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Herbicidal effects of *Satureja hortensis* L. and *Melissa officinalis* L. essential oils on germination and root length of *Lollium rigidum* L. and *Phalaris brachystachys* L. grass weeds

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Summary The herbicidal effect of *Satureja hortensis* L. and *Melissa officinalis* L. essential oils was tested on germination and root length of two grass weed species, *Lollium rigidum* L. and *Phalaris brachystachys* L., under laboratory conditions. Carvacrol and citral were the main constituents of the essential oil of *S. hortensis* and *M. officinalis*, respectively. *Melissa officinalis* essential oil showed higher inhibitory effect on germination and root length of *L. rigidum* and *Ph. brachystachys*, respectively. The phytotoxic effects were more pronounced on the latter weed species.

Additional keywords: allelopathy, carvacrol, geranial, neral

Introduction

The continuous use of the synthetic herbicides for weed control apart from their high efficacy, selectivity and relatively inexpensive cost to manufacture has raised concerns about their potential health and environmental impact (Dayan *et al.,* 2009) and the development of herbicide resistance among weed species (Batish *et al.,* 2004). Natural compounds with practical use as biocontrol agents are increasingly adopted in agriculture (Dayan *et al.,* 1999; Duke *et al.,* 2002; Singh *et al.,* 2003). Essential oils obtained from aromatic plants have been reported to exhibit herbicidal activity against seed germination (Muller *et al.,* 1964; Vaughn & Spencer, 1993; Dudai *et al.,* 1999). For example, it has been demonstrated that the essential oils of various aromatic plants, such as *Carum carvi* L. (Apiaceae), *Mentha spicata* L. (Lamiaceae), *Origanum vulgare* L. (Lamiaceae), *Thymbra spicata* L. (Lamiaceae), *Ocimum basilicum* L. (Lamiaceae), *Lavandula spp.* (Lamiaceae) and other members of *Lamiaceae* inhibited seed germination and/or root elongation of various weed species and crops (Vaughn & Spencer, 1993; Dudai *et al.,* 1999; Angelini *et al.,* 2003; Vasilakoglou *et al.,* 2007; Argyropoulos *et al.,* 2008; Azirak & Karaman, 2008).

Lemon balm (*Melissa officinalis* L.) is a perennial plant that belongs to *Lamiaceae* family, native to southern Europe and the Mediterranean region (Simon *et al.,* 1984). Lemon balm has been used in medicine, food, perfume and cosmetic industry (Simon *et al.,* 1984; Tagashira & Ohtake, 1998; Duke *et al.,* 2002; De Almeida *et al.,* 2010). De Almeida *et al.* (2010) found that the essential oil of *M. officinalis* was one of the four most active oils against both germination and radicle elongation of *Raphanus sativus* L. (Brassicaceae), *Lactuca sativa* L. (Asteraceae) and *Lepidium sativum* L. (Brassicaceae).

The genus *Satureja* L. (Lamiaceae) comprises numerous species growing wild in the Mediterranean area (Beziĉ *et al.,* 2005). In Europe, summer savory (*S. hortensis* L.) and winter savory (*S. montana* L.) are the most important species for cultivation (Askun *et al.,* 2012). Previous studies have revealed antioxidant, antibacterial, antifungidal activ-

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ity of summer savory (Gulluce *et al.,* 2003). Concerning the herbicidal activity of savory, Tworkoski (2002) reported that the essential oil of *S. hortensis* was phytotoxic and caused electrolyte leakage resulting in cell death when applied to detached leaves of dandelion *Taraxacum officinale* F. H. Wigg (Asteraceae) in the laboratory. Moreover, Angelini *et al*. (2003) showed that the essential oil of *S. montana* completely inhibited germination both of three crops [*Raphanus sativus*, *Capsicum annuum* L. (Solanaceae), *Lactuca sativa*] and three different annual weeds [*Chenopodium album* L. (Amaranthaceae), *Portulaca oleracea* L. (Portulacaceae), *Echinochloa crus-galli* L. Beauv. (Poaceae)]. Up to now there is limited information about the effect of the essential oil of *S. hortenis* and *M. officinalis* on grass weed species. The objective of this study was to evaluate in laboratory conditions the allelopathic activity of *S. hortensis* and *M. officinalis* essential oils on germination and root elongation of annual ryegrass [*Lollium rigidum* L. (Poaceae)] and short spiked canarygrass [*Phalaris brachystachys* L. (Poaceae)].

Materials and methods

Essential oils isolation

The plant material, aerial parts of *M. officinalis* and *S. hortensis,* was collected at bloom stage from experimental plots of Medicinal and Aromatic Plants Department of the National Agricultural Research Foundation (NAGREF) in Thessaloniki, Greece (40° 32΄ 16.32΄΄ B, 23° 00΄ 00.65΄΄ E).

Dried upper leaves, 100g and 30 g of *M. officinalis* and *S. hortensis,* respectively, were hydrodistilled for two hours with a distillation rate of 3 to 3.5 mL/min by using a Clevenger–type apparatus (Chatzopoulou *et al.,* 2006).

Essential oil analyses

The essential oil samples were analyzed by Gas Chromatograph Hewlett Packard 5890 Series II connected to a chromatographic integrator (Hewlett Packard 3396 Series II Dual Channel). Two fused silica columns of different polarity were used: Durabond-DB 1 and DB-Wax. Temperature program: 45 to 220°C at 3.5°C/min, carrier gas nitrogen: 140Kpa, injection temperature: 220°C, detector temperature 300°C. Sample injection: 0.2–0.3μl of a 10% essential oil solution in pentane; split 1:20. The percentage compositions were computed after 3 GC runs of each sample from the peak areas without correction factors. The GC/MS analysis was performed on a fused silica column DB-5, using a Gas Chromatograph 17A Ver. 3 interfaced with a Mass Spectrometer Shimadzu QP-5050A supported by the Class 5000 software. Injection temperature: 260°C, interface heating: 300°C, ion source heating: 200°C, EI mode: 70eV, scan range: 41-450 amu, and scan time 0.50s. Oven temperature programs: a) 55-120°C (3°C/min), 120-200°C (4°C/min), 200-220°C (6°C/min) and 220°C for 5min and b) 60-240°C at 3°C/min, carrier gas He, 54.8kPa, split ratio 1:30.

Identification of the essential oils components

The identification of the constituents was based on comparison of their Kovats indices (RI) relative to n-alkanes with corresponding literature data, as far as by matching a) their spectra with those from MS libraries (NIST 98) (Adams, 1995) and b) the RT of co-eluting reference compounds – peak enrichment technique (authentic samples by Roth and Sigma Aldrich).

Petri dish bioassay

Petri dish bioassay was performed to compare the germination and the radical length of annual ryegrass and shortspiked canarygrass treated with different concentrations of essential oils obtained from *S. hortensis* and *M. officinalis*. Twenty seeds of each species were placed separately at the bottom of 10cm glass Petri dishes and were covered with 5g of perlite. Deionized water (12ml) was added in each Petri dish. Each essential oil was loaded on a piece of filter paper, which was attached to the inner side of a small aluminum cup placed in the centre of each Petri dish, at 0, 8, 16, 32

and 64μl. Taking into account that the volume of the Petri dishes was approximately 100 cm^3, the above quantities were equal to concentrations (v/v) of 0, 0.08, 0.16, 0.32 and 0.64μl of essential oil per ml of Petri dish, respectively. Each Petri dish was sealed by film so as to prevent escape of the volatile compounds and all the Petri dishes were placed in a growth chamber in the dark at 22°C. The Petri dishes were arranged in a completely randomized design. After seven days of incubation, the germinated seeds were counted and their radical length was recorded. Each treatment was replicated three times and the bioassay trial was repeated twice and data were averaged. Bioassay data were analyzed over repetition time by using a factorial approach (essential oil x essential oil concentration). Data was expressed in % of control and was arcsine square root transformed before the ANOVA to reduce the heterogeneity, but means presented are back-transformed. LSD (5%) values were employed for the mean comparison. The ANOVA was performed separately for each weed species and oil.

Results and Discussion

Essential oil composition

The main essential oils' constituents and their percentage yield are presented in Table 1. In *S. hortnesis* oil, carvacrol (46.94%) and *γ*-terpinene (29.14%) were the main components, followed by *a*-terpinene (5.16%) and *p*-cymene (4.62%). In *M. officinalis* oil the dominant components were geranial (47.16%) and neral (36.10%).

Inhibitory effects

Both germination and root length of each species were significantly affected by essential oil concentration ($P<0.001$), however not by the interaction of essential oil x oil concentration. Statistically significant difference in the effect of the two essential oils was recorded. In particular, increased inhibition of *L. rigidum* germination and *Ph. brachystachys* root length were recorded when treated with *M. officinalis* essential oil (Table 2). Since germination and root length was significantly affected by oil concentration, ANOVA was performed separately for each weed species and oil. More specifically, the germination of *L. rigidum* ranged from 70 to 99% and 57 to 91% of control after the application of *S. hortensis* and *M. officinalis* essential oils, respectively, while, the root length of this weed species ranged from 28 to 54% and 25 to 66% of control, respectively (Table 3). Inhibition of *Ph. brachystachys* germination was more pronounced and ranged from 28 to 75% and from 21 to 85% of the control when treated with *S. hortensis* and *M. officinalis* oil, respectively (Table 4). The root length of this weed species ranged from 24 to 71% and from 8 to 74% of control, respectively (Table 4).

The main components of the essential oil of *M. officinalis* are citral (neral and geranial), citronellal, linalool, geraniol and *β*-caryophyllene-oxide (Adzet *et al.,* 1992). Citral is a mixture of two monoterpenes: geranial (citral A) and neral (citral B). It is a volatile essential oil component of lemongrass [*Cymbopogon citrate* (Poaceae)] and other aromatic plants and shows allelopathic traits (Dudai *et al.,* 1999). In a previous study, citral exhibited low phytotoxicity to soybeans and high phytotoxicity to velvetleaf [*Abutilon theophrasti*, Medik. (Malvaceae)], large crabgrass [*Digitaria sanguinalis*, L. Scop. (Poaceae)], redroot pigweed [*Amaranthus retroflexus*, L. (Amaranthaceae)] and italian ryegrass [*Lollium multiflorum*, Lam. (Poaceae)] (Vaughn & Spencer, 1993). Citral was found to be a strong inhibitor of wheat, *Amaranthus palmeri* S. Wats. (Amaranthaceae) and *Brassica nigra* L. (Brassicaceae) seed germination (Dudai *et al.,* 1999). Dudai (2007) reported that citral is absorbed by wheat seed through the abscission layer, and that it reaches highest concentration in the embryo, where it accumulates in the aleurone, scutellum and parts of the endosperm. As reported by Chaimovitsh *et al.* (2012) citral, as an allelochemical or a potential herbicide interferes with cell division cytokinesis and cell elongation. More specifically, they reported that at lower concentrations citral in-

Table 1. Chemical composition of *Satureja hortensis* and *Melissa officinalis* essential oils.

S. hortensis essential oil constituents	% yield	*M. officinalis* essential oil constituents	% yield
a-thujene	2.10	2-hexenal	0.27
α-pinene	1.90	1-octen-3-ol	0.59
β-pinene	1.05	5-hepten-2one-6-methyl	1.10
β-myrcene	3.20	*β*-myrcene	0.17
a-phellandrene	0.58	trans *b*-ocimene	0.13
a-terpinene	5.16	linalool	0.59
p-cymene	4.62	citronellal	0.32
sylvestrene	0.90	neral	36.10
γ-terpinene	29.14	geranial	47.16
carvacrol	46.94	methylgeranate	0.31
β-caryophyllene	1.29	geranyl acetate	2.98
b-bisabolene	0.70	*β*-caryophyllene	2.62
		caryophyllene oxide	1.57

Table 2. Effect of essential oil (% of control) of *Satureja hortensis* and *Melissa officinalis* on germination and root length of *Lollium rigidum* and *Phalaris brachystachis.*

Essential oil	*L. rigidum*		*Ph. brachystachys*	
	germination	root length	germination	root length
	% of control			
S. hortensis	82[1] a[2]	38 a	54 a	47a
M. officinalis	72 b	41 a	47 a	35 b
	**P<0.05	NS[3]	NS	***P<0.001

[1] Data were arcsine square root transformed before statistical analyses, De-transformed data are presented.
[2] Means within each column followed by the same letter are not significantly different at P = 5% level of significance
[3] NS= not significant

terferes with cell division by disrupting mitotic microtubules and cell plates while at higher concentrations it inhibits cell elongation by disrupting cortical microtubules. Carvacrol has also been reported to exhibit potential for use in weed control (Azirak & Karaman, 2008).

In the present study, the essential oil of *M. officinalis*, with citral as main constituent, showed greater phytotoxicity on *L. rigidum* germination and root length of *Ph. brachystachys* compared to *S. hortensis* essential oil, with carvacrol being its main component. The greater effect of *M. officinalis* essential oil could be attributed to higher citral concentration (83%), as compared with carvacrol concentration (47%) in *S. hortensis* essential oil. Our findings come in agreement with the results of Dudai *et al.* (2000) which revealed that inhibition of wheat germination was more pronounced by citral compared to carvacrol. Apart from the higher activity of *M. officinalis*, different response of the two grass weed species was evident, with the *Ph. brachystachys* germination more affected by both essential oils. However, previous studies reported that whatever activity has been found against a certain target species will not necessarily be maintained against another target species, even at the same family or genus (Vokou *et al.,* 2003). Summarizing, the results of this study indicated that both essential oils exhibited herbicidal activity on both grass weed species, with the essential oil of *M. officinalis* to be more active and *Ph. brachystachys* to be more suscepti-

Table 3. Effect of *Satureja hortensis* and *Melissa officinalis* essential oil concentration on *Lolium rigidum* germination and root length (% of control).

Concentration (µl/ml)	Essential oil			
	S. hortensis	*M. officinalis*	*S. hortensis*	*M. officinalis*
	germination		root length	
	% of control			
0.08	99 b	91 c	54 b	66 c
0.16	83 ab	78 b	38 a	43 b
0.32	74 a	64 ab	33 a	30 ab
0.64	70 a	57 a	28 a	25 a

Same letters within each column indicate no significant difference between means at P= 5%.

