In many cases, heavy attack on various crops by these grasshoppers resulted in the declaration of large areas under a special regime by the state where sprayings with insecticides were conducted. This results in an increased cost of crop production and can create serious ecological problems.

In high population densities, grasshoppers consume large amounts of plant mass (Weiland *et al.*, 2002; Antonatos *et al.*, 2013). Additionally, they often cut plant parts without consuming them (Holmberg and Hardman, 1984) and contaminate with their bodies and faecal material the harvested crops (Amarasekare and Edelson, 2004). Numerous insect predators of orthopterans, which may be useful in grasshoppers' control, have been reported in old studies (Merton, 1959; Dempster, 1957), however control of grasshopers nowadays depends mainly on application of insecticides.

Several insecticides (organochlorine, organophosphate or carbamate) have been used successfully for the chemical control of grasshoppers (Tharp *et al.*, 2000; Weiland *et al.*, 2002) but due to change in pesticide reg-

[1] Department of Entomology and Agricultural Zoology, Benaki Phytopathological Institute, 8 St. Delta Str., GR-145 61 Kifissia, Attica, Greece
[2] Laboratory of Agricultural Zoology and Entomology, Agricultural University of Athens, 75 Iera odos Str., GR-118 55 Athens, Greece
* Corresponding author: s.antonatos@bpi.gr

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ulations in the European Union the use of most of those has already been banned.

In recent years most of the research concerning the chemical control of orthopterans involves the use of pyrethroids (Amarasekare and Edelson, 2004; Reinert *et al*., 2001; Johnson, 1990; Olaifa, 1986; Ewen *et al*., 1984; Mukeri and Ewen, 1984), neonicotinoids (Wilps *et al*., 2002; Tharp *et al*., 2000), spinosins (Amarasekare and Edelson, 2004), insect growth regulators (IGRs) (Weiland *et al*., 2002; Amarasekare and Edelson, 2004) and entomopathogenic organisms (Frank, 2009; Amarasekare and Edelson, 2004; Sieglaff *et al*., 1998; Milner *et al*., 1994; Moore *et al*., 1992; Johnson and Pavlikova, 1986).

In the present study an attempt was made to determine the toxic effect of various insecticides, with different mode of action, to nymphs and adults of the grasshopper *C. barbarus barbarus* under laboratory conditions. In addition, the lethal time of the 50% and 90% of the grasshopper population by these insecticides was estimated.

Materials and methods

Biological material

The experiments were conducted with nymphs (mixed population of 3rd and 4th instar) as well as with adults of *C. barbarus barbarus*. Nymphs of early instars (1st and 2nd) were collected by sweep netting in a lowland grassland area near Spata (Attica – Greece). After the collection, the insects were transferred to the laboratory and reared in experimental wood-framed cages (30 x 30 x 30 cm), 20-30 nymphs in each cage, at a temperature of 25 ± 1°C, humidity 65 ± 5% and a photoperiod 16: 8 light: dark (Lactin and Johnson, 1995; De Faria *et al*., 1999). Insects were fed with vine leaves, placed in the cages daily. Water was provided through a piece of water soaked cotton placed on the bottom of each cage. The adults were kept in cages of same dimensions and fed also with vine leaves until used in bioassays.

Insecticides

The insecticides that were used in bioassays with *C. barbarus barbarus* were imidacloprid 35% w/v (SC), alpha cypermethrin 10% w/v (EC) and spinosad 48% w/v (SC) for both nymphs and adults. Two IGRs diflubenzuron 25% w/w (WP) and azadirachtin 1% w/v (EC) were tested on nymphs and lambda cyhalothrin 9.43% w/w (CS) tested on adults. The doses of the insecticides used in the experiments were for imidacloprid 7.7gr a.i./100lt water, for alpha cypermethrin 4gr a.i./100lt water, for lambda cyhalothrin 1.2gr a.i./100lt water, for spinosad 16.8gr a.i./100lt water, for diflubenzuron 25gr a.i./100lt water and for azadirachtin 2gr a.i./100lt water. The concentrations of the insecticides solutions were calculated based on the product labels and were similar with those that were used in agricultural practice against Orthoptera or other insect pests.

Bioassays

To test the toxic effect of the insecticides to *C. barbarus barbarus* nymphs and adults, a water solution of each insecticide was prepared (1.5 lt) using the above mentioned doses. Fully expanded mature leaves of vine (*Vitis vinifera*, cv Sultanina) were dipped in each insecticide solution, removed and dried at 30°C for 30-40 min (Amarasekare and Edelson, 2004). Water treated leaves were used as control. To keep the leaf turgid, the stem was placed in a vial of water. Every leaf was placed separately in a clear plastic cage (1lt volume) bearing an opening at the top covered with fine muslin. Eight individuals (4 males and 4 females), nymphs or adults, of *C. barbarus barbarus* were placed in each cage and remained under starvation for 24 hours before their use in bioassays. The treated leaf was kept in the cage for 48 hours and then was replaced with an untreated leaf every 48 hours, for 10 days. Water was provided to the insects via a piece of water soaked cotton placed at the bottom of each cage. The toxicity of insecticides was determined by the mortality of grasshoppers over the 10-day observation period (Amarasekare and Edelson, 2004). Counting

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of live and dead insects was conducted every 24 hours. An insect was considered dead if no movement was observed after a gentle disturbance. The bioassays were conducted in chambers in which the humidity was 65±5% and the temperature 25±1 °C. Cool fluorescent light provided a photoperiod of 16: 8 light: dark. There were 8 replicates for each treatment.

Statistical analysis

Data obtained from the bioassays on nymphs and adults of *C. barbarus barbarus* were analyzed using Kruskal-Wallis H-test and comparison of means were performed with Mann-Whitney U-test for *P*=0.05. The statistical analysis was conducted using the software Statistica 7 (StatSoft Inc., 2004). Moreover, the data of bioassays were subjected to probit analysis to calculate the lethal time required for 50% (LT_{50}) and 90% (LT_{90}) of the insects after their exposure to the treated leaves for 48 hours. The analysis was performed using the software SPSS 14.0 (SPSS, 2004).

Results

Bioassays on nymphs of *Calliptamus barbarus barbarus*

Significant statistical differences were found between the toxicity of the tested insecticides on nymphs of *C. barbarus barbarus* in all the days of the experiment (Kruskal Wallis H-test). The highest level of mortality was caused by spinosad, followed by imidacloprid and alpha cypermethrin (Table 1). One day after treatment, exposure to spinosad, imidacloprid and alpha cypermethrin resulted in 60.9%, 42.2% and 25% mortality of nymphs, respectively, while ten days after treatment the mortalities obtained from the insecticides were 98.5%, 89.1% and 81.3%, respectively.

The mortalities from the insecticides were greater than that obtained from the water control, in all days of the trial. One day after treatment, 3.1% and 1.6% mortality was observed in nymphs exposed to diflubenzuron and azadirachtin, respectively, which did not significantly differ from the level obtained from the water control. Ten days after treatment the mortalities obtained from these insecticides were 53.1% and 29.7% and were significantly higher than the level obtained from the water control. The mortality of grasshopper nymphs that was observed after their exposure to diflubenzuron was different from the mortality obtained from the water control the last six days of the trial, while those of azadirachtin was greater than that of the control only the last day of the trial (Table 1).

The LT_{50} and LT_{90} values for nymphs of *C. barbarus barbarus*, when the insects were exposed to the treated leaves for 48h varied from 0.83 to 15.79 days and from 3.3 to 35.02 days, respectively (Table 2). The shortest time to kill the 50% as well as the 90% of the grasshopper nymphs was recorded after their exposure to spinosad followed in ascending order by imidacloprid, alpha cypermethrin, diflubenzuron and azadirachtin.

Bioassays on adults of *Calliptamus barbarus barbarus*

The toxicity of the tested insecticides on adults of *C. barbarus barbarus* differed significantly between the insecticides in all days of the experiment (Kruskal-Wallis H-test). The highest level of mortality of the grasshopper adults was observed after their exposure to spinosad followed by the mortalities obtained from imidacloprid, alpha cypermethrin and lambda cyhalothrin. One day after treatment, exposure to these insecticides resulted in 48.4%, 28.1%, 7.8% and 4.7% mortality of adults respectively, while ten days after treatment the relative numbers were 98.4%, 71.9%, 67.2% and 43.8%. The mortalities obtained from all insecticides were greater than that from the water control in all the days of the trial, with the exception of lambda cyhalothrin on the first day (Table 3).

The LT_{50} and LT_{90} values for adults of *C. barbarus barbarus*, when the insects were exposed to the treated leaves for 48h, varied from 0.97 to 12.51 days and from 3.18

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Table 1. Mortality % (Mean ± S.E.) of nymphs of *Calliptamus barbarus barbarus* when exposed for 48 hours to grapevine leaves treated with different insecticides.

Treatment	Day of trial									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Spinosad	60,9 ± 9,0a	81,3 ± 7,5a	89,1 ± 6,4a	93,8 ± 4,1a	96,9 ± 2,1a	96,9 ± 2,1a	98,5 ± 1,6a	98,5 ± 1,6a	98,5 ± 1,6a	98,5 ± 1,6a
Imidacloprid	42,2 ± 4,7ab	53,1 ± 6,1b	65,6 ± 5,2b	73,4 ± 5,5b	78,1 ± 4,6b	81,3 ± 4,7b	85,9 ± 5,0ab	89,1 ± 5,0ab	89,1 ± 5,0ab	89,1 ± 5,0ab
Alpha cypermethrin	25,0 ± 7,5b	35,9 ± 10,1b	54,7 ± 10,3b	64,1 ± 9,0b	70,3 ± 7,1b	75,0 ± 6,7bc	75,0 ± 6,7bc	78,1 ± 6,1bc	79,7 ± 5,3bc	81,3 ± 4,7bc
Diflubenzuron	3,1 ± 2,1c	7,8 ± 4,1c	20,3 ± 8,2c	31,3 ± 10,6c	37,5 ± 10,3c	42,2 ± 12,7cd	48,4 ± 11,9cd	48,4 ± 11,9cd	51,6 ± 11,4cd	53,1 ± 11,5cd
Azadirachtin	1,6 ±1,6c	4,7 ± 2,3c	6,3 ± 2,4c	12,5 ± 2,4c	12,5 ± 2,4d	15,6 ± 3,1de	18,8 ± 4,7de	23,4 ± 3,7de	26,6 ± 3,7de	29,7 ± 4,7d
Control	3,1 ± 2,1c	4,7 ± 2,3c	9,4 ± 3,1c	9,4 ± 3,1c	9,4 ± 3,1d	9,4 ± 3,1e	12,5 ± 4,1e	14,1 ± 3,7e	15,6 ± 3,9e	15,6 ± 3,9e
Chi-square	36,49	36,47	35,39	35,38	37,22	34,4	35,29	35,39	35,25	35,05
df	5	5	5	5	5	5	5	5	5	5
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means of the same column followed by different letter differ significantly (Mann-Whitney U-test: $P < 0.05$)

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Table 2. Lethal time of 50% (LT_{50}) and 90% (LT_{90}) of nymphs of *Calliptamus barbarus barbarus* when exposed for 48 hours to grapevine leaves treated with different insecticides.

Treatment	Slope (b) ± S.E.	LT_{50}^{α} (Days)	95% confidence limits	LT_{90}^{α} (Days)	95% confidence limits	*df*	X^2	P
Spinosad	2.14 ± 0.26	0.83	0.48 – 1.16	3.3	2.67 – 4.18	78	108.8	0.012^{β}
Imidacloprid	1.68 ± 0.20	1.89	1.39 – 2.34	10.89	8.62 – 15.42	78	78.46	0.464
Alpha cyper-methrin	1.86 ± 0.22	3.26	2.51 – 3.94	15.95	11.61 – 27.49	78	125.05	0.001^{β}
Diflubenzuron	2.48 ± 0.34	9.16	7.38 – 13.78	30.14	17.98 – 113.48	78	211.79	$<0.0001^{\beta}$
Azadirachtin	3.70 ± 1.19	15.79	11.98 – 48.94	35.02	19.79 – 414.05	78	40.93	1.000

α: LT_{50} and LT_{90} are considered different when the 95% confidence limits fail to overlap
β: Heterogeneity factors were used in the calculation of confidence

to 107.41 days, respectively (Table 4). The shortest time recorded to kill the 50% and the 90% of the adult grasshoppers was that after their exposure to spinosad, followed by imidacloprid and alpha cypermethrin whereas lambda cyhalothrin showed the slowest action of all insecticides.