Table 4. Effect of *Satureja hortensis* and *Melissa officinalis* essential oil concentration on *Phalaris brachystachis* germination and root length (% of control)

Concentration (µl/ml)	Essential oil			
	S. hortensis	*M. officinalis*	*S. hortensis*	*M. officinalis*
	germination		root length	
	% of control			
0.08	75 c	85 c	71 c	74 d
0.16	64 bc	52 b	53 bc	37 c
0.32	50 b	30 a	37 ab	19 b
0.64	28 a	21 a	24 a	8 a

Same letters within each column indicate no significant difference between means at P= 5%.

ble. Further studies could provide more information concerning the concentration of each oil applied in order to achieve high efficacy against weeds.

Literature cited

Adams, R.P. 1995. Identification of Essential Oil Components by Gas Chromatography / Mass Spectroscopy, Allured Publishing Co, Carol Stream, Illinois.

Adzet, T., Ponz, R., Wolf, E. and Schultze, E. 1992. Content and composition of Melissa officinalis oil in relation to leaf position and harvest time. Planta medica, 58: 562-564.

Angelini L.G., Carpanese, G., Cioni, P.L., Morelli, I., Macchia, M., and Flamini, G. 2003. Essential oils from Mediterranean Lamiaceae as weed germination inhibitors. Journal of Agriculture and Food Chemistry, 51: 6158-6164.

Argyropoulos, E.I., Eleftherochorinos, I.G. and Vokou, D. 2008. In vitro evaluation of essential oils from Mediterranean aromatic plants of the Lamiaceae for weed control in tomato and cotton crops. Allelopathy Journal, 22: 69-78.

Askun, T., Tumen, G., Satil, F. and Karaarslan, D. 2012. Active constituents of some Satureja L. species and their biological activities. African Journal of Microbiology Research, 6: 4623-4633.

Azirak, S. and Karaman, S. 2008. Allelopathic effect of some essential oils and components on germination of weed species. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science, 58: 88-92.

Batish, R.D., Setia, N., Singh, H.P. and Kohli, K.R. 2004. Phytotoxicity of lemon-scented eucalypt oil and its potential use as a bioherbicide. Crop Protection, 23: 1209-1214.

Beziĉ, N., Skocibusic, M., Dunkic, V. 2005. Phytochemical composition and antimicrobial activity of Satureja montana L. and Satureja cuneifolia ten. essential oils. Acta Botanica Croatica, 64: 313–322.

Chaimovitsh, D., Rogovoy, (Stelmakh), O., Altshuler, O., Belausov, E., Abu-Abied, M., Rubin, B., Sadot, E., and Dudai, N. 2012. The relative effect of citral on mitotic microtubules in wheat roots and BY2 cells. Plant Biology, 14: 354–364.

Dayan, F.E., Cantrell, C.L. and Duke, S.O. 2009. Natural products in crop protection. Bioorganic and Medicinal Chemistry, 17: 4022-4034.

Dayan, F.E, Romagni, J., Tellez, M., Rimando, A. and Duke, S. 1999. Managing weeds with natural

products. Pesticide Outlook, 10: 185–188.

De Almeida, L.F.R., Frei, F., Mancini, E., De Martino, L. and De Feo, V. 2010. Phytotoxic Activities of Mediterranean Essential Oils. Molecules, 15: 4309-4323.

Dudai, N. 2007. Potential Use of Essential Oils as Bio-Herbicides: Ecology, Physiology and Agrotechnology Aspects. In: Abstracts of Novel and Sustainable Weed Management in Arid and Semi-Arid -Ecosystems, Rehovot, Israel. p. 34.

Dudai, N., Larkov, O., Putievsky, E., Lerner, H. R., Ravid, U., Lewinsohn, E. and Mayer, A.M. 2000. Biotransformation of constituents of essential oils by germinating wheat seed. Phytochemistry, 55: 375-382.

Dudai, N., Poljakoff-Mayber, A., Mayer, A. M., Putievsky, E., and Lerner, H. R. 1999. Essential oils as allelochemicals and their potential use as bioherbicides. Journal of Chemical Ecology, 25: 1079–1089.

Duke, S.O., Dayan, F.E., Rimando, A.M., Schrader, K.K., Aliotta, G., Oliva, A. and Romagni, J.G. 2002. Chemicals from nature for weed management. Weed Science, 50: 138–151.

Gulluce, M., Sokmen, M., Daferera, D., Agar, G., Ozkan, H., Kartal, N., Polissiou, M., Sokmen, A. and Sahin, F. 2003. In vitro antibacterial, antifungal and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of Satureja hortensis L. Journal of Agricuture and Food Chemistry, 51: 3958-3965.

Muller, C. H., Muller, W. H. and Haines. B. L. 1964. Volatile growth inhibitors produced by aromatic shrubs. Science, 143: 471-473.

Chatzopoulou, P. S., Koutsos, T. V., Katsiotis S. T. 2006. Chemical Composition of the essential oils from cultivated and wild grown St John's Wort (Hypericum perforatum). Journal of Essential Oil Research, 18: 643-646

Simon, J.E., Chadwick, A.F. and Craker, L.E. 1984. Herbs: An Indexed Bibliography, 1971-1980. The Scientific Literature on Selected Herbs, and Aromatic and Medicinal Plants of the Temperate Zone. Archon Books. Hamden, CT, USA. pp. 770.

Singh, H.P., Batish, D.R. and Kohli, R.K. 2003. Allelopathic interactions and allelochemicals: new possibilities for sustainable weed management. Critical Reviews in Plant Sciences, 22: 239–311.

Tagashira, M. and Ohtake, Y. 1998. A new antioxidative 1,3-benzodioxole from Melissa officinalis. Planta Medica, 64: 555–558.

Tworkoski, T. 2002. Herbicide effects of essential oils. Weed Science, 50: 425–431.

Vasilakoglou, I., Dhima, K., Wogiatzi, E., Eleftherohorinos I., and Lithourgidis, A. 2007. Herbicidal potential of essential oils of oregano or marjoram (Origanum spp.) and basil (Ocimum basilicum) on Echinochloa crus-galli (L.) P. Beauv. and Chenopodium album L. weeds. Allelopathy Journal, 20: 297–306.

Vaughn, S.T. and Spencer, G.F. 1993. Volatile monoterpenes as potential parent structures for new herbicides. Weed Science, 41: 114-119.

Vokou, D., Douvli, P., Blionis, G.J. and Halley, J.M. 2003. Effects on monoterpenoids, acting alone or in pairs, on seed germination and subsequent seedling growth. Journal of Chemical Ecology, 29: 2281-2301.

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Ζιζανιοκτόνος δράση των αιθερίων ελαίων των *Satureja hortensis* L. και *Melissa officinalis* L. στη βλάστηση και το μήκος ριζιδίου των αγρωστωδών ζιζανίων *Lollium rigidum* L. και *Phalaris brachystachys* L.

Θ.Κ. Γιτσόπουλος, Π. Χατζοπούλου και Ι. Γεωργούλας

Περίληψη Σε συνθήκες εργαστηρίου μελετήθηκε η ζιζανιοκτόνος δράση των αιθερίων ελαίων των *Satureja hortensis* L. και *Melissa officinalis* L. στη βλάστηση και το μήκος ριζιδίου των αγρωστωδών ζιζανίων *Lollium rigidum* L. and *Phalaris brachystachys* L. Η καρβακρόλη και η κιτράλη ήταν τα κύρια συστατικά των αιθερίων ελαίων των *S. hortensis* και *M. officinalis*, αντίστοιχα. Το αιθέριο έλαιο του *M. officinalis* επηρέασε περισσότερο τη βλάστηση του ζιζανίου *L. rigidum* και το μήκος ριζιδίου του ζιζανίου *Ph. brachystachys*. Η ζιζανιοκτόνος δράση των αιθερίων ελαίων ήταν περισσότερο έντονη στο ζιζάνιο *Ph. brachystachys*.

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SHORT COMMUNICATION

First record of the scale insect *Asterodiaspis ilicicola* in Greece and additional data about the scale *Gonaspidiotus minimus* on *Quercus ilex*

G.J. Stathas, E.D. Kartsonas and P.J. Skouras

Summary The scale insect *Asterodiaspis ilicicola* (Targioni Tozzetti) (Hemiptera, Coccoidea, Asterolecaniidae) was recorded for the first time in Greece in February 2013. It was found on *Quercus ilex,* in the National Garden of Athens. On the same infested trees, the scale insect *Gonaspidiotus minimus* (Leonardi) (Hemiptera, Coccoidea, Diaspididae) was also recorded. Both scale species were settled on the upper surface of leaves of *Q. ilex*.

According to ScaleNet Database (Ben-Dov *et al.,* 2013) the scale insects species of the family Asterolecaniidae recorded in Greece include *Asterodiaspis bella* (Russell) (Russell, 1941; Borchsenius, 1960), *A. repugnans* (Russell) (Russell, 1941; Borchsenius, 1960), *A. variolosa* (Ratzeburg) (Milonas *et al.,* 2008) and *Pollinia pollini* (Costa) (Alexandrakis, 1980).

Asterodiaspis ilicicola (Targioni Tozzetti) (Hemiptera, Coccoidea, Asterolecaniidae) was recorded for the first time in Greece on *Quercus ilex* (Fagaceae) in the National Garden of Athens in February 2013. On the same trees, the scale insect *Gonaspidiotus minimus* (Leonardi) (Hemiptera, Coccoidea, Diaspididae) was also recorded. The colonies of both scale species were settled on the upper surface of leaves of *Q. ilex* (Fig. 1 and Fig. 2). The vouchers of the permanent slides of *A. ilicicola* are deposited in collections of the University of Padova and of the Laboratory of Agricultural Entomology & Zoology of the Highest Technological Educational Institute of Kalamata. The vouchers of the permanent slides of *G. minimus* are deposited in collec-

Figure 1. *Asterodiaspis ilicicola* on leaves of *Quercus ilex* (Photo by G. Stathas).

Figure 2. *Gonaspidiotus minimus* on leaves of *Quercus ilex* (Photo by G. Stathas).

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tions of the Plant Protection Institute of the Hungarian Academy of Sciences and of the Laboratory of Agricultural Entomology & Zoology of the Highest Technological Educational Institute of Kalamata.

Asterodiaspis ilicicola is a species of Palaearctic Region, recorded in Algeria, Croatia, France, Italy, Portugal, Spain and Turkey (Ben-Dov *et al.,* 2013; Pellizzari & Camporese, 1991; Pellizzari and Fontana, 1996). The host plant species of *A. ilicola* involve *Quercus coccifera, Q. ilex, Q. rotundifolia* and *Q. suber* (Ben-Dov *et al.,* 2013).

Gonaspidiotus minimus was recorded for the first time in Greece by Koroneos (1934) as the synonym *Aspidiotus minimus* (Leonardi) Cockerell, which was rarely found on *Q. ilex* in the regions Milies and Neokhorion of Magnesia (Central Greece) and more often on *Q. coccifera* in Magnesia and Attica. Later, Jansen *et al.* (2010) and Pellizzari *et al.* (2011) recorded *G. minimus* on *Q. coccifera* in Crete. This is a species of Palaearctic Region, referred as scale insect pest of *Chamerops humilis* (Arecaceae) and species of Fagaceae (*Quercus coccifera, Q. ilex, Q. ilicis, Q. incana, Q. ithaburensis* and *Q. suber*). It is recorded in Algeria, Croatia, France, Greece, Israel, Italy, Lebanon, Morocco, Spain and Turkey (Ben-Dov *et al.,* 2013).

Although the infestation level of *A. ilicicola* on *Q. ilex* cannot be considered significant, it is wise to encourage the study of the scale's distribution in other areas of the country. The reason is the possibility that this scale may infest *Q. coccifera*, which is a very important forest species for Greece. Similar studies were made in the past on other forest species e.g. *Physokermes inopinatus* Danzig & Kozár (Hemiptera: Coccidae) (Hungarian spruce scale) found on *Abies cephalonica* and *Planococcus vovae* (Nasonov) found on various Cupressus species in Greece (Milonas *et al.*, 2008; Stathas *et al.*, 2010). The scale *G. minimus* was found on *Q. ilex* in a higher population density than *A. ilicicola*. The fact that *G. minimus* was recorded on a different host plant than the one found by Koroneos, underlines the necessity to support regular surveys on scale insects in order to complete the missing parts of existing data of Coccoidea fauna in Greece.

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Literature cited

Alexandrakis, V. 1980. Donnıes bio-ecologiques sur *Pollinia pollini* (Hom. Coccoidea, Asterolecanidae) sur olivier en Crète. [Bio-ecological data on *Pollinia pollini* (Homoptera, Coccoidea, Asterolecanidae) on olive trees in Crete, Greece.] (In French, Summary In English). *Annales de la Société Entomologique de France* 16: 9-17.

Ben-Dov, Y., Miller, D.R. and Gibson, G.A.P., 2013. *ScaleNet: a database of the scale insects of the World.* Available in: http://www.sel.barc.usda.gov/scalenet/query.htm (accessed 17 04 2013).

Borchsenius, N.S. 1960. (In Russian). In: Fauna of USSR, Homoptera, Kermococcidae, Asterolecaniidae, Lecanidodiaspididae, Aclerdidae. Akademiia Nauk SSSR, Zoologicheskii institut (Series), Leningrad. 282 pp.

Jansen, M.G.M., Ben-Dov, Y. & Kaydan, M.B. 2010. New records of scale insects from Crete Island, Greece (Hem., Coccoidea). *Bulletin de la Société Entomologique de France* 115 (4): 483-484.

Koroneos, J. 1934. (In French). In: Les Coccidae de la Grèce surtout du Pılion (Thessalie). I. Diaspinae., Athens. 95 pp, 70 plates.

Milonas, P.G., Kozár, F. & Kontodimas, D.C. 2008. List of scale insects of Greece. 143-147 In: Branco, M., Franco, J.C. & Hodgson, C.J. (Editors), Proceedings of the XI International Symposium on Scale Insect Studies, Oeiras, Portugal, 24-27 September 2007. ISA Press, Lisbon, Portugal. 322 pp.

Milonas, P.G., Kozár, F. 2008. Check list of mealybugs (Homoptera: Pseudococcidae) in Greece: three new records. *Hellenic Plant Protection Journal* 1: 35-38.

Pellizzari Scaltriti, G. & Camporese, P. 1991. [Contribution to the knowledge of scale insects (Homoptera, Coccoidea) living on oaks in Italy.] Contributo alla conoscenza delle cocciniglie (Homoptera, Coccoidea) delle querce in Italia. (In Italian; Summary In English). 193-209.

Pellizzari, G. & Fontana, P. 1996. [Contribution to the knowledge of Homoptera Coccoidea of Sardinia with description of a new species.] (Summary In Italian). *Bollettino di Zoologia Agraria e di Bachicoltura* (Milano) 28: 119-140.

Pellizzari, G., F. Porcelli, G. Seljak, and F. Kozár (2011): Some additions to the Scale insect fauna (Hemiptera: Coccoidea) of Crete with a check list of the species known from the island *J. Ent. Acar. Res*. Ser. II, 43: 291-300.

Russell, L.M. 1941. A classification of the scale insect genus *Asterolecanium*. United States Department of Agriculture, Miscellaneous Publications 424: 1-319.

Stathas G.J., Kozar F., 2010. First record of *Physokermes inopinatus* Danzig & Kozar 1973 (Hemiptera: Coccidae) in Greece. *Hellenic Plant Protection Journal*, 3 (1): 7-8.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή του κοκκοειδούς *Asterodiaspis ilicicola* στην Ελλάδα και στοιχεία για την παρουσία του κοκκοειδούς *Gonaspidiotus minimus* στο *Quercus ilex*

Γ.Ι. Σταθάς, Ε.Δ. Κάρτσωνας και Π.Ι. Σκούρας

Περίληψη Καταγράφηκε για πρώτη φορά στην Ελλάδα το κοκκοειδές έντομο *Asterodiaspis ilicicola* (Targioni Tozzetti) (Hemiptera, Coccoidea, Asterolecaniidae) το Φεβρουάριο του 2013. Το έντομο βρέθηκε επί του *Quercus ilex,* στον Εθνικό Κήπο της Αθήνας. Στα ίδια προσβεβλημένα δένδρα βρέθηκε και το κοκκοειδές έντομο *Gonaspidiotus minimus* (Leonardi) (Hemiptera, Coccoidea, Diaspididae). Και τα δύο αυτά είδη βρέθηκαν εγκατεστημένα στην άνω φυλλική επιφάνεια του *Q. ilex*.

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Density-dependent predation of *Propylea quatuordecimpunctata* L. (Coleoptera: Coccinellidae) larval instars on *Aphis fabae* Scopoli (Hemiptera: Aphididae)

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Summary The predation of the fourteen-spotted ladybird beetle, *Propylea quatuordecimpunctata* L. (Coleoptera: Coccinellidae), larval instars on several densities of the black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), was investigated under laboratory conditions. The experimental arena consisted of a single plant of *Vicia faba* L. bearing different prey densities (different instars of *A. fabae*), where a single, starved from food for 12 h, *P. quatuordecimpunctata* larva was allowed to prey for 12 h. Logistic regression analysis on the proportion of aphids consumed as a function of the initial aphid density showed that predator larvae cause an inverse density dependent mortality on *A. fabae* (type II functional response). Therefore, data fitted to the random predator equation. Estimated attack rates were 0.21, 0.23, 0.18 and 0.26 h^{-1}, and handling times 5.15, 2.40, 0.83 and 0.35 h for the first, second, third and fourth instars, respectively. The results of this study may be indicative of the predation ability of *P. quatuordecimpunctata* under field conditions.

Additional keywords: attack rate, fourteen-spotted ladybird beetle, functional response, maximum attack rate, random predator equation

Introduction

Aphids are recognized as serious pests of cultivated and wild growing plants worldwide, having a direct and indirect effect on plant viability. Biological control and integrated management of aphid pests often includes the use of aphidophagous coccinellid species, due their ability to suppress aphid populations (Dixon, 2000; Obrycki *et al*., 2009). The fourteen-spotted ladybird beetle, *Propylea quatuordecimpunctata* L. (Coleoptera: Coccinellidae), is a Palearctic coccinellid, common in Greece (Kavallieratos *et al*., 2002, 2004a,b; Katsarou *et al*., 2008). Based on its intrinsic characteristics such as the rate of increase and population doubling time, is considered promising in biological control practice (Kontodimas *et al*., 2008; Pervez and Omkar, 2011).

Solomon (1949) used the term 'functional response' in order to describe the number of prey consumed per predator as a function of prey density and Holling (1959) classified functional responses into three types: density independent predation occurs via a linear response of prey consumption to its density (type I functional response), where density dependence occurs when prey consumption increases with a deceleration rate (type II functional response) or exhibits a sigmoid response to prey density (type III functional response). All types of predation reach a plateau at high prey densities. Functional responses are frequently used in order to predict the effectiveness of predators in biological control (Cabral *et al*., 2009).

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Type II functional response seems to be common among aphidophagous coccinellidae species. Thus, a type II functional response is recorded for *Cheilomenes sulphurea* Olivier male adults on *Aphis fabae* Scopoli (Hodek *et al*. 1984), *Coelophora inaequalis* F. fourth instar larvae and female adults on *Toxoptera citricida* Kirkaldy (Wang and Tsai, 2001), *Propylea dissecta* Mulsant fourth instar larvae and adults on *Aphis gossypii* Glover (Omkar and Pervez, 2004), *Harmonia axyridis* Pallas larval instars and female adults on *Myzus persicae* Sulzer (Lee and Kang, 2004), *Coccinella septempunctata* L. fourth instars and adults on *M. persicae* (Cabral *et al*., 2009), *H. axyridis* and *C. septempunctata* third instar larvae and adults on *Aphis glycines* Matsumura (Xue *et al*., 2009), *Hippodamia variegata* Goeze larval instars and adults on *A. fabae* (Forhadi *et al*., 2010) and *Adalia bipunctata* L. female adults on *M. persicae* (Jalali *et al*., 2010). However, few exceptions have been reported, as Messina and Hanks (1998) showed that plant architecture may alter the type of functional response of *P. quatuordecimpunctata* L. fourth instars and adults, where Lou (1987) reported type I and Hu *et al*. (1989) type III functional responses for *Harmonia axyridis* Pallas adults.

Coccinellids' handling time, i.e. the time a predator spends on pursuing, subduing, eating and digesting its prey, is not constant through functional response experiments (Hassell *et al*., 1976). Hodek (1996) stated that hungry aphidophagous coccinellids completely devour the first prey items they encounter and then become less competent at exploiting prey. According to Papanikolaou (unpublished data) this trait results from the digestion process. Nevertheless, the estimation of handling time based on mean values is substantial for developing general models for the description of density-dependent predation in a realistic manner (Hassell *et al*., 1976).

In a previous study, Papanikolaou *et al*. (2011) showed that larval instars of *P. quatuordecimpunctata* exhibit a type II functional response on *A. fabae*, when allowed to prey for 24 h. The present study investigated the functional response of the predator on *A. fabae* at 12 h taking into account that handling time is not constant through functional response experiments. Furthermore, the appropriate model was fitted to the data in order to predict the predation ability of *P. quatuordecimpunctata* on several densities of *A. fabae*.

Materials and Methods

An *A. fabae* colony (originally from a stock colony from the Biological Control Laboratory, at the Benaki Phytopathological Institute) was reared on potted fava bean, *Vicia faba* L., plants at 20 ± 1ºC (65 ± 2% RH, 16:8 (L:D) h). *Propylea quatuordecimpunctata* was collected in summer 2006 from corn, *Zea mays* L., plants infested by the corn leaf aphid, *Rhopalosiphum maidis* Fitch, in Arta County (Northwestern Greece, 21°0′0′′/39°10′0′′) and was reared on *A. fabae* in cylindrical Plexiglass cages (50 x 30 cm) at 25 ± 1ºC, 65 ± 5% RH and a photoperiod of 16:8 (L:D) h.

Functional response experiments were carried out at 20 ± 1°C, 65 ± 2% R.H., with a photoperiod of 16:8 (L:D) h. The experimental arena consisted of a plastic container (12 cm height x 7 cm diameter) with a potted *V. faba* plant (top growth was cut in order to provide plants of 8-9 cm height), bearing different densities of *A. fabae* (3-3.5 day-old immature aphids to avoid reproduction during the experiments). A single predator larva of a certain instar, which was previously starved from food for 12 h, was placed into the experimental arena for 12 h. The prey densities tested were 2, 4, 8, 16 and 32 aphid nymphs for the 1st instar larvae of the predator, 2, 4, 8, 16, 32 and 64 nymphs for the 2nd instar larvae, and 4, 8, 16, 32, 64 and 128 aphids for the 3rd and 4th instar larvae.

In order to test for density-dependent prey mortality, a logistic regression analysis was conducted on the proportion of prey eaten as a function of the initial prey density, fitting the polynomial function (Juliano, 2001):

$$\frac{N_e}{N_0} = \frac{exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}{1 + exp(P_0 + P_1N_0 + P_2N_0^2 + P_3N_0^3)}$$

where N_e is the number of prey consumed, N_0 the initial prey number available and P_0, P_1, P_2 and P_3 are the intercept, linear, quadratic and cubic coefficients estimated using the method of maximum likelihood. Regression outcome suggested a type II functional response. The visual inspection of the observed *vs* expected probabilities showed good agreement, as the proportion of prey eaten decreased as prey density increased.

Data fitted to the random predator equation proposed by Rogers (1972). In order to derive more stable estimates of calculated parameters, the Lambert *W* function was used, which provides a numerical solution of the equation. In terms of Lambert *W*, the random predator equation can be written as (Bolker, 2008):

$$N_e = N_0 - \frac{W\left(abN_0 e^{(abN_0 - aT)}\right)}{ab}$$

where *a* is the attack rate, i.e. the per capita prey mortality at low prey densities; *b* the handling time, *T* the total time that prey was exposed to predator; *T*/*b* the maximum number of prey which can be attacked by the predator during the time interval (maximum attack rate). Fitting was performed using the method of maximum likelihood (R Development Core Team, 2010). The mortality of the prey was assumed negligible in the absence of predator. Significant differences between estimated parameters were evaluated using 95% confidence intervals.

Results

The estimated parameters of the logistic regression analysis on the proportion of prey eaten as a function of initial prey density are presented in Table 1. Fitting *vs* observed probabilities of the proportion of *A. fabae* prey eaten by each of the larval instars of *P. quatuordecimpunctata* suggest a continuous decrease in prey consumption with increasing prey density (Fig. 1). Thus, an inverse density-dependent prey mortality exhibited by *P. quatuordecimpunctata* larvae (type II functional response).

The random predator equation was used to describe the predation rates of *P. quatuordecimpunctata* larval instars. The model fitted the observed data reasonably well, as the fitted probabilities of the number of prey consumed lie within the main bulk of the data (Fig. 2). Although the estimated attack rates did not differ among larval instars, maximum attack rates increased at the fourth instar larvae. Thus, the maximum numbers of prey which can be attacked were 2.3, 5.0, 14.5 and 34.5 for the first, second, third and fourth instar larvae, respectively (Table 2).

Discussion

Our results show that predation ability for each larval instar of *P. quatuordecimpunctata* increases with a deceleration rate, as *A. fabae* density increases, indicating a type II functional response. Papanikolaou *et al.* (2011) showed that a type II functional response was also exhibited by *P. quatuordecimpunctata* larval instars to *A. fabae* when exposure time was 24 h. It is expected that type II functional responses affect negatively the population dynamics of prey and predator (Oaten and Murdoch, 1975). As the intrinsic rate of increase and population doubling time of *P. quatuordecimpunctata* support the potential of a successful biological control agent (Kontodimas *et al.*, 2008; Pervez and Omkar, 2011), these data should also be taken into account for final conclusions on the performance of the predator in biological control practice.

Our study supported that the handling times of *P. quatuordecimpunctata* of the older larval instars were shorter than those of the younger ones, hence the predation ability increased at the fourth instar. This is due to the higher energy requirements of the fourth instar larvae and their ability to pursue, capture and consume faster the prey items. Papanikolaou *et al.* (2011) reported the same trend in handling times of larval instars of *P. quatuordecimpunctata*, where the mean

Table 1. Estimated parameters of the logistic regression analysis of the proportion of *Aphis fabae* prey eaten by the larval instars of *Propylea quatuordecimpunctata*, as a function of initial prey density.

Parameter	1st instar	2nd instar	3rd instar	4th instar
P_0	-1.03 ± 0.14	0.48 ± 0.12	-0.24 ± 0.08	0.91 ± 0.11
P_1	-6.88 ± 0.89	-9.79 ± 0.77	-8.96 ± 0.53	-9.27 ± 0.66
P_2	2.79 ± 0.91	4.56 ± 0.77	3.26 ± 0.51	2.37 ± 0.62
P_3	-1.19 ± 0.94	-2.25 ± 0.74	-1.16 ± 0.48	0.86 ± 0.55

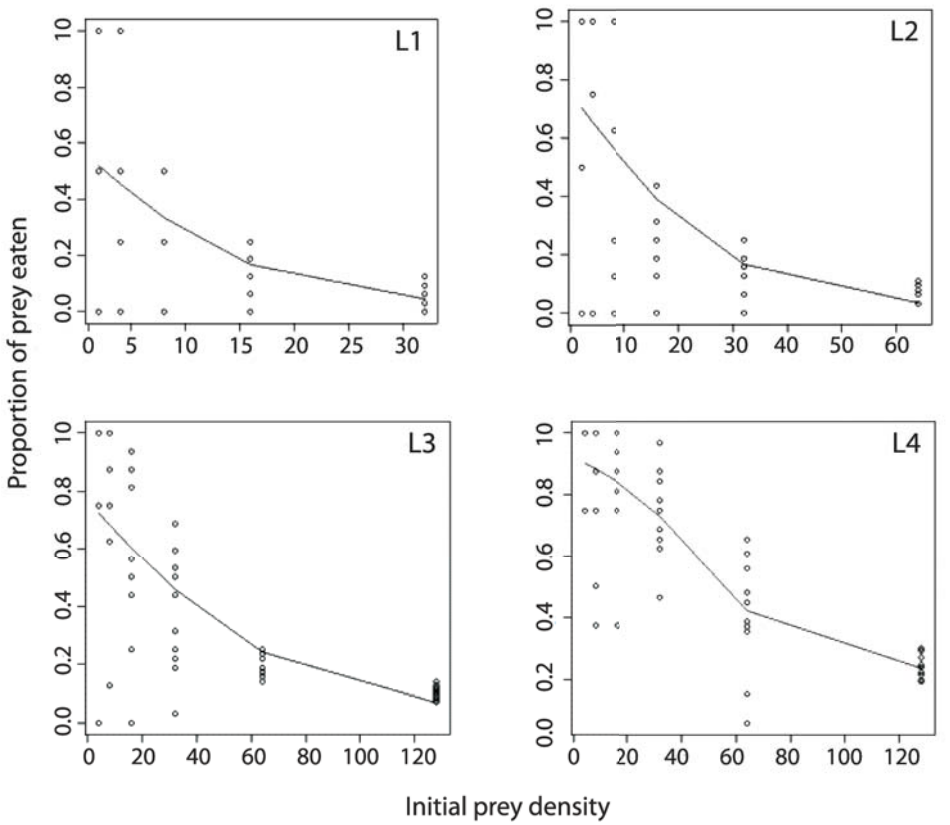

Figure 1. Observed *vs* fitted probabilities of *Aphis fabae* prey eaten by the larval instars of *Propylea quatuordecimpunctata*. L1: first instar larvae, L2: second instar larvae, L3: third instar larvae and L4: fourth instar larvae.

values from first to fourth larval instar were 6.18, 2.37, 1.06 and 0.44 h respectively.