Discussion

The results of the present study showed that azadirachtin had a minimal toxicity to nymphs of *C. barbarus barbarus*. The mortality induced by this insecticide reached the level of less than 30% and it was greater than that obtained from the water control only 10 days after treatment. Similar conclusions about the effectiveness of this insecticide for the control of *Melanoplus differentialis* Thomas (Orthoptera: Acrididae) have been reported by Amarasekare and Edelson (2004) although higher dose had been used in their experiments.

Diflubenzuron had a moderate toxicity by causing approximately 50% mortality in nymphs of *C. barbarus barbarus* 10 days after treatment. Significant difference in the mortality of grasshopper nymphs obtained by their exposure to diflubenzuron compared to the mortality obtained from the water control was observed after the fourth day after treatment. Ingestion of diflubenzuron by immature insects results in disruption of chitin formation and deposition that affects the cuticle and the molting process (Weiland *et al.*, 2002). For this reason it is possible that diflubenzuron will be more toxic in higher temperatures when molting rates increase (Lactin and Johnson, 1995). From the insecticides tested, diflubenzuron and azadirachtin were also those that required the longest time to kill the 50% of the grasshopper exposed population and particularly long time to kill the 90% of the insects. Weiland *et al.* (2002) reported that the use of diflubenzuron resulted in maximum control of Acrididae, 14 days after its application in a field experiment. Similar results about the effectiveness of diflubenzuron on the control of nymphs of *M. differentialis* have also been reported by Amarasekare and Edelson (2004).

The pyrethroid insecticide alpha cypermethrin showed high toxic effect and relatively high speed of action to *C. barbarus barbarus* nymphs and adults. However, lambda cyhalothrin showed a moderate toxicity and a slower action than the other insecticides on the grasshopper adults. In contrast with our results, Reinert *et al.* (2001) reported very good efficacy of lambda cyhalothrin on the control of *M. differentialis* adults but the dose they used was higher than the dose used in the present study.

Spinosad exhibited the most rapid ac-

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Table 3. Mortality % (Mean ± S.E) of adults of *Calliptamus barbarus barbarus* when exposed for 48 hours to grapevine leaves treated with different insecticides.

Treatment	Day of trial									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Spinosad	48.4 ± 8.0a	79.7 ± 5.8 a	93.8 ± 4.7a	95.3 ± 3.3a	96.9 ± 2.1 a	96.9 ± 21.0a	98.4 ± 1.6a	98.4 ± 1.6a	98.4 ± 1.6a	98.4 ± 1.6a
Imidacloprid	28.1 ± 3.9b	35.9 ± 3.7 b	43.8 ± 2.4b	53.1 ± 3.9b	56.3 ± 4.7 b	59.4 ± 4.6b	60.9 ± 4.4b	67.2 ± 4.1b	70.3 ± 4.7b	71.9 ± 3.9b
Alpha cypermethrin	7.8 ± 3.3c	23.4 ± 6.0 b	35.9 ± 7.3b	45.3 ± 9.7b	53.1 ± 10.2b	56.3 ± 10.3b	59.4 ± 10.5b	60.9 ± 9.9bc	65.6 ± 9.4bc	67.2 ± 8.8bc
Lambda cyhalothrin	4.7 ± 2.3cd	18.8 ± 6.7 b	25.0 ± 7.1b	29.7 ± 9.1b	32.8 ± 9.7b	34.4 ± 9.7b	39.1 ± 9.9b	39.1 ± 9.9c	43.8 ± 8.8c	43.8 ± 8.8c
Control	0.0 ± 0.0d	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	1.6 ± 1.6c	3.1 ± 2.1c	3.1 ± 2.1d	3.1 ± 2.1d	4.7 ± 2.3d
Chi-square	26,73	29,31	30,77	30,27	29,98	29,13	29,05	29,61	29,96	30,21
df	4	4	4	4	4	4	4	4	4	4
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means of the same column followed by different letter differ significantly (Mann-Whitney U-test: $P < 0.05$)

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Table 4. Lethal time of 50% (LT_{50}) and 90% (LT_{90}) of adults of *Calliptamus barbarus barbarus* when exposed for 48 hours to grapevine leaves treated with different insecticides.

Treatment	Slope (b) ± S.E.	$LT_{50}{}^{\alpha}$ (Days)	95% confidence limits	$LT_{90}{}^{\alpha}$ (Days)	95% confidence limits	*df*	X^2	P
Spinosad	2,49 ± 0,26	0,97	0,66 – 1,25	3,18	2,66 – 3,89	78	107,00	0,016$^{\beta}$
Imidacloprid	1,24 ± 0,18	3,90	3,12 – 4,71	41,82	24,91 – 104,28	78	33,75	1,000
Alpha cypermethrin	1,86 ± 0,21	5,40	4,45 – 6,64	26,43	17,17 – 59,19	78	155,46	<0,0001$^{\beta}$
Lambda cyhalothrin	1,37 ± 0,23	12,51	8,80 – 28,26	107,41	40,54 – 1471,18	78	155,01	<0,0001$^{\beta}$

α: LT_{50} and LT_{90} are considered different when the 95% confidence limits fail to overlap
β: Heterogeneity factors were used in the calculation of confidence

tion and it was the most toxic insecticide to the grasshopper nymphs and adults in the laboratory experiments. However, under field conditions its application may not have exactly the same effect as spinosad undergoes rapid decomposition by sunlight when it is on the leaf surface of plants. Photolysis is the main degradation mechanism of spinosad. Its half-life ranges from 2.61 to 6.31 days depending on the species of plant on which has been applied (DPR, 1995a, DPR, 1995b). Our results on both the speed of action and the toxicity of spinosad are in agreement with the study of Amarasekare and Edelson (2004) on *M. differentialis* nymphs.

Imidacloprid exhibited very high toxicity and speed of action on the experiments with *C. barbarus barbarus* nymphs and adults. The mortality of the grasshoppers caused by the application of imidacloprid and its speed of action were lower than those caused by spinosad and similar to those caused by alpha cypermethrin. Tharp *et al.* (2000) reported that the LD_{90} of imidacloprid to 4th instar nymphs of *Melanoplus sanguinipes* Fabricius (Orthoptera: Acrididae) was 93 ppm. In the present study 89% mortality of 3rd and 4th instar nymphs of *C. barbarus barbarus* was achieved over 10 days with a concentration of 77 ppm of this insecticide. Moreover, Wilps *et al.* (2002) reported high mortality (90%) of individuals of *C. italicus* in field experiments using low dose of imidacloprid (7.5 gr a.i./ha).

The results of the laboratory bioassays showed that from the insecticides tested at the labeled use rates, spinosad had the most toxic effect and required the least time to control nymphs and adults of *C. barbarus barbarus*. Imidacloprid and alpha cypermethrin exhibited also high toxicity. However, it must be mentioned that the exposure of the grasshopper's individuals to the insecticides under laboratory conditions is not the same as in field conditions and consequently the actual mortality in the field may be different.

Literature cited

Amarasekare, K.G. and Edelson, J.V. 2004. Effect of temperature on efficacy of insecticides to differential grasshopper (Orthoptera: Acrididae). *Journal of Economic Entomology*, 97(5): 1595-1602.

Antonatos, S.A., Emmanuel, N.G. and Fantinou, AA. 2013. Effect of temperature and species of plant on the consumption of leaves by three species of Orthoptera under laboratory conditions. *European Journal of Entomology*, 110(4): 605-610.

Aragón, P., Coca-Abia, M.M., Llorente, V. and Lobo, J.M. 2013. Estimation of climatic favourable areas for locust outbreaks in Spain: integrating species' presence records and spatial information on outbreaks. *Journal of Applied Entomology*, 137(8): 610-623.

Bei-Bienko, G.Ya. and Mishcheno, L.L. 1963. *Locusts and grasshoppers of the U.S.S.R. and adjacent countries* (Translated from Russian). Part 1, Israel program for scientific translations, 400 p.

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De Faria, M., Almeida, D. and Magalhães, B. 1999. Food consumption of *Rhammatocerus schistocercoides* Rehn (Orthoptera: Acrididae) infected by the fungus *Metarhizium flavoviride* Gams and Rozsypal. *Anais da Sociedade Entomológica do Brasil*, 28(1): 91-99.

Dempster, J.P. 1957. The population dynamics of the Moroccan locust (*Dociostaurus maroccanus* Thunberg) in Cyprus. *Anti-Locust Bulletin*, 27: 1-66.

DPR. 1995a. (1) Nature of residue study in apple leaves using (14C)-XDE-105 (Factor A and Factor D); (2) Nature of (14C)-XDE-105 residues resulting from a single application of compound to cabbage. Volume No. 52050-047, Department of Pesticide Regulation, Sacramento, California.

DPR. 1995b. Nature of residue study in turnips using (14C)-XDE-105 (Factor A and Factor D. Volume No. 52050-049, Department of Pesticide Regulation, Sacramento, California.

Ewen, A.B., Mukerji, M.K. and Hinks, C.F. 1984. Effect of temperature on the toxicity of Cypermethrin to nymphs of the migratory grasshopper, *Melanoplus sanguinipes* (Orthoptera: Acrididae). *Canadian Entomologist*, 116: 1153-1156.

Frank, J.H. 2009. *Steinernema scapterisci* as a biological control agent of *Scapteriscus* Mole Crickets. In HAJEK, A.E. *et al.* (eds). *Use of microbes for control and eradication of invasive arthropods.* Springer Science and Business Media B.V., p. 115-132.

Holmberg, R.G. and Hardman J.M. 1984. Relating feeding rates to sex and size in six species of grasshoppers (Orthoptera: Acrididae). *Canadian Entomologist*, 116: 597-606.

Johnson, L.D. 1990. Influence of temperature on toxicity of two pyrethroids to grasshoppers (Orthoptera: Acrididae). *Journal of Economic Entomology*, 83(2): 366-373.

Johnson, L.D. and Pavlicova, E.1986. Reduction of consumption of grasshoppers (Orthoptera: Acrididae) infected with *Nosema locustae* Canning (Microsporida: Nosematidae). *Journal of Invertebrate Pathology*, 48: 232-238.

Lactin, J.D. and Johnson, D.L. 1995. Temperature-dependent feeding rates of *Melanoplus sanguinipes* nymphs (Orthoptera: Acrididae) in laboratory trials. *Environmental Entomology*, 24: 1291-1296.

Larrosa, E., García, M.D., Clemente, M.E. and Presa, J.J. 2008. Sound production in *Calliptamus barbarus* Costa 1836 (Orthoptera: Acrididae: Catantopinae). *Annales de la Société Entomologique de France*, 44(2): 129-138.

Merton, F.H., 1959. Studies in the ecology of the Moroccan locust (*Dociostaurus maroccanus* Thunberg) in Cyprus. *Anti-Locust Bulletin*, 34, 123 p.

Milner, R.J., Hartley, T.R., Lutton, G.G. and Prior, C. 1994. Control of *Phaulacridium vittatum* (Sjöstedt) (Orthoptera: Acrididae) in field cages using an oil-based spray of *Metarhizium flavoviride* Gams and Rozsypal (Deuteromycotina: Hyphomycetes). *Journal of the Australian Entomological Society*, 33: 165-167.

Moore, D., Reed, M., Le Patourel, G., Abraham, Y.J. and Prior, C. 1992. Reduction of feeding by the desert locust, *Schistocerca gregaria*, after infection with *Metarhizium flavoviride*. *Journal of Invertebrate Pathology*, 60: 304-307.

Mukerji, M.K. and Ewen, A.B. 1984. Field evaluation of cypermethrin and carbaryl as sprays and baits for grasshopper (Orthoptera: Acrididae) control in Saskatchewan. *Canadian Entomologist*, 116: 5-9.

Olaifa I.J. 1986. Toxicity of some insecticides to the grasshopper *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae). *International Journal of Tropical Insect Science*, 7: 135-138.

Reinert, A.J., Wayne, A.M., Steve, W.G., James, R., Engelke, M.C. and Steven, J.M. 2001. Residual chemical control for *Melanoplus differentialis* (Orthoptera: Acrididae) in urban landscapes. *Florida Entomologist*, 84(3): 380-384.

Sieglaff, D.H., Pereira, R.M., Capinera, J.L. 1998. Microbial control of *Schistocerca americana* (Orthoptera: Acrididae) by *Metarhizium flavoviride* (Deuteromycotina): instar dependent mortality and efficacy of ultra low volume application under greenhouse conditions. *Journal of Economic Entomology*, 91(1): 76-85.

SPSS 2004. SPSS 14 for Windows User's Guide. Copyright 2004 by SPSS Inc., SPSS Chicago, IL.

StatSoft Inc. 2004. Statistica (data analysis software system), version 7, Tulsa, USA.

Tharp, I.C., Gregory, D.J. and Jerome, A.O. 2000. Laboratory and field evaluations of imidacloprid against *Melanoplus sanguinipes* (Orthoptera: Acrididae) on small grains. *Ecotoxicology*, 93(2): 293-299.

Weiland, R.T., Judge, F.D., Pels T. and Grosscurt, A.C. 2002. A literature review and new observations on the use of diflubenzuron for control of locusts and grasshoppers throughout the world. *Journal of Orthoptera Research*, 11: 43-54.

Willemse, F. 1984. *Catalogue of the Orthoptera of Greece*. Hellenic Zoological Society, 275 p.

Willemse, F. and Willemse, L. 2008. An annotated checklist of the Orthoptera -Saltatoria from Greece, including an updated bibliography. *Articulata,* Beiheft, 13: 1-91.

Wilps, H., Levchenko, V. and Vernigor, A. 2002. Field trials with imidacloprid (Confidor) on the Italian locust *Calliptamus italicus* in Kazakhstan. *Journal of Applied Entomology*, 126: 436-443.

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Τοξικότητα εντομοκτόνων σε νύμφες και ενήλικα άτομα του *Calliptamus barbarus barbarus* Costa (Orthoptera: Acrididae)

Σ.Α. Αντωνάτος και Ν.Γ. Εμμανουήλ

Περίληψη Στην παρούσα εργασία μελετήθηκε η τοξική δράση διαφόρων εντομοκτόνων σε νύμφες και ενήλικα άτομα του *Calliptamus barbarus barbarus* (Orthoptera: Acrididae) σε εργαστηριακές βιοδοκιμές. Τα εντομοκτόνα ήταν τα imidacloprid, spinosad, alpha cypermethrin, lambda cyhalothrin, diflubenzuron και azadirachtin. Το lambda cyhalothrin χρησιμοποιήθηκε μόνο στις βιοδοκιμές με ενήλικα άτομα ενώ τα diflubenzuron και azadirachtin χρησιμοποιήθηκαν μόνο στις βιοδοκιμές με νύμφες. Το εντομοκτόνο με την πιο τοξική δράση σε νύμφες και ενήλικα άτομα του *C. barbarus barbarus* ήταν το spinosad ακολουθούμενο από τα imidacloprid και alpha cypermethrin. Δέκα ημέρες μετά την εφαρμογή η θνησιμότητα που προκλήθηκε από τα προαναφερθέντα εντομοκτόνα στις νύμφες ήταν 98,5%, 89,1% και 81,3% και στα ενήλικα 98,4%, 71,9% και 67,2%, αντιστοίχως. Η τοξικότητα του lambda cyhalothrin στα ενήλικα άτομα του Ορθοπτέρου ήταν μέτρια (ποσοστό θνησιμότητας 43,8% δέκα ημέρες μετά την εφαρμογή του). Το diflubenzuron προκάλεσε μέτριο και το azadirachtin μειωμένο επίπεδο θνησιμότητας στις νύμφες του Ορθοπτέρου (θνησιμότητες 53,1% και 29,7%, αντιστοίχως, δέκα ημέρες μετά την εφαρμογή τους). Η ίδια τάση παρατηρήθηκε και στον χρόνο θανάτωσης του εντόμου από τα εντομοκτόνα, με το spinosad να έχει την ταχύτερη δράση.

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SHORT COMMUNICATION

Invasive insect pests and their associated parasitoids on ornamental urban plants on Corfu island - *Phytoliriomyza jacarandae* Steyskal and Spencer 1978 (Diptera, Agromyzidae) a new record in Greece

S. Bella

Summary In this study the results of recent surveys on alien insect pests of ornamental urban plants on the island of Corfu are reported. Overall seven alien species associated with allochthonous ornamental plants were recorded: *Acizzia jamatonica* (Kuwayama 1908), *Glycaspis brimblecombei* Moore 1964 (Hemiptera, Psyllidae), *Corythucha ciliata* (Say 1832) (Hemiptera, Tingidae), *Obolodiplosis robiniae* (Haldeman 1847) (Diptera, Cecidomyiidae), *Phytoliriomyza jacarandae* Steyskal and Spencer 1978 (Diptera, Agromyzidae), *Cacyreus marshalli* Butler 1898 (Lepidoptera, Lycenidae) and *Leptocybe invasa* Fisher and La Salle 2004 (Hymenoptera, Eulophidae). Particularly, *Phytoliriomyza jacarandae*, a leafminer of the Blue jacaranda tree *Jacaranda mimosifolia* D. Don. (Bignoniaceae) is reported for the first time from Greece. Two associated parasitoids, *Platygaster robiniae* Buhl and Duso 2008 (Hymenoptera, Platygastridae) and *Psyllaephagus bliteus* Riek 1962 (Hymenoptera, Encyrtidae) obtained from *Obolodiplosis robiniae* and *Glycaspis brimblecombei*, respectively, are also reported. Details on current distribution, host plants and biological remarks are given for each species.

Additional keywords: alien insects, first record, urban environment

Introduction

The introduction of alien insects is a growing phenomenon especially in countries with intensive international movement of goods and people. This is particularly evident in the Mediterranean Basin where climatic conditions are more favourable for the numerous tropical and subtropical species to establish themselves. The continuously increasing use of numerous species of plants native to different regions of the planet in European parks and gardens has been enriching the European fauna with mainly alien species that develop on the same host plants (Bella, 2013). In the last decade, several invasive insect pests have been introduced, have spread rapidly in the Mediterranean area and are causing serious damage to agricultural, forest and ornamental plants. Biological invasions by alien insect species are a great ecological and economic threat for their direct and indirect impact on indigenous biodiversity.

The aim of this work was to identify alien insect species on urban ornamental plants on Corfu. Field observations were made by the author in the late summer 2013 in public gardens in various towns.

Materials and methods

Samplings were carried out in August 2013 throughout Corfu. Ornamental exotic plants were investigated to ascertain the presence of possible pests, by beating or by careful vis-

Dipartimento di Gestione dei Sistemi Agroalimentari e Ambientali (DiGeSA), sez. Entomologia applicata, Università degli Studi di Catania, Via S. Sofia, 100. 95123 Catania, Italy
Email: sbella@unict.it

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ual search of specimens or by examination of symptoms. Species identification was based on the morphology of adults and preimaginal stages; important was the observation of the type of galls and leafmines and the association with the host plants. Part of the samples was conserved in 75% ethanol and labelled. The collected material was studied in the laboratory, dissected under a binocular microscope and prepared when necessary. Examined material is preserved in the private collection of the author and partly in the entomological collection of the Department of Agri-food and Environmental Systems Management, University of Catania.

Results

Detected species

Hemiptera, Psyllidae

***Acizzia jamatonica* (Kuwayama 1908)**

Native range: species of oriental origin (China, Korea and Japan).

Distribution: in Europe, it was first detected in Italy and later recorded in numerous European countries, including the United Kingdom, Portugal, Spain, France, Corsica, Switzerland, Germany, Slovenia, Croatia, Montenegro, Serbia, Slovakia, Greece, Bulgaria and Hungary. Since 2006, it has been found in the United States of America (Bella, 2013).

Host plant: different species of *Albizia* (Fabaceae, Mimosoideae); in Europe on the Persian silk tree, *A. julibrissin* (Willdenow) Durazzini.

Material examined: Corfu city, 39°37′N, 19°55′E, 10 m a.s.l., 17.VIII.2013, adults on *A. julibrissin*.

Biological remarks: the life cycle of *A. jamatonica* includes numerous overlapping generations; the psyllid overwinters in the adult stage. Leaves and shoots can be completely colonised by juvenile and adult stages with serious damage, leading to total or partial desiccation. Large amounts of honeydew are produced and can cause some inconvenience in urban environments (Sánchez García and Burckhardt, 2009).

***Glycaspis brimblecombei* Moore 1964**

Native range: species described from Australia.

Distribution: this species has shown invasive behaviour in the last 15 years and has spread across several continents outside its native range. It was first detected in the United States of America (California, Florida and the Hawaiian Islands) and subsequently recorded from Mexico, Central and South America (Brazil, Uruguay, Ecuador, Venezuela, Colombia, Peru, Chile and Argentina) and Africa (Morocco, Algeria, Tunisia and South Africa). It is also found in the Canary Islands, New Zealand and Mauritius (Bella, 2013). In Europe, *G. brimblecombei* is reported from Portugal, Spain, France, Corsica, Italy, Sicily, Sardinia, Greece, Corfu and Montenegro (Bella and Rapisarda, 2013; Reguia and Peris-Felipo, 2013; Ben Attia and Rapisarda, 2014; Milonas and Partsinevelos, 2014; Tsagkarakis *et al.*, 2014).

Host plant: associated with different species of Eucalyptus (Myrtaceae) (Brennan *et al.*, 2001); in the Mediterranean area mainly on the River red gum, *E. camaldulensis* Dehnh.

Material examined: 16-20 August 2013; Benitses, 39° 32′N, 19° 54′E, 6 m a.s.l.; Lefkimmi 39° 24′N, 20° 04′E, 6 m a.s.l.; Kassiopi, 39° 47′N, 19° 45′E, 8 m a.s.l.; Corfu city, 39° 37′N, 19° 55′E, 10 m a.s.l.; Roda, 39° 47′N, 19° 48′E, 27 m a.s.l.; Agios Mattheos, 39° 29′N, 19° 52′E, 140 m a.s.l.; Sinarades, 39° 34′N, 19° 50′E, 151 m a.s.l.; Kato Garouna, 39° 32′N, 19° 51′E, 225 m a.s.l.; Pelekas, 39° 35′N, 19° 49′E, 247 m a.s.l.; nymphal stage and adults have been observed on *E. camaldulensis*.

Biological remarks: nymph instars construct white conical lerps using wax and honeydew secretions, while the adults are highly mobile and live freely on the foliage. It produces copious amounts of wax and honeydew on the infested leaves, causing desiccation and premature leaf drop (Laudonia and Garonna, 2010).

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Hemiptera, Tingidae

Corythucha ciliata **(Say 1832)**
Native range: species of Nearctic origin.
Distribution: now widespread across Europe in the United Kingdom, Portugal, Spain, France (including Corsica), Italy (including Sardinia and Sicily), Belgium, Netherlands, Germany, Austria, Switzerland, Croatia, Slovenia, Serbia, Montenegro, Greece, Turkey, Czech Republic, Slovakia, Romania, Hungary and Bulgaria. It has been found in Chile, southern Russia, Korea, China, Japan and Australia (Bella, 2013).
Host plant: feeds primarily on Sycamore trees, *Platanus* (Platanaceae), especially *P. occidentalis* L.; other host plants are *Broussonetia papyrifera* (L.) Vent., *Carya ovata* (Mill.) Koch, *Tila* sp., *Chamaedaphne* sp., *Fraxinus* sp., and *Quercus laurifolia* Michx.
Material examined: Corfu city, 39°37′N, 19°55′E, 10 m a.s.l., 17.VIII.2013, adults on Platanus sp.
Biological remarks: *C. ciliata* feeds on the underside of leaves desiccating the tissue, which may drop prematurely. A single female can lay up to 350 eggs along the leaf veins. There are five immature instars, and in Europe one life cycle is completed in just 20 to 50 days and several generations can occur each year (Malumphy *et al*., 2006).

Diptera, Cecidomyiidae

Obolodiplosis robiniae **(Haldeman 1847)**
Native range: species of Nearctic origin.
Distribution: in Europe, it was first noticed during 2003 in Italy, and subsequently it has rapidly spread throughout a large part of Europe. It has also been observed in South Korea, China, Japan, New Zealand, Ucraina and Russia (Bella, 2013). For the Corfu the species is reported by Skuhravá and Skuhravý (2006).
Host plant: different species of *Robinia* (Fabaceae: Papilionoideae).
Material examined: Benitses, 39°32′N, 19°54′E, 6 m a.s.l., 16.VIII.2013; Corfu city, 39°37′N, 19°55′E, m 10 a.s.l., 17.VIII.2013. Galls and larvae have been observed on the Black locust tree, *R. pseudoacacia* L.
Biological remarks: the larvae form characteristic leaf-margin roll galls. Usually 1-2 larvae can be found in a gall and 1-3 galls per leaflet. The larvae of the summer generations pupate inside the galls on the trees, while those of the autumn generation pupate in the soil after leaf fall. Several generations (2, 3 or even 4) of the gall midge may develop in a year depending on climatic conditions (Bella, 2007).