Our study also revealed that the mean attack rates of *P. quatuordecimpunctata* were 0.21-0.26 h^{-1} and did not differ among the larval instars. This further indicates that the per capita prey mortality at low prey densities did not differ among larval instars, displaying similar ability to respond to increasing prey densities. The estimated mean values of the predator's attack rates at 24 h exposure time of prey were 0.06 h^{-1} and did not differ among the larval instars (Papanikolaou *et al*. 2011).

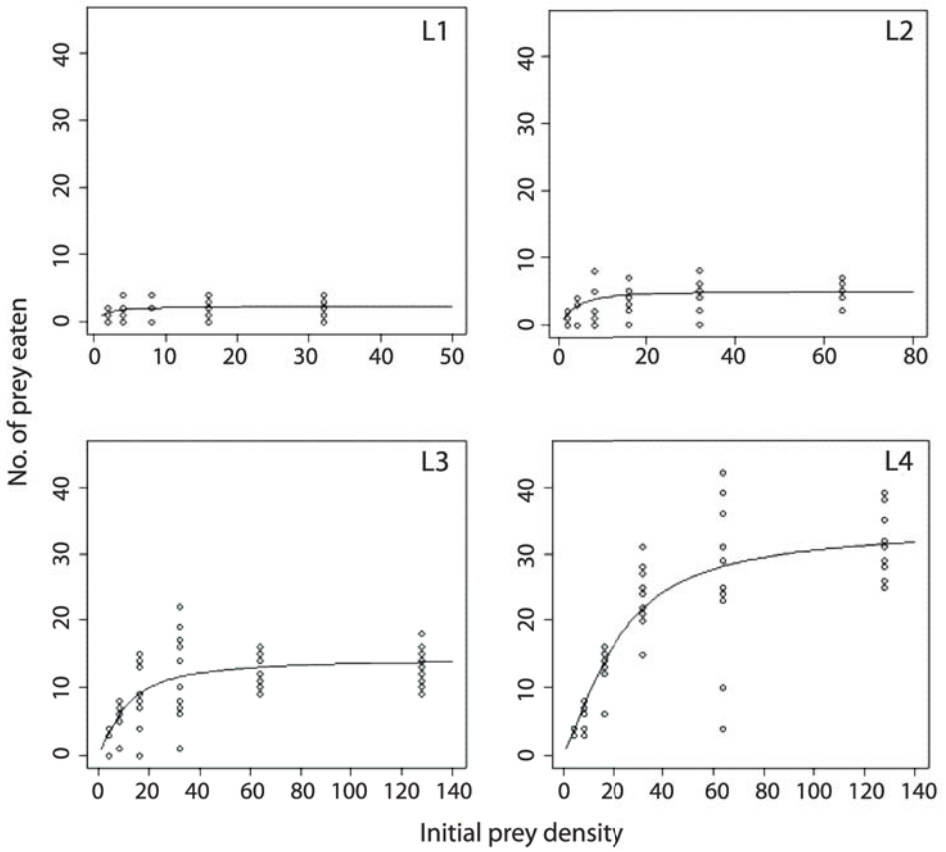

Figure 2. Functional responses of the larval instars of *P. quatuordecimpunctata* to *A. fabae* on potted *V. faba* plants. L1: first instar larvae, L2: second instar larvae, L3: third instar larvae and L4: fourth instar larvae.

Table 2. Handling times, attack rates (mean, 95% confidence intervals) and maximum attack rates of *Propylea quatuordecimpunctata* larval instars fed on *Aphis fabae*.

	attack rate (hours^{-1})		handling time (hours)		maximum attack rate	AIC
	mean	95% C.I.	mean	95% C.I.	mean	
1st instar	0.21	0.08-1.53	5.15	3.74-6.84	2.33	171.6
2nd instar	0.23	0.14-0.37	2.40	2.03-2.83	4.99	271.2
3rd instar	0.18	0.14-0.24	0.83	0.74-0.92	14.50	399.3
4th instar	0.26	0.22-0.31	0.35	0.32-0.38	34.54	390.0

The utility of laboratory estimated functional responses is questionable because complex environmental conditions are not illustrated in laboratory conditions (Murdoch, 1973). Additionally, foraging behavior is different among coccinellid's larvae and adults, due to adults' flight ability. Larvae may stay within a patch, where adults may not (Kindlmann and Dixon, 2001; Dostalkova *et al.*, 2002). However, our results on *P. quatuordecimpunctata* larvae functional responses may be indicative to their voracity

to *A. fabae* and may be used in developing deterministic predator-prey models which allow the prediction of population fluctuations through time (Arditi *et al.*, 2004).

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Literature cited

Arditi, R., Callois, J.M., Tyutyunov, Y. and Jost, C. 2004. Does mutual interference always stabilize predator-prey dynamics? A comparison of models. *C. R. Biologies,* 327: 1037-1057.

Bolker, B.M. 2008. *Ecological models and data in R*. Princeton University Press, Princeton, 408 p.

Cabral, S., Soares, A.O and Garcia, P. 2009. Predation by *Coccinella undecimpuctata* L. (Coleoptera: Coccinellidae) on *Myzus persicae* Sulzer (Homoptera: Aphididae): Effect of prey density. *Biological Control,* 50: 25-29.

Dixon, A.F.G. 2000. *Insect predator-prey dynamics. Ladybird beetles and biological control*. Cambridge, UK: Cambridge University Press, 257 p.

Dostalkova, I., Kindlmann, P. and Dixon, A.F.G. 2002. Are classical predator-prey models relevant to the real world? *Journal of Theoretical Biology,* 218: 323-330.

Forhadi, R. Allahyari, H. and Juliano, S.A. 2010. Functional response of larval and adult stages of *Hippodamia variegata* (Coleoptera: Coccinellidae) to different densities of *Aphis fabae* (Hemiptera: Aphididae). *Environmental Entomology,* 39: 1586-1592.

Hassell, M.P., Lawton, J.H. and Beddington, J.R. 1976. The components of arthropod predation. I. The prey death rate. *Joirnal of Animal Ecology,* 45: 135-164.

Hodek, I. 1996. *Food relationships*. In Hodek I. and Honěk A. (eds.). *Ecology of Coccinellidae*. Kluwer Academic Publishers, Netherlands, p. 143-238.

Hodek, I., Chakrabarti, S. and Rejmanek, M. 1984. The effect of prey density on food intake by adult *Cheilomenes sulphurea* (Col.: Coccinellidae). *Entomophaga,* 29: 179-184.

Holling, C.S. 1959. Some characteristics of simple types of predation and parasitism. *Canadian Entomologist,* 91: 385-398.

Hu, Y.S., Wang, Z.M., Ning, C.L., Pi, Z.Q. and Gao, G.Q. 1989. The functional response of *Harmonia (Leis) axyridis* to their prey of *Cinara sp. Natural Enemies of Insects,* 11: 164-168.

Jalali, M.A., Tirry, L. and De Clercq, P. 2010. Effect of temperature on the functional response of *Adalia bipunctata* to *Myzus persicae*. *BioControl,* 55: 261-269.

Juliano, S.A. 2001. *Nonlinear curve fitting: predation and functional response curve*. In Scheiner S.M. and Gurevitch J. (eds.). *Design and analysis of ecological experiments*. Oxford University Press, New York, p. 178-196.

Katsarou, I., Martinou, A., Papachristos, D.P. and Zoaki, D. 2009. Toxic effects of insecticide residues on three aphidophagous coccinellid species. *Hellenic Plant Protection Journal,* 2: 101-106.

Kavallieratos, N.G, Stathas, G.J., Athanassiou, C.G. and Papadoulis, G.Th. 2002. *Dittrichia viscose* and *Rubus ulmifolius* as reservoirs of aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) and the role of certain coccinellid species. *Phytoparasitica,* 30: 231-242.

Kavallieratos, N.G., Stathas, G.J. and Tomanovic, Z. 2004a. Seasonal abundance of parasitoids (Hymenoptera: Braconidae, Aphidiinae) and predators (Coleoptera: Coccinellidae) of aphids infesting citrus in Greece. *Biologia Bratislava,* 59: 191-196.

Kavallieratos, N.G., Athanassiou, C.G., Tomanovic, Z., Papadopoulos, G.D., and Vayias, B.J. 2004b. Seasonal abundance and effect of predators (Coleoptera, Coccinellidae) and parasitoids (Hymenoptera: Braconidae, Aphidiinae) on Myzus persicae (Hemiptera, Aphidoidea) densities on tobacco: a two-year study from Central Greece. *Biologia Bratislava,* 59: 613-619.

Kindlmann, P. and Dixon, A.F.G. 2001. When and why top-down fails in arthropod predator-prey systems. *Basic and Applied Ecology,* 2: 333-340.

Kontodimas D.C., Milonas, P.G., Stathas, G.J., Papanikolaou, N.E., Skourti, A. and Matsinos, Y.G. 2008. Life table parameters of the aphid predators *Coccinella septempunctata*, *Ceratomegilla undecimnotata* and *Propylea quatuordecimpunctata* (Coleoptera: Coccinellidae). *European Journal of Entomology,* 105: 427-430.

Lee J.H. and Kang, T.J. 2004. Functional responses of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) to *Aphis gossypii* Glover (Homoptera: Aphididae) in the laboratory. *Biological Control,* 31: 306-310.

Lou, H.H. 1987. Functional response of *Harmonia axyridis* to the density of *Rhopalosiphum prunifoliae*. *Natural Enemies of Insects,* 9: 84-87.

Messina, F.J. and Hanks, J.B. 1998. Host plant alters the shape of the functional response of an aphid predator (Coleoptera: Coccinellidae). *Environmental Entomology,* 27: 1196-1202.

Murdoch, W.W. 1973. The functional response of aphid predators. *Journal of Applied Ecology,* 10: 335-342.

Oaten, A. and Murdoch, W.W. 1975. Functional response and stability in predator-prey systems. *American Naturalist,* 109: 289-298.

Obrycki, J.J., Harwood, J.D., Kring, T.J. and O'Neil, R.J. 2009. Aphidophagy by Coccinellidae: Application of biological control in agroecosystems. *Biological Control,* 51: 244-254.

Omkar and Pervez, A. 2004. Functional and numerical responses of *Propylea dissecta* (Col., Coccinellidae). *Journal of Applied Entomology,* 128: 140-146.

Papanikolaou, N.E., Martinou, A.F., Kontodimas, D.C., Matsinos, Y.G. and Milonas, P.G. 2011. Functional responses of immature stages of *Propylea quatuordecimpunctata* (Coleoptera: Coccinellidae) to *Aphis fabae* (Hemiptera: Aphididae). *European Journal of Entomology,* 108: 391-395.

Pervez, A. and Omkar. 2011. Ecology of aphidophagous ladybird *Propylea* species: A review. *Journal of Asia-Pacific Entomology,* 14: 357-365.

R Development Core Team. 2008. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org

Rogers, D. 1972. Random search and insect population models. *Journal of Animal Ecology,* 41: 369-383.

Solomon, M. E. 1949. The natural control of animal populations. *Journal of Animal Ecology,* 18: 1-35.

Wang, J. J. and Tsai, J.H. 2001. Development and functional response of *Coleophora inaequalis* (Coleoptera: Coccinellidae) feeding on brown citrus aphid, *Toxoptera citricida* (Homoptera: Aphididae). *Agricultural and Forest Entomology,* 3: 65-69.

Xue, Y., Bahlai, C.A., Frewin, A., Sears, M.K., Schaafsma, A.W. and Hallett, R.H. 2009. Predation by *Coccinella septempunctata* and *Harmonia axyridis* (Coleoptera: Coccinellidae) on *Aphis glycines* (Homoptera: Aphididae). *Environmental Entomology,* 38: 708-714.

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Πυκνοεξαρτημένη θήρευση των προνυμφικών ηλικιών του *Propylea quatuordecimpunctata* L. (Coleoptera: Coccinellidae) επί του *Aphis fabae* Scopoli (Hemiptera: Aphididae)

Ν.Ε. Παπανικολάου, Γ. Ματσίνος, Δ.Π. Παπαχρήστος, Δ.Χ. Κοντοδήμας και Π.Γ. Μυλωνάς

Περίληψη Μελετήθηκε η επίδραση της πυκνότητας της λείας (μαύρη αφίδα των κουκιών, *Aphis fabae* Scopoli (Hemiptera: Aphididae)) στο ρυθμό θήρευσης των τεσσάρων προνυμφικών ηλικιών του αφιδοφάγου αρπακτικού *Propylea quatuordecimpunctata* L. (Coleoptera: Coccinellidae). Τα αποτελέσματα έδειξαν ότι οι προνύμφες όλων των ηλικιών του αρπακτικού εμφανίζουν συνεχώς μειωμένο ρυθμό θήρευσης σε αυξανόμενη πυκνότητα του *A. fabae* (λειτουργική ανταπόκριση τύπου ΙΙ). Ο ρυθμός επιθέσεων του αρπακτικού, ο οποίος εκφράζει την κατανάλωση λείας σε χαμηλές πυκνότητες αυτής, δεν διέφερε μεταξύ των προνυμφικών ηλικιών. Σε αντίθεση, ο χρόνος χειρισμού της λείας μειώθηκε για τις προνύμφες μεγαλύτερης ηλικίας.