Diptera, Agromyzidae

Phytoliriomyza jacarandae **Steyskal and Spencer 1978**
Native range: species of South American origin.
Distribution: it is widespread in Argentina (Córdoba), the United States of America (California), Australia, New Zealand and South Africa (Spencer, 1990). In the Palaeartic region, the only records are in Italy (Liguria and Sicily regions) and Portugal (Bella *et al*., 2007; Bella, 2013).
Host plant: monophagous leafminer of the Blue jacaranda tree, *Jacaranda mimosifolia* D. Don. (Bignoniaceae).
Material examined: Kato Garouna, 39°32′N, 19°51′E, 216 m a.s.l., 16.VIII.2013.
Biological remarks: the young larva produces a short linear brown mine in a single leaflet developing into an irregular blotch. The affected leaves drop and the larva pupate in the soil. The canopy quickly yellows and defoliates (Bella *et al*., 2007).

Lepidoptera, Lycaenidae

Cacyreus marshalli **Butler 1898**
Native range: species of South African origin (Swaziland, Lesotho, Botswana, Mozambique, Zimbabwe and South Africa).
Distribution: it is widespread in the Balearic Islands, Portugal, Spain, Netherlands, Norway, Finland, Sweden, France, Corsica, Belgium, Germany, Switzerland, Great Britain, Italy, Sardinia, Sicily, Malta, Slovenia, Croatia, Czech Republic, Greece, Romania, Slovakia, Bulgaria, Turkey, Israel, Estonia, Ukraine,

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Uzbekistan, the Canary Islands and Morocco (CABI, 2014).

The first record for mainland Greece is that of Martinou *et al.* (2011), while from the island of Corfu the species is recorded by Parker (2010).

Host plant: *C. marshalli* is a pest of cultivated *Geranium* spp. and *Pelargonium* spp. (Geraniaceae), but the butterfly also has the capacity to infest native *Geranium* spp., and could cause problems for the wild species.

Material examined: Benitses, 39°32′N, 19°54′E, 6 m a.s.l., 16.VIII.2013, adults on flowers of Geranium sp.

Biological remarks: eggs are laid near the flower buds or less frequently on the leaves; the hatched larvae penetrate inside the stems of the host plant, where they bore galleries and emerge at the fourth and final larval stage to form light-green to dark-brown pupae. In favourable conditions they can produce up to six generations per year. The flight period occurs from the first half of April to the first half of November (Longo, 2004).

Hymenoptera, Eulophidae

Leptocybe invasa **Fisher and La Salle 2004**

Native range: species described from Australia.

Distribution: it is widespread in Europe (Balearic Islands, Portugal, Spain, France, Corsica, Italy, Sardinia, Sicily, Greece and Canary Islands); also found in Africa (Morocco, Algeria, Uganda, Ethiopia, Kenya, Tanzania, Mozambique, Zimbabwe and South Africa); in Asia (Turkey, Israel, Jordan, Syria, Kurdistan, Iran, Iraq, India, Thailand, Vietnam, Cambodia and China); in Oceania (Australia and New Zealand); in South America (Brazil and Argentina) and in the USA (Florida) (Maatouf and Lumaret, 2012).

Host plant: the pest attacks different species of *Eucalyptus* (Myrtaceae).

Material examined: Benitses, 39°32′N, 19°54′E, 6 m a.s.l., 16.VIII.2013, galls and adults on *Eucalyptus camaldulensis*.

Biological remarks: *L. invasa* is particularly damaging to new growth, due to its preference for young leaves (including petioles) and stems of new shoots for oviposition: plants may become deformed, and growth may be stunted due to heavy galling. The wasp produces two or three overlapping generations per year. A female lays about 80-100 eggs shallowly beneath the epidermis; the larvae complete their development within the gall (Kim, 2008).

Associated parasitoids

Hymenoptera, Platygastridae

Platygaster robiniae **Buhl and Duso 2008**

Native range: species of Nearctic origin.

Distribution: the parasitoid wasp is present in France, Italy, Sicily, Switzerland, Denmark, Croatia, Montenegro, Serbia, Slovakia, Czech Republic, Bulgaria, Ukraine, South Korea and China (Jørgensen, 2009; Sviridov and Bazhenova, 2009; Lu *et al.*, 2010; DAISIE, 2014).

Host: specific parasitoid of the locust gall midge, *Obolodiplosis robiniae* (Diptera, Cecidomyiidae).

Material examined: Benitses, 39°32′N, 19°54′E, 6 m a.s.l., 16.VIII.2013; Corfu city, 39°37′N, 19°55′E, 10 m a.s.l., 17.VIII.2013, found to parasitize larvae of *O. robiniae* infesting *Robinia pseudoacacia* L. (Fabaceae).

Biological remarks: *P. robiniae* is a gregarious, koinobiont endoparasitoid of *O. robiniae*; it is an egg-larval parasitoid, parasitising eggs of *O. robiniae* and emerging from the host larvae. After hatching, the parasitoid undergoes only two larval instars, and development from egg to adult takes about 28 days to complete. The life cycle of a parasitoid generation is synchronised with that of its host; the adult wasps' emergence coincides with that of the host, so that they can parasitise the host eggs (Kim *et al.*, 2011).

Observed parasitisation: based on personal observations conducted in the town of Corfu, 55 galls were observed (1-3 for single leaflets) on a totally of 30 attacked preleved leaves, with 32 emerged specimens of *P. robiniae*.

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Hymenoptera, Encyrtidae

***Psyllaephagus bliteus* Riek 1962**

Native range: species native to Australia.

Distribution: it spread to New Zealand, Brazil, Spain, Italy (including Sicily and Sardinia), Greece (Corfu), Morocco and Algeria and is due to an accidental introduction, probably together with its host (Bella and Rapisarda, 2013; Reguia and Peris-Felipo, 2013). For psyllid biological control programmes, *P. bliteus* has been deliberately introduced to the USA (California), Mexico and Chile (Bella, 2013).

Host: *P. bliteus* is a specific parasitoid of the invasive Red gum lerp psyllid, *Glycaspis brimblecombei*, a pest on different species of *Eucalyptus* (Myrtaceae).

Material examined: Benitses, 39°32′N, 19°54′E, 6 m a.s.l., 16.VIII.2013, found to parasitize nymphal instars of *G. brimblecombei* infesting *Eucalyptus camaldulensis*.

Biological remarks: *P. bliteus* is an endoparasitoid that delays development until the psyllid host reaches the late fourth or fifth instars. It pupates within the remains of the host. Adults have a metallic green body, the female with darker antennae and more pubescent than the male. Their lifespan depends on the temperature. The biological cycle completes between 16 and 41 days, depending greatly on weather conditions (Bella and Rapisarda, 2013).

Observed parasitisation: personal observations made during summer 2013 in the town of Benitses: from a total of 713 lerps (500 examined leaves of *E. camaldulensis*), 168 nymphs (23.6%) of *G. brimblecombei* result were parasitised.

Discussion

In the present contribution, seven alien insect species (1 Hemiptera, Psyllidae; 1 Hemiptera, Tingidae; 1 Diptera, Cecidomyiidae; 1 Diptera, Agromyzidae; 1 Lepidoptera, Lycaenidae; 1 Hymenoptera, Eulophidae) associated with non-indigenous ornamental plants on Corfu (Greece) are recorded. One of these pests, the agromyzid *Phytoliriomyza jacarandae*, is new to the Greek fauna. Moreover, the presence of two parasitoid wasps, *Platygaster robiniae* (Hymenoptera, Platygastridae) and *Psyllaephagus bliteus* (Hymenoptera, Encyrtidae) obtained from *Obolodiplosis robiniae* and *Glycaspis brimblecombei*, respectively, are also reported. The introduction of all new recorded alien insects must be considered accidental; also the two associated antagonists were most probably introduced together with their hosts, as has already happened in other European countries.

In the Palaeartic region, the only records of *Phytoliriomyza jacarandae* are in Italy (mainland and Sicily) and Portugal; it was probably imported to these countries and to Greece with infested ornamental Blue jacaranda trees (Bella *et al.*, 2007; Bella, 2013).

The detected species show a high level of host-plant specificity, and their dispersion is related to the presence of their exotic host plants, thus they should remain restricted to artificial habitats, such as nurseries, parks, gardens and urban areas. However, two species, *Corythucha ciliata* and *Cacyreus marshalli*, can spread to natural environments and attack spontaneous plants.

The rapid colonisation in Mediterranean countries by the psyllid *Glycaspis brimblecombei* can represent a more serious threat, with both economic and ecological impacts, because of the large diffusion of *Eucalyptus* trees used for extensive reforestations (Bella and Rapisarda, 2013). The spontaneous dispersal of the psyllid's exotic parasitoids also in Greece leads to a remarkable interest in the biological control of the pest, as already shown in the countries where it has been found. The rapid colonisation of European countries by alien pests requires continuous investigation of their possible impacts and population dynamics.

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Literature cited

Bella, S. 2007. Presenza di *Obolodiplosis robiniae* (Haldeman, 1847) in Italia centro meridionale e in Sicilia (Diptera Cecidomyiidae). *Bollettino di Zoologia agraria e di Bachicoltura*, Ser. II 39 (3): 239-242.

Bella, S. 2013. New alien insect pests to Portugal on urban ornamental plants and additional data on recently introduced species. Annales de la Société entomologique de France (N.S.), 49(4): 374-382.

Bella, S. and Rapisarda, C. 2013. First record from Greece of the invasive red gum lerp psyllid *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae) and its associated parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae). *Redia*, XCVI, 33-35.

Bella, S., Mazzeo, G. and Süss, L. 2007. First record for the European fauna of *Phytoliriomyza jacarandae* Steyskal & Spencer 1978 (Diptera Agromyzidae) leafminer of *Jacaranda mimosifolia* D. Don. (Bignoniaceae). *Bollettino di Zoologia agraria e di Bachicoltura*, Ser II. 39(1): 75-78.

Ben Attia, S. and Rapisarda, C. 2014. First record of the red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hemiptera Psyllidae), in Tunisia. *Phytoparasitica*, DOI 10.1007/s12600-014-0391-8.

Brennan, E.B., Hrusa, G.F., Weinbaum, S.A. and Levison, W. 2001. Resistance of *Eucalyptus* species to *Glycaspis brimblecombei* (Homoptera: Psyllidae) in the San Francisco Bay area. *Pan-Pacific Entomologist*, 77(4): 249-253.

CABI 2014. *Cacyreus marshalli*. http://www.cabi.org/isc/search/?q=Cacyreus+marshalli&rows=25 (accessed april 2014).

DAISIE 2014. *Platygaster robiniae*. http://www.europe-aliens.org/speciesFactsheet.do?speciesId=105445 (accessed april 2014).

Jørgensen, J. 2009. *Obolodiplosis robiniae* (Haldeman, 1847) (Cecidomyiidae) og dens parasitoid *Platygaster robiniae* Buhl et Duso, 2007 (Platygastridae) to nye arter for Danmark. *Journal Entomologiske Meddelelser*, 77(2): 141-144.

Kim, I.K., Mendel, Z., Protasov, A., Blumberg, D. and Lasalle J. 2008. Taxonomy, biology and efficacy of two Australian parasitoids of the eucalyptus gall wasp, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae: Tetrastichinae). *Zootaxa*, 1910: 1-20.

Kim, I.K., Park, J.D., Shin, S.C. and Park I.K. 2011. Prolonged embryonic stage and synchronized life-history of *Platygaster robiniae* (Hymenoptera: Platygastridae), a parasitoid of *Obolodiplosis robiniae* (Diptera: Cecidomyiidae). *Biological Control*, 57 (1): 24-30.

Laudonia, S. and Garonna, A.P. 2010. The red gum lerp psyllid, *Glycaspis brimblecombei*, a new exotic pest of *Eucalyptus camaldulensis* in Italy. *Bulletin of Insectology*, 63: 233-236.