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Hellenic Plant Protection Journal **6:** 67-68, 2013

SHORT COMMUNICATION

First record of *Rhodania occulta* Schmutterer (Hemiptera: Pseudococcidae) in Greece

P.G. Milonas[1], G.K. Partsinevelos[1] and Y. Ben-Dov[2]

Summary The mealybug *Rhodania occulta* has been recorded for the first time in Greece. Adult females were collected from leaves sheaths of *Lolium* sp. (Poaceae) at Parnitha mount in Attica. This is the first record of the genus *Rhodania* in Greece.

The genus *Rhodania* (Hemiptera: Pseudococcidae) includes the following six species that are distributed in the Palaearctic region: *Rh. aeluropi* Williams & Moghaddam, 2007; *Rh. festucae* Hadzibejli, 1959; *Rh. flava* Goux, 1936; *Rh. hypogea* Leonardi, 1908; *Rh. occulta* Schmutterer, 1952 and *Rh. porifera* Goux, 1934 (Gavrilov-Zimin, 2011; Ben-Dov *et al*., 2013). All stages of these mealybug species feed on roots and at leaf-bases of grasses (Poaceae) that belong to the genera *Agrostis*, *Corynephorus* and *Festuca* (Kosztarab and Kozar, 1988).

Rhodania occulta has been recorded in Germany (Schmuterrer, 1952), Netherlands (Reyne, 1957) and Poland (Koteja & Zak Ogaza, 1966). This is the first record of the species in the Mediterranean region and in Greece in particular (Milonas *et al*., 2008).

In Greece, *Rh. occulta* was collected in January 2007, at Parnitha mount, Attica, off leaves of *Lolium sp*. (Poaceae). The population consisted of adult females, suggesting that it overwinters at this stage. Specimens are deposited at the Biological Control Laboratory of BPI Greece and at the Department of Entomology, Agricultural Research Organization, in Israel

Adult females of *Rh. occulta* are elongate oval, light yellow and approximately 1.9 mm long, 0.95 mm wide; the antenna is 6-segmented. It develops one generation per year. In central Europe adult females overwinter and lay their eggs at the end of April-early May. Eggs hatch after two weeks and nymphs reach maturity in September. They feed on the underside of the leaf-blades or at the base of stem of grasses (Kosztarab and Kozar, 1988; Schmutterer, 1952).

The present new records of *R. occulta* and the host plant species may indicate that this mealybug species is more common in Greece and in Europe than the current records suggest. Further studies are required to determine its distribution in Palaearctic region.

Literature cited

Ben-Dov Y., Miller, D.R. and Gibson G.A.P. 2013 - ScaleNet: a database of the scale insects of the world. Available from http://www.sel.barc.usda.gov/scalenet/scalenet.htm (accessed May 2013).

Gavrilov-Zimin, I.A. 2011. Mealybugs of the genus *Rhodania* Goux, 1934 (Homoptera: Coccinea: Pseudococcidae) from Russia and neighbouring countries *Zoosystematica Rossica* 20(1):40-44.

Goux, L. 1934. Notes sur les coccides [Hem.] de la France. (10e note). Étude d'une espèce nouvelle constituant un genre nouveau. *Bulletin de la So-*

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ciété Entomologique de France, 39: 291–296.

Goux, L. 1936. Notes sur les coccides [Hem.] de la France. (13e note). Étude d'une deuxieme *Rhodania nouvelle*. *Bulletin de la Société Entomologique de France*, 41: 35–40.

Hadzibejli, Z.K. 1959. New species of scale insect pests of pastures of Eastern Georgia. *Notes of Agricultural Academy of Georgian SSR*, 2(3): 61–71. (In Georgian).

Kosztarab, M. and Kozár, F. 1988. Scale insects of central Europe. Budapest. 456 p.

Koteja, J. and Zak-Ogaza, B. 1966. Investigations on scale insects (Homoptera, Coccoidea) of the Pieniny Klippen Belt. *Acta Zoologica Cracoviensia* 11: 305-332.

Leonardi, G. 1908. Seconda contribuzione alla conoscenza della cocciniglie Italiane. *Bollettino del Laboratorio di Entomologia Agraria di Portici*, 3: 150–191.

Milonas, P.G., Kozár, F. and Kontodimas, D.C. 2008: New data on the scale insects (Homopterra: Coccoidea) of the Greek Entomofauna. *Hellenic Plant Protection Journal*, 1: 32-34.

Reyne, A. 1957. Snavelinsecten - Rhynchota, I, Nederlandse schildluizen (Coccidae). *Wetenschappelijke Mededlingen van de Koninklijke Nederlandes Natuurhistorische Vereniging* 22: 1-44.

Schmutterer, H. 1952. Über drei neue an Gräsern lebende Schildlausarten der deutschen Fauna. Beiträge zur Entomologie (Berlin), 2: 554–566. (In German)

Williams, D.J. and Moghaddam, M. 2007. A new species of *Rhodania* Goux (Hemiptera, Coccoidea, Pseudococcidae) and new records of two other mealybug species from Iran. *Zootaxa*, 1398: 37–44.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή του *Rhodania occulta* Schmutterer (Hemiptera: Pseudococcidae) στην Ελλάδα

Π.Γ. Μυλωνάς, Γ.Κ. Παρτσινέβελος και Υ. Ben-Dov

Περίληψη Το είδος *Rhodania occulta* καταγράφεται για πρώτη φορά στην Ελλάδα. Ενήλικα θηλυκά άτομα συλλέχθηκαν από φύλλα φυτού *Lolium* sp. (Poaceae) σε περιοχή του όρους Πάρνηθα στην Αττική. Πρόκειται για πρώτη καταγραφή του γένους *Rhodania* στην Ελλάδα.

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Biodegradation of diazinon by the epiphytic yeasts *Rhodotorula glutinis* and *Rhodotorula rubra*

E.D. Bempelou[1,3], J.G. Vontas[2], K.S. Liapis[3] and V.N. Ziogas[1]

Summary The capability of the epiphytic yeasts *Rhodotorula glutinis* and *Rhodotorula rubra* to biodegrade diazinon in pure cultures and tomato fruits was investigated. Higher biodegradation rates were observed as the concentration of diazinon and the inoculum of the microorganisms were increased, and the yeasts proved to be more active at 25°C and 15°C. The presence of glucose in the mineral nutrient medium, as an extra source of carbon, delayed the biodegradation by *Rh. glutinis*, while *Rh. rubra* biodegrade diazinon faster in the presence of glucose. The detection and quantification of diazinon and its degradation product 2-isopropyo-6-methyl-4-pyrimidinol (IMP) were achieved successfully in the LC-MS/MS chromatographic system. The in vitro enzymatic assays applied suggested that glutathione-S-transferases may be involved in the biodegradation of diazinon, a fact that was further enhanced after the addition of the synergists triphenyl phosphate, diethyl maleate and piperonyl butoxide in the biodegradation trials. The decrease of diazinon residues on tomato fruits confirmed the corresponding on pure cultures, resulting in the suggestion that the yeasts *Rh. glutinis* and *Rh. rubra* can possibly be used successfully for the removal or detoxification of diazinon residues ontomatoes.

Additional keywords: biodegradation, diazinon, epiphytic yeasts, glutathione-S-transferases, synergists

Introduction

The excessive and frequent application of pesticides in agricultural practice has resulted in high levels of pesticide residues accumulated on vegetables. This matter poses a potential health risk to consumers (Bolognesi and Morasso, 2000), particularly in countries where highly toxic insecticides are still in use. An increased interest in the development of means for removing pesticide residues from vegetables, using environmental friendly approaches, such as microorganisms or recombinant enzyme systems has been observed.

Diazinon, *[O, O-diethyl O-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] ester,* has mainly been used extensively as an insecticide or nematicide for the control of a broad range of insects and pests, playing a distinguished role in agriculture and veterinary. It is a non systemic pesticide with action through stomach or suffocation. The main paths of its degradation in the environment are oxidation in air (Schomburg *et al*., 1991; Seiber *et al*., 1993) and hydrolysis, photolysis and biodegradation in water and soil (Domagalski and Kuivila, 1993; Frank *et al*., 1991; Howard, 1991). The half-life of diazinon in plants has been measured from 1 to 14 days while in tissues with high fat concentration has been found to be more persistent (Bartch, 1974). The corresponding time in animals is too short with a maximum of 12 hours half-life. The pesticide is rapidly excreted though urine and feces, with the 70% of the total excretion to be consisted of metabolites (US Public Health Service, 1995). Moreover, diazinon has an intermediate toxicity to mammals and may affect the central nervous system, the cardiovascular system and the

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respiratory system as well as may cause skin and eye irritation (Gallo and Lawryk, 1991).

The contamination of aquatic and terrestrial ecosystems with diazinon, even though no longer registered for use in the European Union (Desicion 2007/393/EC), has increased the public concern to establish an efficient and safe method to detoxify diazinon residues from contaminated environments. Generally, the microorganisms able to degrade diazinon belong to the genus *Streptomyces*, *Arthrobacter*, *Flavobacter* and *Pseudomonas*. Gunner and Zuckerman first reported (1968) the synergistic action of the bacteria *Arthrobacter* sp. and *Streptomyces* sp. in the degradation of diazinon. A year later (1969), Sethunathan and MacRae observed that *Streptomyces* sp., isolated from rice fields, consumed diazinon only on the presence of glucose. Sethunathan and Pathak (1972) also reported the degradation of diazinon in water, rhizosphere and soil of rice fields by *Arthrobacter* sp. and *Flavobacter* sp. The degradation of diazinon, as a sole source of carbon, by *Pseudomonas* was mediated by hydrolase activity according to Rosenberg and Alexander (1979) and Barik and Munnecke (1982). *Flavobacterium* sp. ATCC27551 was responsible for the hydrolysis of diazinon and its transformation to 2-isopropyl-4-methyl-6-hydroxypyrymidine (IMHP) and carbon dioxide (Adhya *et al*., 1981; Sethunathan, 1989) with the biodegradation to be attributed to the enzymatic system of phosphorotriesterases. *Arthrobacter* sp. and *Enterobacter* strain B14 (used for the bioremediation of soil contaminated with chlorpyrifos) and *Alcaligenes faecalis* DSP3 also degraded diazinon (Ohshiro *et al*., 1996). Cycon *et al*. (2009) reported that the bacteria *Pseudomonas* sp., *Serratia liquefacienes* and *Serratia mascencens* depleted diazinon in the presence of glucose.

In the present study, the capability of the epiphytic yeasts *Rh. glutinis* and *Rh. rubra* to degrade diazinon was examined, aiming to explore the feasibility of using these naturally present microorganisms to detoxify organophosphate residues and improve food safety.

Materials and Methods

Chemicals and reagents

Analytical standards of diazinon (97.5%) and piperonyl butoxide (92.5%) were purchased from Dr Ehrenstofer (Augsburg, Germany). The metabolite 2-isopropyl-6-methyl-4-pyridinol (IMP) (99.1%), triphenyl phosphate (99.5%) and diethyl maleate (95%) were obtained from ChemService (West Chester, UK). Ethyl acetate, acetone, dichloromethane, acetonitrile and hexane all of pesticide residues grade, methanol and water of LC-MS grade and petroleum ether (40-60°C) of analytical reagent grade, were all obtained from Lab Scan (Dublin, Ireland). Stock solutions of 1000 μg/mL and 10000 μg/mL were made in methanol or acetonitrile (enzymatic assays) and were stored at –20°C. Working solutions were prepared in order to be used in analytical procedures of the study. Formic acid purchased from Sigma (Greece) was also used. The reagents used in the enzymatic assays were p-Nitrophenyl acetate (PNPA), a-naphthyl acetate, b-naphthyl acetate and 1-chloro-2,4-benzene (CDNB).

Chromatographic analysis and validation of analytical methods

A Varian [2x prostar 210 (LC) and 1200 L (quadrupole MS/MS)] LC-MS/MS was used for the determination of diazinon and its metabolite 2-isopropyl-6-methyl-4-pyrimidinol (IMP) with a Varian Polaris C18-A column (5 cm length, 2 mm internal dimension and 5 μm particle size) at ambient temperature (25 ± 4°C). Elution solvents were the mixtures of methanol / water supplemented with 1 mM $HCOONH_4$ (10/90) (Solvent A) and methanol / water supplemented with 1 mM $HCOONH_4$ (90/10) (Solvent B). A flow of 0.25 mL/min and an injection volume of 5 μL (full loop) were applied. The elution program used was gradient, starting with 90% of solvent A and 10% of solvent B, reaching the 100% of solvent B at 14 min, remaining there for 6min and returning to its first constitution at 25 min. The mass spectrometry multiplier was set at 1500 V voltage, ion

source at 50°C and drying gas (N_2) at 19 psi pressure and 250°C temperature. The electrospray ionization mode was in positive mode using nitrogen as nebulizer gas at 50 psi. The needle voltage was 5000 V while the spray shield voltage was 600 V.

The European guideline SANCO 2011/12495 document (Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Foods and Feeds) was followed for the validation of the analytical methods. Different known concentrations of diazinon and IMP were fortified in the mineral salts medium (0.05 μg/mL, 0.1 μg/mL and 0.5 μg/mL) and tomato fruits (only parent compound) with 5 replicates.

Yeast cultures

Rhodotorula glutinis was isolated from tomato plants while *Rh. rubra* from strawberry plants. Cultures were developed in mineral salts medium containing in g/L: 12 NH_4NO_3, 8 KH_2PO_4, 2 Na_2SO_4, 4 KCl, 1$MgSO_4 \cdot 7H_2O$, 0.5 $CaCl_2$, 0.26 $ZnSO_4 \cdot 7H_2O$, in 250mL Erlenmeyer flasks supplemented with diazinon and cells (each yeast in a separate flask) and incubated in an orbital incubator at 25°C and 150 rpm.

Effects of various factors on diazinon degradation in liquid cultures

Incubations of the yeasts in 250 mL flasks containing 50 mL mineral salts medium (NH_4NO_3, KH_2PO_4, Na_2SO_4, KCl, $MgSO_4 \cdot 7H_2O$, $CaCl_2$, $ZnSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$ and $Na_2MbO_4 \cdot 7H_2O$) with 10^2, 10^4 and 10^6 cells/mL, in the presence of 10 μg/mL diazinon, were conducted at 25°C in order to examine the effect of yeast inoculum on the degradation. To examine the effect of concentration on the biodegradation rates, the experiments were conducted in 250 mL flasks containing 50 mL mineral salts medium supplemented with diazinon at concentrations of 10 and 25 μg/mL. To confirm the influence of temperature, degradation trials were conducted in 250 mL flasks containing 50 mL of the mineral salts medium supplemented with 10 μg/mL diazinon at 15°C, 25°C and 35°C, respectively. The influence of glucose in the nutrient mineral medium, as an extra source of carbon, was also investigated simultaneously with all the above parameters. In each trial there were additional 250 mL flasks containing 50 mL mineral salts medium supplemented with glucose under the certain experimental conditions and the influence of this factor was also estimated. All treatments were in triplicate. Flasks were incubated in an orbital incubator at 25°C and 150 rpm. At time intervals of 2 days (samples were taken aseptically and analyzed for diazinon residues.

Extraction of diazinon and IMP from pure cultures and tomato fruits

Subsamples of 5 mL of the of mineral salts nutrient medium (50 mL) were taken aseptically and filtered through a Whatman No 2 filter above a 100 mL separating funnel. Liquid- liquid extraction was carried out with 20 mL dichloromethane: acetone (1:1) for three times. Extracts were passed through anhydrous sodium sulfate, collected in a 100 mL flat bottom flask and evaporated to dryness on a rotary evaporator. The dry residue was diluted to 2 mL methanol: water (30:70) and filtered through a 0.2 μm film filter in order to be analyzed in the LC-MS/MS chromatographic system.

The method used for the sample processing of tomato fruits has been described by Bilthoven (1996). 30mL of acetone were added in an aliquot of 15 g of the homogenated sample in a 250 mL PTFE centrifuge bottle (Nalgene, Rochester, NY, USA) and stirred for 1 min in an ultra-turrax homogenizer at 15000 rpm. 30 mL of dichloromethane and 30 mL of petroleum ether (40-60°C) were added following by a new stirring step for 1 min. The sample was centrifuged at 4000 rpm for 2 min. 25 mL of the supernatant were evaporated to dryness on a water bath at 65-70°C and afterwards 3 mL of methanol: water (30:70) was added. The extract was placed in an ultrasonic bath for 30 s, filtered through a 0.2 μm film filter and transferred in a vial with teflon septa, ready for chromatographic analysis.

Enzymatic assays

One mL of liquid culture was added in an

eppedonrf tube (1.5 mL) and centrifuged in 5000 rpm for 5 min. The supernatant was removed and 1 mL of sodium phosphate buffer solution 100 mM, pH 7, was added in the tube. Following the same procedure, the final sediment was redissolved in 0.5 mL sodium phosphate buffer solution 100 mM, pH 7.2, supplemented with 0.2% Triton.

The activity of esterases and glutathione-S-transferases (GSTs) was determined spectrophotometrically on an UVmax mictotitre plate reader, as previously described (Vontas *et al*., 2001). A-naphthyl and b-naphthyl acetate were used as substrates for esterases and for glutathione-S-transferases (GSTs) the corresponding 1-chloro-2,4-dintrobenzene (CDNB). All treatments were carried out in triplicate.

Protein was assayed by using the Bio-Rad protein assay kit (Bio-Rad, Hemel Hempstead, Herts, UK) with BSA as a standard protein (Bradford *et al*., 1976).

Inhibition studies were conducted by incubation of the homogenates for 10 min with diazinon concentrations of 23, 46, 59, 66 and 79 μM in the presence of acetonitrile. The remaining activity was determined in triplicate as in the standard assays mentioned above and expressed as units (U) per mg of proteins (IU = μmol of substrate hydrolyzed per minute).

Addition of synergists in the biodegradation trials

10μg/mL of the synergists triphenyl phosphate (inhibitor of esterases) piperonyl butoxide (inhibitor of monoxygonases and esterases) and diethyl maleate (inhibitor of monoxygonases and glutathione- S- transferases) were added (one in a time) in the biodegradation trials and the biodegradation procedure was studied. Previously, the sensitivity of the two yeasts in the synergists was studied. 250 mL flasks containing 50 mL mineral salts medium (NH_4NO_3, KH_2PO_4, Na_2SO_4, KCl, $MgSO_4 \cdot 7H_2O$, $CaCl_2$, $ZnSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$ and $Na_2MbO_4 \cdot 7H_2O$) were inoculated with 10^6cells/mL under sterile conditions and supplemented with triphenyl phosphate, piperonyl butoxide and diethyl maleate (one in a time) at concentrations 1, 5, 10 and 15 μg/mL and incubated in an orbital incubator at 25°C and 150 rpm.

Biodegradation on tomato fruits

In order to estimate the activity of the yeasts to biodegrade diazinon in plant surfaces, trials were conducted in tomato fruits of Noa variety. Fruits were washed and disinfected with an ethanolic solution 90% and sprayed with 10^8 cells/mL of yeast and one day later with 2 mg/kg of diazinon. Control samples were treated only with the insecticide. All treatments were in triplicate. Fruits were stored in an incubator at 20°C with high relative humidity and 12 hours photoperiod. Samplings occurred in 5 days and 20 days. Each sample, constituting of 5 fruits, was homogenated and then analyzed as described above.

Results and Discussion

Chromatographic determination and validation results

The qualitative and quantitative determination of diazinon and its metabolite IMP was successfully achieved in the LC-MS/MS chromatographic system (Figure 1) with the technique of multiple reaction monitoring (MRM), using the ions 305.1>169.1 m/z and 305.1>96.6 m/z for diazinon and 153>84 m/z and 153>70 m/z for IMP, applying electrospray ionization in positive mode ESI (+), collision energy at 31V for diazinon transitions and 22 for the transitions of IMP. The corresponding values in capillary were 21 V and 76 V. Confirmation was based on the criteria of retention time and ion abundance of qualitative and quantitative ions according to the European guideline SANCO 2011/12495 and the method was found to be effective for the extraction of the tested compounds.

The method was validated by assessing the basic parameters such as sensitivity, mean recovery (as a measure of trueness) and repeatability (as a measure of precision). The transitions 305.1>169.1 m/z and 153>84

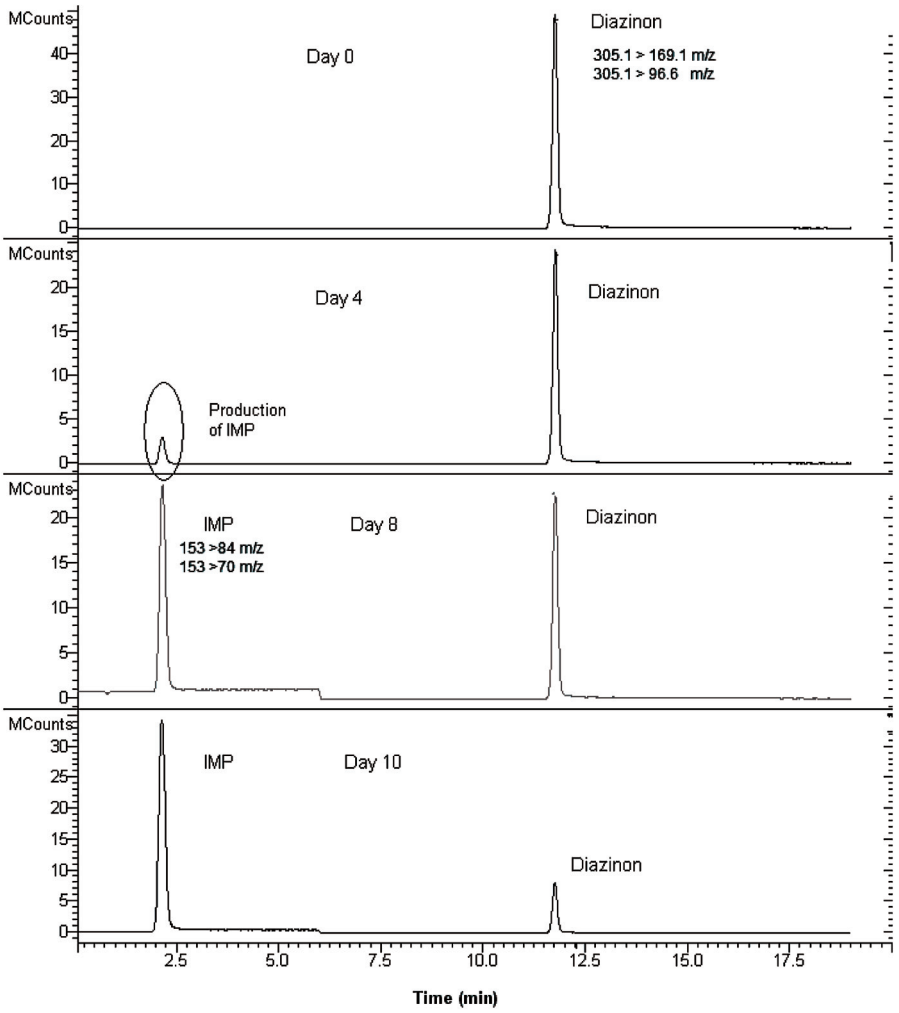

Figure 1. Determination of diazinon and its metabolite 2-isopropyl-6-methyl-4-pyridinol (IMP) in the LC-MS/MS chromatographic system with ESI (+) during a time period of 10 days. The determination of the two compounds was achieved with the MRMs 305.1> 169.1 m/z and 305.1>96.6 m/z for diazinon and 153> 84 m/z and 153>70 m/z for IMP.

m/z were used for the quantification of diazinon and IMP, respectively. After the fortification of 250 mL flasks containing 50 mL mineral salts medium with diazinon and IMP, the obtained recoveries for the two compounds ranged from 85.2 to 100%, values which were accepted according to SANCO Document 2011/ 12495 (Table 1). Relative standard deviations (RSDs) values ranged from 0.34 to 3.55 %, all lower to 20%, also accepted. Validation results of tomato fruits sprayed with 0.05 0.1 and 0.5 μg/mL diazinon gave average recoveries from 97.6% to 102% and mean RSDs from 1.7% to 4.7% (Table 1), respectively. The limit of quantification (LOQ) was set for both substrates in the lowest validation level, 0.05 μg/mL and the limit of detection (LOD) was calculated to be 0.02 μg/mL. The above results indicate the efficiency of the analytical methods for the

Table 1. Mean recoveries (R, %), relative standard deviations (RSD, %) and signal to noise (S/N) ratio for the determination of diazinon and 2-isopropoyl-6-methyl-4-pyrimidinol (IMP) in mineral salts medium and diazinon in tomato fruits, in 5 replicates (n) at 3 fortification levels in the LC-MS/MS chromatographic system.

Compound	Mineral salts medium (n=5)				Tomato fruits (n=5)			
	C (μg/mL)	Mean Recovery (%)	RSD (%)	S/N	C (μg/mL)	Mean Recovery (%)	RSD (%)	S/N
Diazinon	0.05	95.6	0.34	47	0.05	97.6	1.7	32
	0.1	91.4	2.8	53	0.1	91.4	2.8	93
	0.5	100	1.6	112	0.5	102	4.7	124
IMP	0.05	85.2	3.55	17	-	-	-	-
	0.1	93	2.79	32	-	-	-	-
	0.5	96.6	2.06	51	-	-	-	-

determination of diazinon and its metabolite IMP from the mineral salts medium and diazinon from tomato fruits and ensure the accuracy of the results from the biodegradation trials.

Up to now, the simultaneous determination of the two compounds has been reported in various substrates (water, soil, plants, products of animal origin, urine and blood plasma mainly using high performance liquid chromatography with UV detector (HPLC/UV) (Abu- Qare *et al*., 2001; Abu- Qare and Abou- Donia, 2001), as well chromatography with FID detector (Bavcon *et al*., 2003). In agreement to our analytical method Rain and Sun (2008) reported the determination of diazinon and IMP by liquid chromatography-positive ion electrospray tandem mass spectrometry.

Biodegradation of diazinon by *Rhodotorula glutinis* and *Rhodotorula rubra*

Effect of yeast inoculum on biodegradation

No biodegradation was observed when 10^2 cells/mL and 10^4 cells/mL of *Rh. glutinis* and *Rh. rubra* were supplemented in the mineral salts nutrient medium, up to the end of the experimental period. On the contrary, degradation did occur after the incubation of each culture with the initial inoculum of 10^6 cells/mL and in this case IMP was detected as a degradation product. In contrast to our study Sethunathan (1989), who also verified degradation products of diazinon after the incubation with the bacterium *Flavobacterium* sp. ATCC27551 in rice fields, found the metabolite 2-isopropyl-4-methyl-6-hydroxypirimidine (IMHP) was the major metabolite.