Longo, S. 2004. Note sul Licenide sud-africano dei gerani *Cacyreus marshalli* (Lepidoptera Lycenidae) nell'Italia meridionale. *Tecnica Agricola*, 55(4) (2003): 3-11.

Lu, C.K., Buhl, P.N., Duso, C., Zhao, C.M., Zhang, J.S., Ji, Z.X., Gao, S.H., Yu, J.Y. and Wen X.L. 2010. First discovery of *Platygaster robiniae* (Hymenoptera: Platygastridae) parasitizing the invasive *Obolodiplosis robiniae* (Diptera: Cecidomyiidae), a gall maker in China. *Acta Entomologica Sinica*, 53(2): 233-237.

Maatouf, N. and Lumaret, J.P. 2012. Eco-ethology of new invasive pest species on eucalyptus plantation of Morocco. *Annales de la Société Entomologique de France*, 48(3/4) : 289-297.

Malumphy, C., Reid, S. and Eyre, D. 2006. Platanus lace bug *Corythucha ciliata*. *Plant pest notice*, 46: 4.

Martinou, A.F., Papachristos, D. and Milonas, P.G. 2011. Report of the geranium bronze butterfly, *Cacyreus marshalli* for mainland Greece. *Hellenic Plant Protection Journal*, 4(2): 31-34.

Milonas, P.G. and Partsinevelos, G.K. 2014. First record of *Glycaspis brimblecombei* Moore, 1964 (Hemiptera: Psyllidae) in Greece. *Hellenic Plant Protection Journal*, 7: 19-23.

Parker, R. 2010. *Cacyreus marshalli* Butler, 1898 (Lepidoptera, Lycaenidae) newly recorded for Corfu, with notes on other butterflies on the island in September 2008. *Entomologist's Gazette*, 61(1): 40-42.

Reguia, K. and Peris-Felipo, F.J. 2013. *Glycaspis brimblecombei* Moore, 1964 (Hemiptera Psyllidae) invasion and new records in the Mediterranean area. *Biodiversity Journal*, 4(4): 501-506.

Sánchez García, I. and Burckhardt, D. 2009. First Record of *Acizzia jamatonica* (Kuwayama, 1908) (Hemiptera: Psylloidea) for the Iberian Peninsula. *Boletín de la Sociedad Entomológica Aragonesa*, 45: 525-526.

Skuhravá, M. and Skuhravý, V. 2006. Gall midge (Diptera: Cecidomyiidae) of the islands Corfu and Samos (Greece). *Acta Universitatis Carolinae-Biologica*, 50: 109-123.

Spencer K. A. 1990. Host specialization in the world Agromyzidae (Diptera). Dordrecht: Kluwer Academic Publishers.

Sviridov, S.V. and Bazhenova, T.N. 2009. The first record of *Platygaster robiniae* (Hymenoptera, Platygastridae), parasitoid of *Obolodiplosis robiniae* (Diptera, Cecidomyiidae) for Ukraine. *Vestnik Zoologii*, 43(2): 106.

Tsagkarakis, A.E., Kalaitzaki, A.P. and Balotis G.N. 2014. Note on *Glycaspis brimblecombei* Moore (Hemiptera Psyllidae): A new pest of *Eucalyptus* in Greece. *Advances in Entomology*, 2(1): 57-59.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Χωροκατακτητικοί εντομολογικοί εχθροί και τα παρασιτοειδή τους σε καλλωπιστικά φυτά του αστικού περιβάλλοντος της Κέρκυρας - *Phytoliriomyza jacarandae* Steyskal and Spencer 1978 (Diptera, Agromyzidae) νέα καταγραφή στην Ελλάδα

S. Bella

Περίληψη Η παρούσα εργασία παρουσιάζει τα αποτελέσματα πρόσφατων επισκοπήσεων για ξενικά είδη εντόμων σε καλλωπιστικά φυτά στο αστικό περιβάλλον της Κέρκυρας. Συνολικά καταγράφηκαν επτά ξένα είδη τα οποία σχετίζονται με αλλόχθονα καλλωπιστικά φυτά: *Acizzia jamatonica* (Kuwayama 1908), *Glycaspis brimblecombei* Moore 1964 (Hemiptera, Psyllidae), *Corythucha ciliata* (Say 1832) (Hemiptera, Tingidae), *Obolodiplosis robiniae* (Haldeman 1847) (Diptera, Cecidomyiidae), *Phytoliriomyza jacarandae* Steyskal and Spencer 1978 (Diptera, Agromyzidae), *Cacyreus marshalli* Butler 1898 (Lepidoptera, Lycenidae) και *Leptocybe invasa* Fisher and La Salle 2004 (Hymenoptera, Eulophidae). Αυτή είναι η πρώτη αναφορά του *Phytoliriomyza jacarandae*, υπονομευτής του *Jacaranda mimosifolia* D. Don. (Bignoniaceae), στην Ελλάδα. Επίσης καταγράφηκαν δύο υμενόπτερα παρασιτοειδή, *Platygaster robiniae* Buhl and Duso 2008 (Hymenoptera, Platygastridae) και *Psyllaephagus bliteus* Riek 1962 (Hymenoptera, Encyrtidae), των *Obolodiplosis robiniae* και *Glycaspis brimblecombei*, αντίστοιχα. Παρουσιάζονται λεπτομέρειες για την τρέχουσα γεωγραφική κατανομή, ξενιστές και βιολογικά χαρακτηριστικά κάθε είδους.

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Weed control benefit to cost ratio and labour return value in crops of southern European countries with the use of herbicide pendimethalin

E. Marinan-Arroyuelo[1], A. Marchi[2], F. Marchal-Rubio[3], C. Bozoglou[4], D. Servis[5] and C.N. Giannopolitis[6*]

Summary Specially designed field trials were conducted in transplanted processing tomatoes, direct-seeded onions (dry bulb production), cotton and broccoli (fresh vegetable market) considered as four of the core crops determining the importance of using pendimethalin for weed control in southern European countries. Based on yield data and actual farmer's prices for costs and products, the expected weed control benefit/cost ratio was determined when pendimethalin, some alternative herbicides, hand weeding or a combination was used. In most cases pendimethalin, being sufficiently effective as a single treatment, provided the most favourable benefit/cost ratio, which was by far better than the ratio obtained with hand weeding. Evidence is also provided that alternative weed control methods, like inter-row cultivations and on the row polyethelene mulching, which were not used in this study, are by no means able to provide a ratio equivalent to that of pendimethalin. The labour return value analysis further revealed that hand weeding, which as a weed control method is not economically justified by itself in any case, can become justifiable as a supplementary measure following a pendimethalin application in many cases. These results clearly show that pendimethalin (or any herbicide acting similarly) has the potential to provide a favourable benefit/cost ratio for the control of weeds in crops, like the ones examined in this study, that are associated with an increasing demand for the lowest production costs.

Additional keywords: broccoli, cotton, onion, processing tomato, weed control costs, weed control profits

Introduction

Pre-emergence herbicides, having a residual activity in soil, can often offer satisfactory weed control applied as a single treatment in situations where emergence of weeds continues for a longer period. Availability of such herbicides, however, has become limited in recent years, because of the withdrawal of many older active ingredients during the re-registration process in the EU according to Directive 91/414 (eg. EU, 2008). European farmers, thus, have turned more to multiple applications of post-emergence herbicides.

Increased selection pressure associated with repeated multiple applications of post-emergence herbicides has already led to weed species becoming resistant to many of these herbicides including the ACCase-inhibitors (Bravin *et al*., 2001; Papapanagiotou *et al*., 2012), the ALS-inhibitors (Scarabel *et al*., 2004; Kaloumenos and Eleftherohorinos, 2008), glyphosate (Collavo and Sattin, 2012; Nol *et al*., 2012) and others. Multiple applications are also very likely to increase the cost of weed control but clear evidence with specific crops is not available in the literature up to now.

In this study specially designed field trials were conducted that allowed determination

[1] Technical market development, BASF Espanola SA
[2] Field Trial Services, BASF Italia SA
3 Field Trial Services, BASF Espanola SA
[4] Field Trial Services, BASF Hellas SA
[5] Product Development, BASF Hellas SA, Athens, Greece
[6] Currently Editorial Committee of AgroTypos SA, former Director of Weed Science Department at the Benaki Phytopathological Institute, Greece
* Corresponding author: editor@agrotypos.gr

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of the benefit/cost ratio, at actual farmer's prices, when using a pre-emergence herbicide (pendimethalin) versus hand weeding and alternative herbicides for weed control in specific crops. The benefit/cost analysis has been used by some researchers to determine if a weed management practice is economically justifiable (Culliney, 2005; Magani *et al.*, 2012). The hand weeding labour return value was also determined.

One objective of this study was to appraise the importance of using a proper pre-emergence herbicide for weed control in crops of Southern European countries, since appropriate benefit/cost studies have not been done so far. In these countries, the variety of weed species is in general greatest and climatic conditions favour their prolonged emergence, thus northern European farmers are the ones mostly affected by the limited availability of pre-emergence herbicides. Four diverse crops were therefore selected that are important for Southern European countries, namely processing tomatoes, onions for dry bulb production, cotton and broccoli. Furthermore, market prices for processing tomatoes (WPTC, 2012), cotton (USDA, 2012) and onions (Eurostat, 2012) are affected by strong international competition and Southern European farmers continuously try to keep weed control and production costs low so that they do not loose access to market. An important minor crop, broccoli, was also included in the study since weed control in minor crops is even more difficult due to the unwillingness of herbicide manufacturers to seek registration for such crops (Gast, 2008).

Pendimethalin was used as the reference pre-emergence herbicide in this study, as it is known for its good efficacy on many weed species with a good duration of control and no resistance problems. Furthermore, it is one of the few members of the low resistance-risk group of the dinitroaniline or K1 herbicides-inhibiting microtubule assembly (HRAC, 2014), that have remained available to the farmers (EU, 2003) and are expected to play an important role in the anti-resistance strategy in next years. It is worth mentioning that, although dinitroaniline and other microtubule-inhibiting herbicides have been used for several decades, they have selected resistance in only 12 weed species (Heap, 2014). This is because the target-site resistance for the dinitroaniline herbicides is inherited as a recessive single nuclear gene, which makes this mechanism of resistance more difficult to evolve as the initially rare heterozygous individuals are killed at normal herbicide dose. Also, this helps explain the limited evolution of this mechanism of resistance, especially in cross-pollinated species (Powles and Yu, 2010).

Pendimethalin is registered and used in many major crops and it is the only herbicide registered in as many as 20 minor crops in the Southern European countries. The reason for its broad use as a selective herbicide is the limited acropetal translocation from the root tissues to the above plant organs (products), which prevents the presence of pendimethalin residues on the products above the prescribed MRL limits (Sondhia, 2013; WSSA, 2007). In addition, its strong adsorption on soil colloids reduces its leaching potential (it is practically immobile in the soil) and makes this herbicide environmentally friendly regarding water contamination (WSSA, 2007). Being one of the few herbicides registered in several crops, the scenario of a possible withdrawal of these pendimethalin uses from the market has to be evaluated from the farmer's economic perspectives. The second objective of this study was therefore to provide a basis for such an evaluation.

Materials and methods

General

For the purpose of this study field trials were conducted in major growing areas for the respective crops in either Greece or Italy. Treatments in all trials included pendimethalin (at one or two rates) as registered and commonly used by farmers, an untreated control and a hand-weeded control. Depending on the crop, treatments also includ-

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ed some other herbicides that are registered and commonly used by local farmers.

For a better evaluation of the actual herbicide efficacy and weed control costs, in all experiments herbicide treatments were used alone or combined to hand weeding at the proper time to control escaping weeds. The labour input was quantified by recording the time and cost required for this supplemental hand-weed control. All treatments in an experiment were replicated three times and arranged in a randomized complete block design (RCBD).

With the exception of weed control practices, the crops in the trials received all other locally adopted practices of fertilization, irrigation and plant protection. Crops were grown to maturity and harvested. For each treatment the marketable yield (kg/ha) was recorded and the yield value (€/ha) was estimated based on actual farmer's prices. For each treatment, the cost of weed control (€/ha, including value of the herbicide, cost of application and/or cost of hand weeding, depending on the treatment) was also estimated based on local prices. The benefit/cost ratio in €/€, for each treatment, was calculated by taking the difference in yield value between a treatment and the untreated control and dividing it by the respective weed control cost. The benefit/cost ratio is used in this study as a more useful expression of the weed control efficacy related directly to farmer's economical interests.