Effect of presence of glucose on mineral salts liquid medium

The addition of glucose in the mineral salts nutrient medium, as an extra source of carbon, affected the action of *Rhodotorula* yeasts as degraders of diazinon in a different way. Under the presence of glucose, *Rh. glutinis* did not consume diazinon during the first days of the trials, while after the fourth day the insecticide was decreased rapidly (Figure 2). On the contrary, in the flasks without glucose degradation was observed from the first sampling (day 2) of the trials and diazinon was finally depleted. In agreement with our results, Muncnerova and Augustin (1995) had reported the degradation of benzoate by *Rh. glutinis* only after the consumption of glucose in the medium. According to Ruiz-Amil *et al.* (1965); Torrontegui *et al.* (1966), Fernadez *et al.* (1967) and Medrano *et al.* (1969) the presence of glucose in the nu-

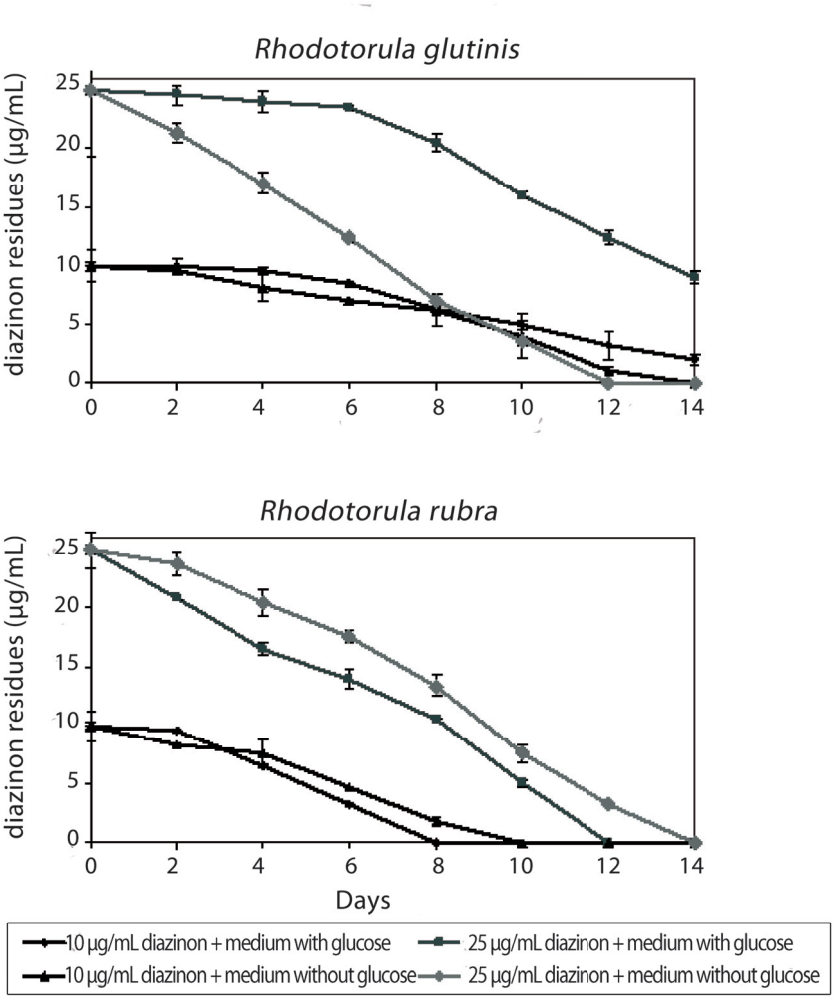

Figure 2. Biodegradation of 10 and 25 µg/mL diazinon by the yeasts *Rhodotorula glutinis* and *Rhodotorula rubra* in mineral salts nutrient medium with and without glucose. Each value is the mean of three replicates with error bars representing the standard deviation of the mean.

trient medium prevents the assimilation or the metabolism of another substance from the cells of the yeast until the depletion of the levels of glucose. In the case of *Rh. rubra*, the addition of glucose in the nutrient medium as an extra source of carbon did not affect its ability to degrade diazinon. Biodegradation was observed from the beginning of the trials in both media (Figure 2) and in particular the medium supplemented with glucose gave higher biodegradation rates, 1.67 µg/mL/day and 2.5 µg/mL/day comparing to 1.25 µg/mL/day and 2.08 µg/mL/day on the medium without glucose for 10 µg/mL and 25 µg/mL diazinon, respectively. By the end of the experiment diazinon was fully degraded in both media. This has also been reported by Cycon *et al.* (2009) during the degradation of diazinon by the bacteria *Pseudomonas* sp., *Serratia liquefaciens* and *Serratia marcescens* and by Sethunathan and MacRae (1969), who reported that *Streptomyces* sp., isolated from soil of rice fields, degrade diazinon only under the presence of

glucose in the cultivation medium. We have to mention that the above results are attributed to biodegradation of diazinon with a maximum concentration of glucose in the nutrient medium of 5 g/L, since Muncnerova and Augustin (1995) reported that in the concentration of glucose of 10 g/L *Rh. rubra* also started the degradation only after the depletion of the saccharide.

Effect of diazinon concentration on biodegradation

To investigate the effect of diazinon concentration on degradation, degradation trials of diazinon with different concentrations were conducted in the mineral salts medium at 25°C. *Rh. glutinis* and *Rh. rubra* proved to be capable degraders of diazinon. Both yeasts degrade fully 25 μg/mL diazinon in 12 to 14 days (Figure 2). As it was observed the higher the concentration it was, the more active the yeasts were. In particular, although *Rh. glutinis* maintained the delayed action under the presence of glucose, it presented higher biodegradation rates when it was incubated with 25 μg/mL diazinon in both media. In flasks supplemented with glucose the rate of 0.57 μg/mL/day (10 μg/mL diazinon) increased to 1.14 μg/mL/day (25 μg/mL diazinon). The corresponding values in the flasks without glucose in the mineral salts medium were 0.83 μg/mL/day and 2.5 μg/mL/day (Figure 2). *Rhodotorula rubra* depleted diazinon in all cases presenting higher biodegradation rates in the concentration of 25 μg/mL, 2.5 μg/mL/day and 2.08μg/mL/day in comparison with 1.67 μg/mL/day and 1.25 μg/mL/day in media with or without glucose, respectively. The hydrolysis percentages of diazinon were less than 5% in all controls.

The metabolite 2-isopropyl-6-methyl-4--pyrimidinol (IMP) was produced as a degradation product from both yeasts (Figure 3) in increasing levels up to the depletion of the parent compound. Both yeasts produced bigger concentrations of IMP after their incubation with 25 μg/mL diazinon. As we can observe in Figures 2 and 3, after the consumption of diazinon the levels of IMP remained almost stable and did not decrease until the end of the experimental. Having in mind that at that time IMP was the only source of carbon available for the microorganisms, we can suspect that the two yeasts can not mineralize this compound.

Similar studies have been conducted. Yang *et al*., 2005 reported that *Alcaligenes faecalis* DSP3 degraded 90% of 100μg/mL diazinon in 10 days. Moreover, the bacterium *Enterobacter* strain B-14 degraded 25 μg/mL diazinon in 2 days (Singh *et al*., 2004). Singh has also reported (2006) the degradation of high concentration of diazinon by soil microorganisms. Barik and Munnecke (1982) reported the depletion of diazinon in 24 hours. *Arthrobacter* and *Flavobacterium* transformed 14-15 ppm diazinon in rice fields (soil, water, rhizosphere) in 10 days (Sethunathan and Pathak, 1972). Finally Cycon *et al*. (2009) described the degradation (80-90%) of 50 μg/mL insecticide in 14 days by *Pseudomonas* sp., *Serratia liquefaciens* and *Serratia marcescens*. The majority of the microorganisms reported to be able to degrade diazinon are soil bacteria. No reference has been found describing the biodegradation of diazinon by yeasts and moreover by the epiphytic yeasts *Rh. glutinis* and *Rh. rubra*.

Effect of temperature on biodegradation of diazinon

The effect of different temperatures, 15°C, 25°C and 35°C, on diazinon biodegradation in mineral salts medium is presented in Figure 4. As shown after 14 days of incubation no degradation took place at 35°C, while the degradation rates at 25°C and 15°C were 0.67 μg/mL/day and 0.4 μg/mL/day for *Rh. glutinis* and 0.83 μg/mL/day and 0.56 μg/mL/day for *Rh. rubra*, respectively. The results are considered acceptable taking into account that *Rhodotorula* yeasts belong to psychrophyllic microorganisms.

Enzymatic assays

Inhibition studies were conducted by incubation of homogenates of the two yeasts with diazinon as described above. As it can

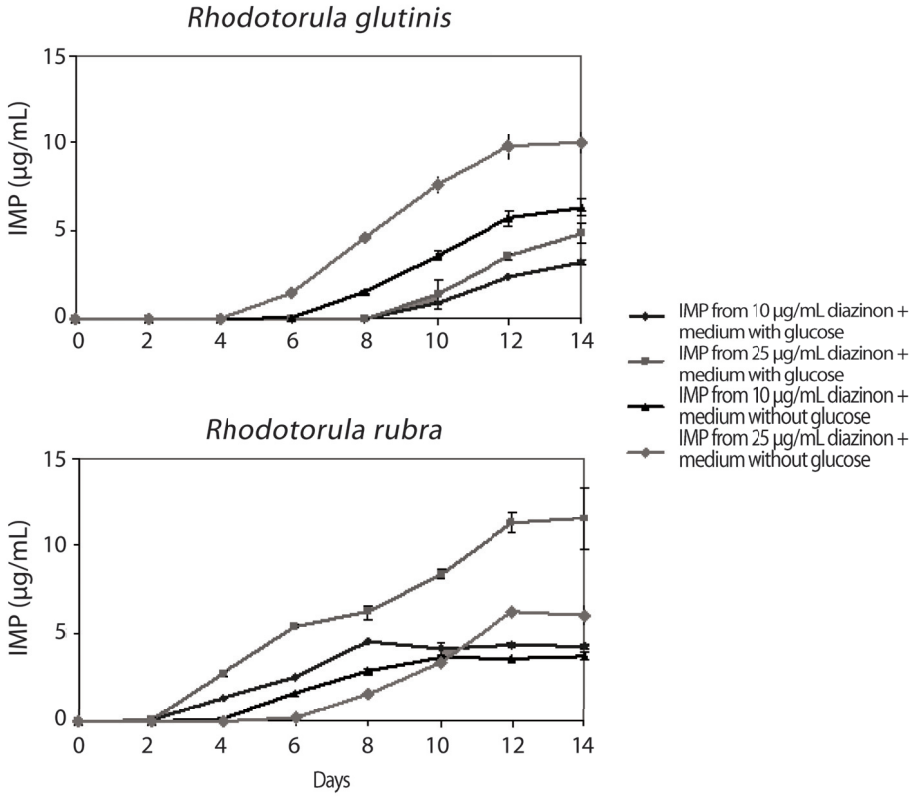

Figure 3. Production of the metabolite 2-isopropyl-6-methyl-4-pyrimidinol (IMP) after the biodegradation of 10 and 25 µg/mL diazinon by the yeasts *Rhodotorula glutinis* and *Rhodotorula rubra* in mineral salts nutrient medium with and without glucose. Each value is the mean of three replicates with error bars representing the standard deviation of the mean.

be seen in Figure 5, the interaction of diazinon in the activity of glutathione-S-transferases (GSTs) of both yeasts was obvious. Diazinon inhibited GSTs activity reaching the maximum inhibition of 100% at concentration of 79 µM. The IC_{50} (half maximal inhibition concentration- IC_{50}) values calculated were 39 µM for *Rh. glutinis* and 52 µM for *Rh. rubra*. On the other hand, diazinon did not reduce the activity of esterases at the concentrations tested and under assay conditions described (Figure 5). These results provided an indication that GSTs and not esterases mediate the observed biodegradation of diazinon by *Rh. glutinis* and *Rh. rubra*. Although most studies support the esterase-hydrolase-based biodegradation of diazinon in hydrolases (Munnecke, 1976; Barik and Munnecke, 1982 and Sethunathan, 1989), GSTs have been previously shown to be capable of degrading organophosphates (Klonis, 2007).

Effect of synergists in biodegradation rates

The possible effect of enzymatic inhibitors in the biodegradation of diazinon was examined in 250 mL flasks with mineral salts medium without glucose since in this medium both yeasts started the consumption of the insecticide from the beginning of the trials. After the incubation of the microorganisms with 1, 5, 10 and 15 µg/mL of the synergists triphenyl phosphate, dieth-

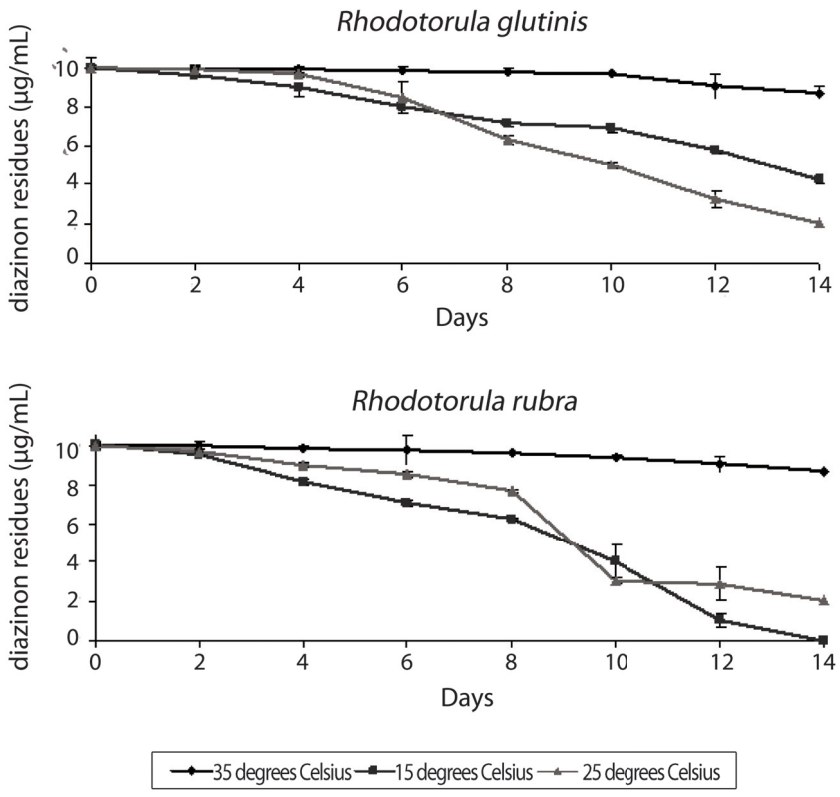

Figure 4. Effect of temperature in the biodegradation of diazinon by the yeasts *Rhodotorula glutinis* and *Rhodotorula rubra*, after the incubation of the microorganisms with 10 µg/mL diazinon in 15°C, 25°C and 35°C, respectively.

yl maleate and piperonyl butoxide (one in a time), no significant inhibition (<5%) was observed in the development of their cultivations. Therefore 10 µg/mL of each enzymatic inhibitor was added in the 250 mL flasks of the biodegradation trials. As it was observed after 10 days of incubation, triphenyl phosphate did not affect the biodegradation of diazinon by *Rh. glutinis* and *Rh. rubra* and similar results were produced after the addition of piperonyl butoxide. On the contrary, diethyl maleate substantially inhibited the biodegradation rate of diazinon. After the end of the experiment 92.2% of the initial concentration of diazinon was recovered. These results enforced the above preliminary statements that GSTs are involved in the biodegradation of diazinon by the two epiphytic yeasts.