Since hand weeding is sometimes used by farmers in these countries as a rescue method in situations of weed control failure and more often as a supplemental measure to situations of erratic herbicidal weed control, the labour return value for each treatment in these trials was also determined. The labour return value (in €/hr) for a treatment was calculated by taking the difference in yield value of the same treatment, with and without hand weeding, and dividing it by the respective time required for hand weeding. This value could then be compared with the actual hourly payment for hand weeding to assess if a treatment has a positive or negative effect on the weed control labour inputs and if hand weeding can be economically justified.

The herbicide formulations used in the trials were Stomp Aqua 455 CS (pendimethalin 45,5% w/v), Goal 48 SC (oxyfluorfen 48% w/v), Dual Gold 96 EC (s-metolachlor 96% w/v), Sencor 35 WG (metribuzin 35% w/w) and Cottonex 50 SC (fluometuron 50% w/v). Herbicide application was made using AZO sprayers, with a spraying boom of variable length and nozzle number, delivering 300-400 L/ha at a pressure of 3 bar.

The trials were conducted, under the authors' supervision, by contracted GEP-certified bodies (Agro Unit and Agrolab) in Greece and the GEP Test Facility of BASF Italia Srl in Italy.

The time of hand weeding and the yield data were subjected to one-way ANOVA and the statistically different means (denoted with different letters in the same column of the result tables) were determined using the Duncan's test at P=0.05. The SPSS statistical pack PASW Statistics 18 was used for all statistical analyses.

The processing tomato trial

The processing tomato trial was conducted during the 2010 growing season in Larissa (Central Greece). Seedlings of the commercial hybrid Heinz 3402 were transplanted in the field on May 6. Experimental plots of 3.1x10 m (4 rows, 25 plants/row) were used.

The two most widely applied pre-transplant herbicides in transplanted processing tomatoes in Greece, namely pendimethalin and s-metolachlor, were used as the basic treatments along with an untreated control. Pendimethalin was applied at the rate of 1365 g/ha eight days before tranplanting and was mechanically incorporated into the soil as recommended. S-metolachlor was applied at the rate of 1248 g/ha the same day as pendimetalin but with no incorporation. The experimental area was irrigated two days after tranplanting to activate the soil applied herbicides.

The other experimental treatments included the untreated control, and hand

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weeding at 23, 39 and 54 days after transplanting (DAT). Pendimethalin and s-metolachlor were used both alone or combined to a post-transplant (23 DAT) application of metribuzin at the rate of 280 g/ha and two hand weedings (39 and 54 DAT). A post-transplant application of metribuzin is commonly used in Greece as a supplemental weed control method in processing tomatoes.

The tomato fruits were harvested on August 20 (104 DAT) from the central two rows of each plot and the yield data recorded.

Weeds present in untreated controls (expressed as plants/m^2, 23 DAT) were: *Portulaca oleracea* (175), *Heliotropium europaeum* (85), *Solanum nigrum* (31), *Amaranthus lividus* (31), *Cyperus esculentus* (1).

The onion trials

The winter-seeded onion trial presented in detail here was conducted in 2011 in the province of Bologna (region of Emilia Romagna, Italy), an area where onion is the main cultivation. Onion seeds of the variety Cometa were sown on February 2 and seedling emergence was completed on March 30. Experimental plots of 10x12 m were used.

The basic treatments in this trial were the untreated control, a pendimethalin pre-emergence treatment, an oxyfluorfen post-emergence treatment and a combined application of the two herbicides (the common practice in the area). Pendimethalin was applied at the rate of 910 g/ha 37 days after sowing (before emergence) of the onions. Oxyfluorfen was applied at the rate of 360 g/ha when onion seedlings reached the 3-leaf stage, 22 days after emergence (DAE). Supplemental weed control for all basic treatments in this trial was made with a hand weeding on the 49 and the 79 DAE.

Onion dry bulbs were harvested on July 19 (109 DAE) from the central 4x1.4 m area of each plot, the bulbs were graded to three classes according to size (<50 mm being not marketable, 50-80 mm being marketable, >80 mm regarded as not marketable) and the marketable yield per plot recorded.

Weed species present in untreated controls (expressed as mean % contribution of each species in the weed coverage about 60 DAE) were: *Polygonum convolvulus* (87%), *Anagallis foemina* (4%), *Anagallis arvensis* (4%), *Polygonum persicaria* (1,3%), *Stellaria media* (1%), *Amaranthus retroflexus* (1,7%), *Solanum nigrum* (1%).

The cotton trial

The cotton trial presented in detail here was conducted in Greece during the 2011 growing period. Cotton seed of the variety Celia was sown on April 20 in a field located at Elefterio, a typical cotton growing area in Central Greece (Larissa). Seedling emergence was completed on May 6. Experimental plots of 3.84x10 m (4 crop rows) were used.

Basic treatments in this trial included a pre-emergence pendimethalin application at the two recommended rates (1638 or 1956.5 g/ha) and the combination pendimethalin (1638 g/ha) plus fluometuron (1250 g/ha), as commonly used in Greece. Pendimethalin was applied four days before sowing and mechanically incorporated into the soil. Fluometuron was applied on the same day after sowing (no incorporation). The experimental area was irrigated two days after sowing to activate the soil applied herbicides and to favor cotton seed germination. All basic treatments were used alone or supplemented with hand weeding at 30, 44 and 60 days after cotton sowing (DAS).

Cotton was harvested on September 26 from the central two rows of each plot and the yield (as seed cotton) recorded.

Main weed species present in the untreated controls (plants/m^2, 30 DAS) were: *Solanum nigrum* 53, *Amaranthus hybridus* 11.3, *Chenopodium album* 8.

The broccoli trials

Two trials are presented that were conducted with transplanted broccoli in Greece. The first was with a crop (cv. Parthenon) transplanted in December 9 2010 and harvested on April 13, 2011 in a field located in the area of Imathia (W. Macedonia, N. Greece). The second was with a crop (cv. Modor 8228) transplanted on March 14, 2011

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and harvested on May 31, 2011, in a field located in Pella (C. Macedonia, N. Greece). Experimental plots of 36x8 m (8 crop rows) were used in both experiments.

In both trials, the basic treatments were the untreated control and pendimethalin at the two recommended rates (1137.5 and 1365 g/ha). Pendimethalin in these trials was applied and incorporated the same day before transplanting of the broccoli.

Supplemental weed control in all treatments was made with hand weeding three times, 31, 51 and 73 DAT for the first trial and 30, 45 and 60 DAT for the second trial.

At harvest, the marketable flower heads were collected from all plants of the central 4 rows in each plot and weighted.

Weed species present in untreated controls (expressed as mean % contribution of each species in the weed coverage, 30 DAT) were:

In the first experiment: *Senecio vulgaris* (12%), *Sonchus oleraceus* (12%), *Papaver rhoeas* (8%), *Stellaria media* (13%), *Sinapis* spp. (8%), *Capsella bursa-pastoris* (10%).

In the second experiment: *Senecio vulgaris* (7%), *Sonchus oleraceus* (8%), *Chenopodium album* (14%), *Stellaria* sp. (15%), *Anthemis* sp. (5%), *Capsella bursa-pastoris* (17%).

Results[1]

Tomato trial

Results from the processing tomato trial are shown in Table 1.

As shown with the yield data, weeds in this trial caused a yield reduction of over 50%. Pendimethalin (applied pre-transplant) was sufficient in preventing yield reduction but s-metolachlor (also pre-transplant) was not. Yield loss in this trial was also prevented when hand weeding (three times, total required time 190 hrs/ha) was used by itself or when s-metolachlor was supplemented with post-emergence metribuzin (common practice by farmers) and the required time of hand weeding (twice, 92 hrs/ha). When pendimethalin was similarly supplemented with metribuzin and the required time of hand weeding (twice, 26 hrs/ha) further yield increase was insignificant.

The best weed control benefit/cost ratio (17.3) in the processing tomato trial was achieved with pendimethalin when used alone. A lower ratio of 7.0 was achieved when pendimethalin was combined with metribuzin and hand weeding but even this ratio is much better than that from all other treatments. The worst benefit/cost ratio (2.1) in this trial was achieved when hand weeding was used alone.

The hand weeding labour return value was 10.5 €/ha when hand weeding was the only weed control method and significantly better, 16.8 and 22.4, when it was used to supplement the treatments of pendimethalin + metribuzin and s-metolachlor + metribuzin, respectively.

Onion trials

Results from a trial with winter-seeded onions are presented in Table 2.

As shown with the yield data, weeds in onion caused a dramatic reduction of marketable yield. Pre-emergence pendimethalin only partly prevented yield loss in this experiment due to the presence of a tolerant weed species (*Polygonum convolvulus*) and post-emergence oxyfluorfen which controlled this weed performed better. The combination of the two herbicide applications (a usual practice by farmers) did not further increase yield. Maximum yield in this experiment was achieved only when the combined herbicide applications were supplemented with hand weeding.

Hand weeding (twice) was about as effective as the herbicides in preventing yield loss when used alone and contributed to a significantly better yield when used as a supplemental treatment to pendimethalin or pendimethalin+oxyfluorfen but not

[1] **Note:** All prices quoted in this document are retail prices, have been obtained by BASF from public sources and/or legal sources believed to be accurate at the time and are not an indication for current or future prices. Prices have been provided for the purposes of this document only and are not intended to be a guidance nor recommendation for the purchase or application of herbicides. Liability for any errors is hereby disclaimed.

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to oxyfluorfen. The time required for hand weeding (Table 2) was significantly shorter in treatments that included pendimethalin and this is explained by the residual activity of the herbicide.

The best benefit/cost ratio (69.1) in this experiment was obtained with post-emergence oxyfluorfen and not with pre-emergence pendimethalin due to the presence of *Polygonum convolulus*. Despite that, a quite favourable ratio was also obtained with pendimethalin (47.1) as well as

Table 1. Results from the processing tomato field trial (Greece, 2010).

Treatments	Yield (Kg/ha)*	Labour for hand weeding (hr/ha)*	Yield value (€/ha)[1]	Weed control costs (€/ha)[2]	Benefit/ Cost ratio (€/€)**	Labour return value (€/hr)***
Untreated control	22580 b	-	1423	-	na	
3 Hand weedings (3 hw)	54193 a	190 a	3414	950	2.1	10.5
Pendimethalin	50280 a	-	3168	101	17.3	
Pendimethalin + metribuzin + 2 hw	57207 a	26 c	3604	313	7.0	16.8
S-metolachlor	25527 b		1608	62	3.0	
S-metolachlor + metribuzin + 2 hw	57853 a	91 b	3645	599	3.7	22.4

[1] The farmer's price in Greece for the 2010 period was 63 €/ton (based on industry sources).
[2] Labour cost for hand weeding (payment 5 €/hr, based on local sources) + Value of herbicide (Stomp 17 €/L, Sencor 65 €/Kg, Dual 24,6 €/L) + Cost of application (50 €/ha for Stomp incorporated, 30 €/ha for Dual or Sencor surface-applied).
* Means of three replicates. Values within a column followed by the same letters are not statistically different at P=0.05.
** Benefit/Cost ratio for a treatment (€/€) = (Yield value of the treatment – Yield value of untreated control)/Weed control costs of the treatment.
*** Labour return value for a treatment (€/hr)= (Yield value of the treatment with hand weeding – Yield value of the treatment without hand weeding)/Labour for hand weeding.

Table 2. Results from the seeded onion trial (Italy, 2011).

Treatments	Marketable yield (Kg/ha)*	Labour for hand weeding (hr/ha)*	Yield value (€/ha)[1]	Weed control costs (€/ha)[2]	Benefit/ Cost ratio (€/€)**	Labour return value (€/hr)***
Untreated control	4292 d	-	773	0	na	
2 Hand weedings (2 hw)	32268 bc	1095 a	5808	12045	0.4	4.6
Pendimethalin pre	21048 c		3789	64	47.1	
Pendimethalin pre + 2 hw	41679 b	476 b	7502	5300	1.3	7.8
Oxyfluorfen post	38851 b		6993	90	69.1	
Oxyfluorfen post + 2 hw	48988 b	1369 a	8818	15149	0.5	1.3
Pendimethalin pre + oxyfluorfen post	43089 b		7756	154	45.3	
Pendimethalin pre + oxyfluorfen post + 2 hw	59542 a	611 b	10718	6875	1.4	0.4

[1] Actual farmer's price 0.18 €/kg (for 50-80 mm bulbs only) (based on market sources).
[2] Labour cost for hand weeding (payment in Italy 11 €/hr, based on local sources) + Herbicide value (Stomp Aqua 455 CS 17 €/L, Goal 48 CS 80 €/L) + Cost of application (30 €/ha surface application of both herbicides).
* Means of three replicates. Values within a column followed by the same letters are not statistically different at P=0.05.
** Benefit/Cost ratio for a treatment (€/€) = (Yield value of the treatment – Yield value of untreated control)/Weed control costs of the treatment.
*** Labour return value for a treatment (€/hr)= (Yield value of the treatment with hand weeding – Yield value of the treatment without hand weeding)/Labour for hand weeding.

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with pendimethalin+oxyfluorfen (45.3). The cost of labour, on the other hand, dramatically reduced the ratio value to 1.4 when pendimethalin+oxyfluorfen was supplemented with hand weeding pointing out that the maximum yield treatment is not necessarily the most profitable one for the farmer.

The hand weeding labour return value was very low (0.4) in onions and was only slightly improved (1.3-1.4) when hand weeding was combined with pendimethalin, indicating that hand weeding can hardly be a justifiable method for weed control in onions. In agreement to this were also the benefit/cost ratio values which were very favourable with the use of herbicides but became dramatically lower when the herbicides were supplemented with hand weeding.

A second field trial with spring-seeded onions in Greece the next year (results not presented) confirmed the above statements. In that trial, pendimethalin (applied pre-emergence or early post-emergence at the whip stage of onions) provided the best benefit/cost ratios (17.0 and 15.1 respectively), post-emergence oxyfluorfen and the combination pendimethalin + oxyfluorfen provided a lower ratio (6.5 and 7.1 respectively) and hand weeding the lowest (1.0). Maximum yield was obtained with pendimethalin early post-emergence. The hand weeding labour return value was better (5.2) in this experiment due to faster growth of onion seedlings in spring and again improved by pendimethalin or pendimethalin + oxyfluorfen (7.0-12.3). It is worthy to note the absence of a pendimethalin tolerant weed species in this trial and the lower benefit/cost ratio values due to a lower farmer's price for onions at this season.

Cotton trials

Results from a cotton field trial are presented in Table 3.

As shown from the yield data, weeds diminished cotton yield and pre-emergence pendimethalin (applied at the lowest and the highest recommended rates) was capable of preventing yield loss significantly. The maximal cotton yield was obtained with either hand weeding applied alone (3 times, total of 503 hrs/ha) or hand weeding applied for a shorter time as a supplemental treatment to pendimethalin. Similar yield was obtained with combined application of the low pendimethalin rate with fluometuron, another pre-emergence herbicide commonly used by farmers as no selective post-emergence herbicide is available for cotton.

Best weed control benefit/cost ratio was provided by the low pendimethalin rate treatment (11.5), followed by the combined pendimethalin+fluometuron (9.1) and the high pendimethalin rate (8.4) treatments. The cost of labour, on the other hand, reduced the ratio value to below 4 when hand weeding was used alone or as a supplementary treatment to herbicides, pointing out that in cotton also the maximum yield treatments are not the most profitable ones for the farmer.

The labour return value in cotton was low (4.5 €/hr) and hand weeding by itself is again not justified. The herbicide treatments, particularly the ones with the low pendimethalin rate and the combination of pendimethalin+fluometuron, improved the labour return value (9.6 and 10.5 €/hr, respectively) and therefore hand weeding may be justified as a supplementary measure to these treatments, in cases where the maximal yield is desired.

Similar trends had been observed in another trial conducted in Greece the previous year (results not presented). The best benefit/cost ratio was provided by pendimethalin at the high or the low rate (4.6 and 3.9 respectively) and the worst by hand weeding or pendimethalin+fluometuron (0.9 and 0.5 respectively).

Lower best ratio values in this trial are due to lower yields that year. The hand weeding labour return value was again low (4.7) and improved most by the low pendimethalin rate (9.0) and the combination pendimethalin+fluometuron (9.2).

Broccoli trials

Results from two trials with broccoli are shown in Table 4.

The yield data indicate that weeds in the

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Table 3. Results from the cotton trial (Greece, 2011).

Treatments	Yield (Kg/ha)*	Labour for hand weeding (hr/ha)*	Yield value (€/ha)[1]	Weed control costs (€/ha)[2]	Benefit/ Cost ratio (€/€)**	Labour return value (€/hr)***
Untreated control	254 c		152	0	na	
3 Hand weedings (3 hw)	4039 a	503 a	2423	2515	0.9	4.5
Pendimethalin 1638 g/ha	1812 b		1087	111	8.4	
Pendimethalin 1638 g/ha + 3 hw	4109 a	143 b	2465	826	2.8	9.6
Pendimethalin 1956.5 g/ha	2513 b		1508	118	11.5	
Pendimethalin 1956.5 g/ha + 3 hw	3364 ab	96 b	2018	598	3.1	5.3
Pendimethalin 1638 g/ha + fluometuron	2743 ab		1646	164	9.1	
Pendimethalin 1638 g/ha + fluometuron + 3 hw	4251 a	86 b	2551	594	4.0	10.5

1 Average price paid to Greek cotton growers in 2011 0.60 €/Kg (based on industry sources).
2 Labour cost for hand weeding (payment 5 €/hr, based on local sources) + Value of herbicide (Stomp 17 €/L, Cottonex 9 €/L) + Cost of application (30 €/ha for Cottonex and 50 €/ha for Stomp).
* Means of three replicates. Values within a column followed by the same letters are not statistically different at P=0.05.
** Benefit/Cost ratio for a treatment (€/€) = (Yield value of the treatment – Yield value of untreated control)/Weed control costs of the treatment.
*** Labour return value for a treatment (€/hr)= (Yield value of the treatment with hand weeding – Yield value of the treatment without hand weeding)/Labour for hand weeding.

Table 4. Results from the two broccoli trials (Greece, 2010-11).

Treatments	Yield (Kg/ha)	Labour for hand weeding (hr/ ha)*	Yield value (€/ha)[1]	Weed control costs (€/ha)[2]	Benefit/ Cost ratio (€/€)**	Labour return value (€/hr)***
Experiment from Dec. 2010 to April 2011						
Untreated control	1607 c		1768	0	na	
3 Hand weedings (3 hw)	1797 c	151a	1977	755	0.3	1.4
Pendimethalin 1137.5 g/ha	2205 b		2425	92	7.1	
Pendimethalin 1137.5 g/ha + hw	2344 b	39b	2578	287	2.8	3.9
Pendimethalin 1365 g/ha	2490 a		2739	101	9.6	
Pendimethalin 1365 g/ha + hw	2594 a	21b	2853	207	5.2	5.4
Experiment from March to May 2011						
Untreated control	1359 c		1495	0	na	
3 Hand weedings (3 hw)	1677 c	134a	1845	670	0.5	2.6
Pendimethalin 1137.5 g/ha	2005 b		2205	92	7.7	
Pendimethalin 1137.5 g/ha + hw	2319 b	32b	2551	252	4.2	10.8
Pendimethalin 1365 g/ha	2444 ab		2688	101	11.8	
Pendimethalin 1365 g/ha + hw	2688 a	14c	2957	171	8.5	19.2

1 Farmer's price for broccoli 1.10 €/kg (based on market sources).
2 Labour cost for hand weeding (5 €/hr based on local sources) + Value of herbicide (Stomp 42 and 51 €/ha depending on rate) + Cost of application (50 €/ha for spraying and incorporation).
* Means of three replicates. Values within a column followed by the same letters are not statistically different at P=0.05.
** Benefit/Cost ratio for a treatment (€/€) = (Yield value of the treatment – Yield value of untreated control)/Weed control costs of the treatment.
*** Labour return value for a treatment (€/hr)= (Yield value of the treatment with hand weeding – Yield value of the treatment without hand weeding)/Labour for hand weeding.

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broccoli trials caused a reduction of marketable yield of about 40-50% and pendimethalin, particularly when used at the high recommended rate, could prevent most of the yield loss. Hand weeding used by itself could not prevent yield loss and when used in combination with pendimethalin contributed to only a small increase of yield. Pendimethalin at the high rate was the most effective treatment since it provided the best yield and required the least labour input for removing escaping weeds in both experiments.

The best benefit/cost ratio values were provided by the high rate of pendimethalin in both trials. A probably satisfactory, although lower, benefit/cost ratio value was also provided by the low rate of pendimethalin. Combining pendimethalin with hand weeding results in lower ratio values but they are in any case much better than when hand weeding is used by itself.

The low hand weeding labour return values (1.4 and 2.6) indicate that hand weeding itself cannot be a justifiable weed control method for broccoli. Hand weeding as a supplementary measure of pendimethalin may sometimes be justified, however, as indicated by the higher values especially in the second trial.

Discussion

In almost all field trials, conducted in this study with diverse annual crops, the best weed control benefit/cost ratio at actual farmers' prices was obtained with a single pre-emergence application of a residual herbicide, namely of pendimethalin. Only in one trial, due to a pendimethalin tolerant weed species, best ratio was rather achieved with a post-emergence herbicide (oxyfluorfen) but still the single pendimethalin treatment kept the benefit/cost ratio at quite favourable levels (Table 2).

In all field trials, hand weeding provided the lowest weed control benefit/cost ratio. The labour return value further indicated that hand weeding by itself cannot even be used as a yield rescue method in all crops examined except possibly the processing tomatoes (Table 1). Hand weeding in processing tomatoes may be justified by itself in countries like Greece where field labour is paid less than 10.5 €/ha and may be further justified in countries where the field labour is more expensive if combined with herbicides like pendimethalin or s-metolachlor. The onion trial, on the other hand, revealed another important advantage offered by a residual herbicide like pendimethalin (Table 2) that even in the presence of a herbicide tolerant weed species, yield loss can be prevented with a relatively shorter time of hand weeding.

Alternative weed control methods to the ones examined in this study, that are used by farmers at least in processing tomatoes, are: a) inter-row cultivations combined with hand weeding or application of a post-transplant herbicide and b) the use of plastic mulch along the rows combined with herbicide application and/or inter-row cultivations between the rows (Anzalone *et al.*, 2010). Expected benefit/cost ratios for the processing tomatoes, under all alternative weed control scenarios, are given as an example in Table 5, assuming that all methods are equally effective in preventing a 50% yield reduction. Due to the high cost of inter-row cultivation and polyethelene (PE) mulching, the expected benefit/cost ratio from these methods is much lower than the one expected from herbicide-based methods. Pendimethalin, usually being sufficiently effective as a single treatment, is expected to provide a better benefit/cost ratio than alternative herbicides in agreement with the actual data from the respective field trial.

Achieving the best weed control benefit/cost ratio is to the farmer's interests since it helps to optimize weed control profitability, but achieving maximal yield may also be important in an effort to overall optimize crop profitability. In most trials, the single pendimethalin treatment provided both best weed control benefit/cost ratio and maximal yield (Tables 1 and 4). In some trials, however, the single pendimethalin treatment significantly reduced yield loss but for maximal

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Table 5. Expected weed control benefit/cost ratio in processing tomatoes using alternative weed control scenarios*.

Scenarios	Benefit (€/ha)[1]	Cost (€/ha)	Benefit/Cost (€/€)
Inter-row cultivations + hand weeding on rows[2]	2697.50	650	4.1
PE mulch on rows + herbicides post- transplanting[3]	2697.50	476	5.7
Pre-transplanting residual broad-spectrum herbicide (pendimethalin)[4]	2697.50	101	26.7
Pre-tranplanting broadleaf herbicide (oxadiazon) + post-transplanting grass herbicide (fluazifop)[5]	2697.50	143.5	18.8
Post-transplanting, two herbicide applications (rimsulfuron and metribuzin+rimsulfuron)	2697.50	215.8	12.5

* Based on 2011 prices in Greece (first year after decoupling of the EU production subsidies, when the industry paid for the tomatoes 20 €/ton higher than the previous year).
1 Assuming a 50% gain (32.5 ton/ha) of an average normal yield (65 ton/ha) x 83 €/ton in all scenarios.
2 Cost: 200 €/ha for two cultivations + 450 €/ha for two hand weedings.
3 Cost: 350 €/ha for the PE plastic + 96 €/ha for the price of herbicides (metribuzin+rimsulfuron) + 30 €/ha for application.
4 Cost: 51 €/ha for the price of herbicide + 50 €/ha for the application.
5 Herbicide prices 46+37,5 €/ha and application cost in Greece 30+30 €/ha.

yield it needed to be supplemented with some hand weeding (Table 3) or with a post-emergence herbicide and some hand weeding (Table 2), measures which increased the weed control costs and decreased the benefit/cost ratio.

With these trials it became clear, therefore, that pendimethalin, being a residual herbicide with a broad weed spectrum (including both broadleaf and grass weed species) and registered for many crop uses in Southern European countries, has the potential to provide maximal yield at the best benefit/cost ratio in many situations. In other situations, in which there may be a need to combine pendimethalin with supplementary measures in order to get the maximal yield, the benefit/cost ratio would mostly depend on the cost of the supplementary measures that will be used.

A properly selected post-emergence herbicide, if available, could be an economical supplement to pendimethalin. Other researchers have already shown that high yield in annual crops is better secured if a pre-emergence herbicide has been used prior to the post-emergence herbicide application (Nurse *et al.* 2006; Fickett *et al.*, 2013). In all trials of this study, on the other hand, pendimethalin significantly reduced the required time of supplemental hand weeding to avoid yield loss and provided better benefit/cost ratios and labour return values than using hand weeding alone (which by itself is not economically justified at current prices in Southern European countries). Hand weeding may therefore be another economically justified supplement to pendimethalin.

Furthermore, other specific reasons have also increased in recent years the importance of using a pre-emergence herbicide particularly for the crops examined in these trials. Most processing tomato growers in Southern European countries, to achieve a better management of specific weeds (eg. *Solanum nigrum*), have abandoned direct seeding and turned to transplanting which allows the pre-transplant application of a pre-emergence herbicide (Tei *et al.*, 2003). The onion dry bulb producers, to reduce production costs and face price competition in the market, have turned to direct seeding but with the young onion seedlings being susceptible to available post-emergence herbicides they need to use a selective pre-emergence herbicide for early season weed control (Tei *et al.*, 1999). In cotton (Giannopolitis, 2013), broccoli and other vegetable crops (Campagna *et al.* 2009), there are no registered broad spectrum post-emergence herbicides currently available to growers and they have to rely exclusively on few pre-emergence herbicides.

Conclusions

Field trials in this study with diverse annual crops (processing tomato, dry bulb onion, cotton and broccoli) demonstrated that pendimethalin, a residual herbicide with a broad weed spectrum, can in many cases be sufficiently effective as a single weed control treatment to provide maximal yield and best weed control benefit/cost ratio. Other alternatives that prevented yield loss, especially hand weeding, provided much lower benefit/cost ratios and cannot be regarded as profitable by farmers.

Determination of hand weeding labour return values further substantiated usefulness of the pendimethalin residual activity. By significantly reducing the required time of hand weeding, pendimethalin improved considerably the labour return value. Hand weeding thus became justifiable as a supplementary measure while it was economically unjustified as a standalone method at actual prices.

It becomes obvious from the results that producers in Southern European countries rely on herbicides like pendimethalin (only few available) for succeeding a profitable crop and remaining competitive in the market. For some crops they have even changed the cropping system to be able to use such herbicides.

Literature cited

Anzalone, A., Cirujeda, A., Aibar, J., Pardo, G. and Zaragoza, C. 2010. Effect of biodegradable mulch materials on weed control in processing tomatoes. *Weed Technol*., 24: 369–377.

Bravin, F., Zanin, G. and Preston, C. 2001. Diclofop-methyl resistance in populations of *Lolium* spp. from central Italy. *Weed Res*., 41: 49-58.

Campagna, G., Geminiari, E. and Rapparini, G. 2009. Pochi erbicidi per il diserbo delle orticole. *L' Inform. Agr.,* 33: 68-74.

Collavo, A. and Sattin, M. 2012. Resistance to glyphosate in Lolium rigidum selected in Italian perennial crops: bioevaluation, management and molecular bases of target-site resistance. *Weed Res.,* 52: 16–24.

Culliney, T.W. 2005. Benefits of classical biological control for managing invasive plants. *Crit. Rev. Plant Sci.,* 24: 131–150.

EU. 2003. Commission Directive 2003/31/EC of 11 April 2003 amending Council Directive 91/414/EEC to include 2,4-DB, beta-cyfluthrin, cyfluthrin, iprodione, linuron, maleic hydrazide and pendimethalin as active substances. Off. J. Eur. Union L. 101/23.4.2003, pp. 3-9.

EU. 2008. Commission Decision 2008/934/EC of 5 December 2008 concerning the non-inclusion of certain active substances in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing these substances. Off. J. Eur. Union L. 333/11.12.2008, pp. 11-14.

Eurostat. 2012. Onion Production in EU. Available at: http://epp.eurostat.ec.europa.eu/tgm/table.do?tab=table&init=1&language=en&pcode=tag00111&plugin=1 (last accessed May 11, 2012).

Fickett, N.D., Boerboom, C.M. and Stoltenberg, D.E. 2013. Soybean yield loss potential associated with early-season weed competition across 64 site-years. *Weed Sci*., 61: 500–507.

Gast, R. 2008. Industry view of minor crop weed control. *Weed Technol.,* 22: 385–388.

Giannopolitis, C.N. 2013. Weed control in cotton. *Georgia – Ktinotrofia*, issue 1/2013, pp. 30-31 (in Greek).

HRAC (Herbicide Resistance Action Committee). 2012. Classification of herbicides according to site of action. Available at: http://www.hracglobal.com/Publications/ClassificationofHerbicideSiteofAction/ tabid/222/Default.aspx

Kaloumenos, N.S. and Eleftherohorinos, I.G. 2008. Corn poppy (*Papaver rhoeas*) resistance to ALS-inhibiting herbicides and its impact on growth rate. *Weed Sci.,* 56: 789-796.

Magani, E.I., Shave, P.A. and Avav, T. 2012. Evaluation of fluazifop-P-butyl and propanil for weed control in sesame (*Sesamum indicum* L.) in Southern Guinea Savanna, Nigeria. *Am. J. Exp. Agr.,* 2: 680-689.

Nol, N., Tsikou, D., Eid, M., Livieratos, I.C. and Giannopolitis, C.N. 2012. Shikimate leaf disc assay for early detection of glyphosate resistance in *Conyza canadensis* and relative transcript levels of EPSPS and ABC transporter genes. *Weed Res*., 52: 233–241.

Nurse, R.E., Swanton, C.J., Tardif, F. and Sikkema, P.H. 2006. Weed control and yield are improved when glyphosate is preceded by a residual herbicide in glyphosate-tolerant maize (*Zea mays*). *Crop Prot.,* 25: 1174–1179.

Papapanagiotou, A.P., Kaloumenos, N.S. and

© Benaki Phytopathological Institute

Eleftherohorinos, I.G. 2012. Sterile oat (Avena sterilis L.) cross-resistance profile to ACCase-inhibiting herbicides in Greece. *Crop Prot.* 35: 118-126.

Powles, S.B. and Q. Yu. 2010. Evolution in Action: Plants resistant to herbicides. *Annual Review of Plant Biology,* 61: 317-347.

Scarabel, L., Carraro, N., Sattin, M. and Varotto, S. 2004. Molecular basis and genetic characterisation of evolved resistance to ALS-inhibitors in *Papaver rhoeas*. *Plant Sci.,* 166: 703-709.

Sondhia, S. 2013. Harvest time residues of pendimethalin in tomato, cauliflower, and radish under field conditions. *Toxicological & Environmental Chemistry,* 95: 254-259.

Tei, F., Ascard, J., Baumann, D.T., Caussanel, J-P., Dobrzanski, A., Froud-Williams, R.J., Kleifeld, Y., Pardo Iglesias, A., Rocha, F., Ruuttunen, P., Rzozi, S.B., Sanseovic, T. and Suso, L. 1999. Weeds and weed management in onion – a review. 11th EWRS Symp. 1999, Basel. Available at: http://www.agr.unipg.it/ewrsveg/onionsummary2.htm#references

Tei, F., Montemurro, P., Baumann, D.T., Dobrzanski, A., Giovinazzo, R., Kleifeld, Y., Rocha, F., Rzozi, S.B., Sanseovic, T., Simončič, A. and Zaragoza, C. 2003. Weeds and weed management in processing tomato. Proc. VIII Int. Symp. Proc. Tomato, Istanbul, Turkey. *Acta Hortic*. 613: 111-121.

USDA - Foreign Agricultural Service. 2012. EU-27 Cotton and Products Annual 2012. GAIN Report N: GR1201.

WPTC (World Processing Tomato Council). 2012. http://www.wptc.to/ (last accessed April 2, 2012).

WSSA (Weed Science Society of America). 2007. Herbicide Handbook. *In* S.A. Senseman 9th ed. WSSA, Lawrence, KS USA. 458 p.

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Αναλογία όφελος/κόστος και ανταποδοτικότητα εργασίας στην αντιμετώπιση των ζιζανίων σε καλλιέργειες της Νότιας Ευρώπης με τη χρήση του ζιζανιοκτόνου pendimethalin

E. Marinan-Arroyuelo, A. Marchi, F. Marchal-Rubio, K. Μπόζογλου, Δ. Σέρβης και K.N. Γιαννοπολίτης

Περίληψη Διεξήχθησαν ειδικά σχεδιασμένα πειράματα αγρού σε μεταφυτευόμενη βιομηχανική τομάτα, σε απευθείας σποράς κρεμμύδι (για παραγωγή ξηρών βολβών), σε βαμβάκι και σε μπρόκολο (για την αγορά νωπών λαχανικών), τέσσερις από τις βασικές καλλιέργειες που θεωρείται ότι καθορίζουν τη σπουδαιότητα της χρήσης του ζιζανιοκτόνου pendimethalin για αντιμετώπιση των ζιζανίων σε χώρες της Νότιας Ευρώπης. Με βάση τα δεδομένα της απόδοσης των καλλιεργειών στα πειράματα και τις πραγματικές τιμές παραγωγού, τόσο για τα έξοδα όσο και για τα έσοδα από την πώληση των προϊόντων, προσδιορίσθηκε η αναμενόμενη αναλογία όφελος/κόστος για την αντιμετώπιση των ζιζανίων όταν χρησιμοποιήθηκε pendimethalin, μερικά άλλα εναλλακτικά ζιζανιοκτόνα, βοτάνισμα ή συνδυασμός αυτών. Στις περισσότερες περιπτώσεις το pendimethalin, επιτυγχάνοντας ικανοποιητική αποτελεσματικότητα με μια απλή εφαρμογή, εξασφάλισε την πιο ευνοϊκή για τον παραγωγό αναλογία όφελος/κόστος, η οποία ήταν κατά πολύ καλύτερη από την αναλογία που πρόκυψε με το βοτάνισμα. Υπολογισμοί με βάση τις τρέχουσες τιμές παραγωγού έδειξαν επίσης ότι άλλες καλλιεργητικές μέθοδοι αντιμετώπισης των ζιζανίων, όπως σκαλίσματα μεταξύ των γραμμών ή εδαφοκάλυψη κατά μήκος των γραμμών με φύλλα μαύρου πλαστικού, οι οποίες χρησιμοποιούνται από τους παραγωγούς (δεν χρησιμοποιήθηκαν στα πειράματα), δεν είναι δυνατόν να οδηγήσουν σε μια εξίσου ευνοϊκή αναλογία όφελος/κόστος. Περαιτέρω, ανάλυση της ανταποδοτικότητας της εργασίας του βοτανίσματος έδειξε ότι ενώ το βοτάνισμα δεν συνέφερε οικονομικά σε καμιά περίπτωση όταν χρησιμοποιήθηκε μόνο του, μπορούσε να είναι οικονομικά συμφέρον ως συμπληρωματικό μέτρο του pendimethalin σε αρκετές περιπτώσεις. Τα αποτελέσματα αυτά δείχνουν ότι το pendimathalin (ή άλλο ζιζανιοκτόνο με παρόμοια δράση) έχει τη δυνατότητα να εξασφαλίσει μία ευνοϊκή αναλογία όφελος/κόστος σε καλλιέργειες που δέχονται αυξανόμενη πίεση για μείωση του κόστους παραγωγής.

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