Biodegradation pathway

The biodegradation of diazinon by the epiphytic yeasts *Rh. glutinis* and *Rh. rubra* is attributed to the cleavage of the parent moiety by the action of the enzymes of glutathione- S- transferases and the conjugation of glutathione with the leaving group. The conjugate is afterwards hydrolyzed and the metabolite 2-isopropoyl-6-methyl-4-pyrimidinol (IMP) is produced (Figure 6).

Biodegradation of diazinon on tomato fruits

The biodegradation of diazinon by the yeasts *Rh. glutinis* and *Rh. rubra* in tomato fruits is shown in Table 2. Obviously the decrease of diazinon in the treated samples is much higher in comparison with the control. In control samples diazinon showed a

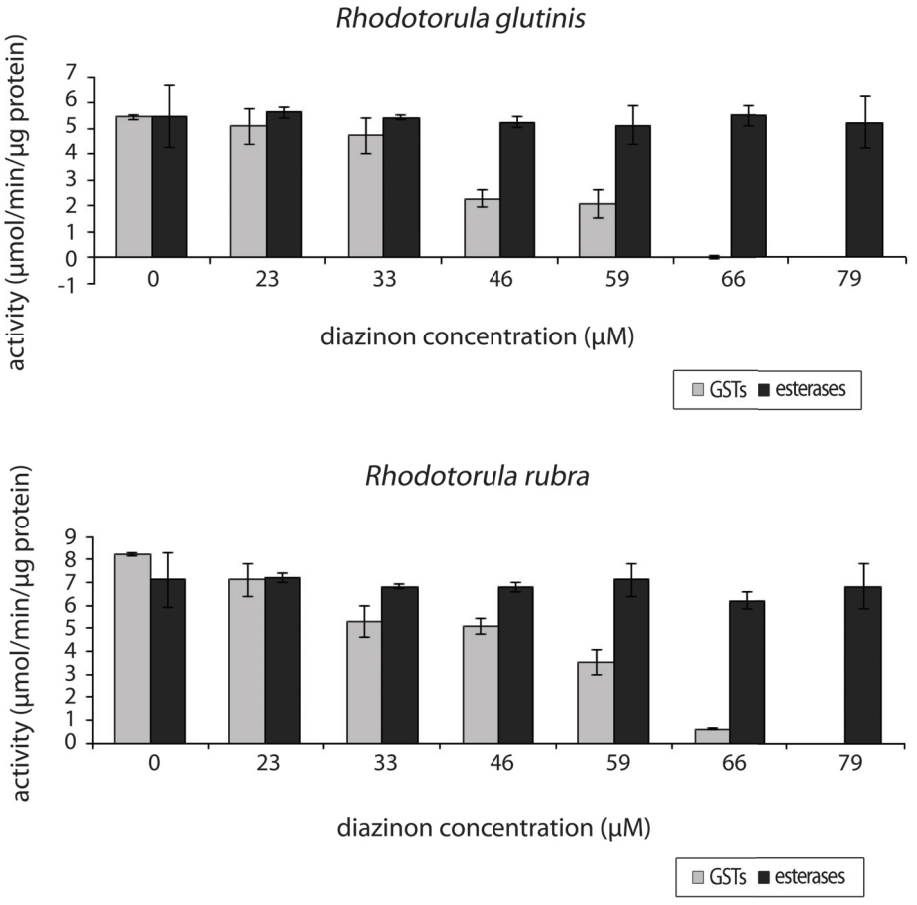

Figure 5. Effect of diazinon on glutathione-S-transferases (gsts) and esterases activity of *Rhodotorula glutinins* and *Rhodotorula rubra*. Each value is the mean of three replicates ± relative standard deviation.

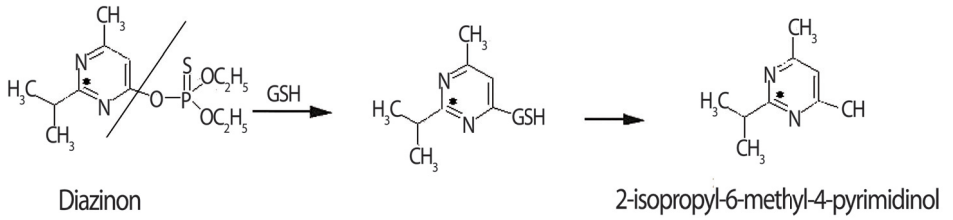

Figure 6. Degradation pathway of diazinon by the epiphytic yeasts *Rhodotorula glutinis* and *Rhodotorula rubra* and production of the metabolite 2-isopropoyl-6-methyl-4-pyrimidinol (IMP).

Table 2. Residues of diazinon (mg/kg) on tomato fruits sprayed with 2mg/kg diazinon and 10^6cells/mL of the yeasts *Rhodotorula glutinis* and *Rhodotorula rubra*. In control the fruits were sprayed only with diazinon and in application the fruits were sprayed with diazinon and the inoculum. Each value is the mean of three replicates ± relative standard deviation.

Days	Concentration of diazinon C (mg/kg)					
	Rhodotorula glutinis			*Rhodotorula rubra*		
	Control	Application	% of the control	Control	Application	% of the control
5	1.68 ± 1.23	1.37 ± 0.55	18.5	1.68 ± 2.04	1.13 ± 1.1	67.3
20	0.83 ± 0.89	0.1 ± 1.63	88	0.83 ± 0.45	Nda	94

[a]Not detected

half life of 20 days. In the same time period the initial concentration of diazinon was decreased up to 88% of the control when *Rh. glutinis* was added, and up to 94% in the case of *Rh. rubra*. These results were in line to our in vitro biodegradation study. It has to be mentioned that the trials were conducted in harvested tomato fruits and therefore are considered as a worst case scenario since the reaction between the microorganism and the insecticide was studied under controlled conditions without the interferences of other factors (biological, environmental) which might contribute to the disappearance of diazinon from the plant surfaces.

Conclusions

Our findings supported that the epiphytic yeasts *Rh. glutinis* and *Rh. rubra* are capable of biodegrading diazinon in liquid cultures and on tomatoes at high rates. The involvement of GSTs in the degradation was indicated by our in vitro and in vivo studies. Therefore, the use of these microorganisms might be a promising method for the removal or detoxification of diazinon residues on tomatoes.

Literature cited

Abu- Qare, A.W. and Abou-Donia, M.B. 2001. Determination of diazinon, chlorpyrifos and their metabolites in rat plasma and urine by high- performance liquid chromatography. *Journal of Chromatographic Science*, 39: 200- 204.

Abu- Qare, A.W. and Abou-Donia, M.B. 2001. High Performance Liquid Chromatographic determination of diazinon, permethrin, DEET (N, N-diethyl-m-toluamide) and their metabolites in rat plasma and urine. *Fresenious Journal of Analytical Chemistry*, 370: 403- 407.

Adhaya, T.K., Sudhakar- Barik, Sethunan N. 1981. Hydrolysis of selected organophosphorous insecticides by two bacteria flooded soil. Journal of Applied Bacteriology, 50: 167-172.

Barik, S., Munnecke, D.M. 1982. Enzymatic hydrolysis of concentrated diazinon in soil. *Environmental Contamination Toxicology*, 29: 235-239.

Bartsch, E. 1974. Diazinon II. Residues in Plants, Soil and Water. *Residue Reviews*, 51: 37-68, 5-59.

Bavcon, M., Trebse, P. and Zupzncic- Kralj 2003. Investigations of the determination of transformations of diazinon and malathion under environmental conditions using gas chromatography coupled with a flame ionization detector. *Chemosphere*, 50: 595- 601.

Bilthoven 1996. Ministry of Public Health, Welfare and Sport. The Netherlands. Analytical methods for pesticide residues in foodstuffs. Sixth edition, P. Van Zoonen Ed.

Bolognesi, C. and Moraso, G. 2000. Genotoxicity of pesticides: potential risk for consumers. Trends in Food Science and Technology, 11: 182-187.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgramquantities of protein utilizing the principle of protein-dye binding. Analytical *Biochemistry*, 72: 248-54.

Cycon, M., Wojcik, M. and Piotrowska-Seget Z. 2009. Biodegradation of the organophosphorus insecticide diazinon by *Serratia sp.* and *Pseudomonas sp.* and their use in bioremediation of contaminated soil. *Chemosphere*, 76: 494-501.

Domagalski, J.L. and Kuivila, K.M. 1993. Distribu-

tions of pesticides and organic contaminants between water and suspended sediment, San Francisco Bay, California. *Estuaries* 16: 416-426.

European Commission 2011. Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed, Document No. SANCO 2011/ 12495. Available from: www.ec.europa.eu/food/plant/protection/resources/qualcontrol_en.pdf

Fernadez, M.J., Medrano, L., Ruiz-Amil, M. and Losada, M. 1967. Regulation and function of pyruvate kinase and malate enzyme yeast. *European Journal of Biochemistry*, 3: 11-18.

Frank, R., Braun, H.E., Chapman, N. and Burchat, C. 1991. Degradation of parent compounds of nine organophosphorous insecticides in Ontario surface waters under controlled conditions. Bulletin of Environmental Contamination and Toxicology, 47: 374-380.

Gallo, M.A. and Lawryk, N.J. 1991. Organic phosphorus pesticides. In Handbook of Pesticide Toxicology. Hayes, W. J., Jr. and Laws, E. R., Jr., Eds. Academic Press, New York, NY:5-3.

Gunner, H.B. and Zuckerman, B.M. 1968. Degradation of diazinon by synergistic microbial action. *Nature* (Lond), 217: 1183- 1184.

Howard, P.H., ed. 1991. Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Vol. 3: Pesticides. Lewis Publishers, Chelsea, MI: 5-14.

Medrano, L. Ruiz-Amil, M and Losada, M. 1969. Effect of glucose on pyruvate utilization by *Rhodotorula glutinis*. *Arcives Microbiology*, 66: 239-249.

Muncnerova, D. and Augustin, J. 1995. Assimilation of benzoate by *Rhodotorula rubra*, *Rhodotorula glutinis* and *Rhodosporidium toluroides*, as affected by glucose or xylose. *World Journal of Microbiology and Biotechnology*, 11: 240-241.

Munnecke, D. M. 1976. Enzymatic hydrolysis of organophosphate insecticides, a possible pesticide disposal method. *Applied and Environmental Microbiology*, 32: 7-13.

Ohshiro, K., Kakuta, T., Sakai. T., Hirota, H. Hoshino, T. and Uchiyama, T. 1996. Biodegradation of organophosphorus insecticides by bacteria isolated from Turf Green Soil. *Journal of Fermentation and Bioengineering*, 82: 299-305.

Raina, R. and Sun, L. 2008. Trace level determination of selected organophosphorus pesticides and their degradation products in environmental air samples by liquid chromatography-positive ion electrospray tandem mass spectrometry. Journal of *Environmental Science and Health*, Part B, 43: 323-332.

Roditakis, E., Grispou, M., Morou, E., Kristoffersen, J.B., Roditakis, N., Nauen, R., Vontas, J. and Tsagkarakou, A. 2009. Current status of insecticide resistance in Q biotype *Bemisia tabaci* populations from Crete. *Pest Management Science*, 65: 313-22.

Rosenberg A. and Alexander M. 1979. Microbial cleavage of various organophosphorus insecticides. *Applied and Environmental Microbiology*. 37: 886-891.

Ruiz-Amil, M., Torrontegui, G., Palacian, E, Catalina, L. and Losada, M. 1965. Properties and functions of yeast *Puryvate Carboxylase*. *Journal of Biological Chemistry*, 240: 3485-3492.

Schomburg, C.J., Glotfelty, D.E. and Seiber, J.N. 1991. *Pesticide Science and Technology*, 25: 155-160.

Seiber, J.N., Wilson, B.W. and McChesney, M.M. 1993. Air and fog deposition residues of four organophosphorous insecticides used on dormant orchards in the San Joaquin Valley, California. *Environmental Science and Technology*, 27: 2236-2243.

Sethunathan, N. and MacRae, T.C. 1969. Persistence and biodegradation of diazinon in submerged soils. *Journal of Agriculture and Food Chemistry*, 17: 221-225.

Sethunathan, N. and Pathak, M.D. 1972. Increased biological hydrolysis of diazinon after repeated application in rice paddies. *Journal of Agriculture and Food Chemistry* 20: 586- 589.

Sethunathan, N. 1989. Biodegradation of pesticides in tropical rice ecosystems. Ecotoxicology and Climate. Edited by P. Bourdeau, J.A. Haines, W. Klein and C.R. Krishna Murti. Published by John Wiley & Sons Ltd, 247-264.

Singh, B.K., Walker, A., Morgan, J.A.W. and Wright, D.J. 2004. Biodegradation of chlorpyrifos by *Enterobacter* strain-B14 and its use in bioremediation of contaminated soils. *Applied and Environmental Microbiology*, 70: 4855-4863.

Singh, B.K., Walker, A. and Wright, D.J. 2006. Bioremedial potential of fenamiphos, chlorpyrifos, degrading isolates: influence of different environmental conditions. *Soil Biology and Biochemistry*, 38: 2682- 2693.

Torrontegui, G., Palacian, E. and Losada, M. 1966. Phosphoenolpyruvate carboxykinase gluconeogenesis and its repression by hexoses in yeasts. *Biochemical and Biophysical Research Communications*, 22: 227-231.

U.S.P.H.S. (U.S. Public Health Service) 1995. Hazardous substance data bank. Washington, DC: 5-9.

Vontas J.G., Small G.J. and Hemingway J. 2001. Glutathione S-transferases as antioxidant defense agents confer pyrethroid resistance in *N. lugens*. *Biochemical Journal,* 357: 65-72.

Yang, L., Zhao, Y.H., Zhang, B.X., Yang, C.H. and Zhang, X. 2005. Isolation and characterization of a chlorpyrifos and 3,5,6-trichloro-2-pyridinol degrading bacterium. *FEMS Microbiology Letters*, 251: 67-73.

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Βιοαποδόμηση του diazinon από τις επιφυτικές ζύμες *Rhodotorula glutinis* και *Rhodotorula rubra*

Ε.Δ. Μπεμπέλου, Γ.Ι. Βόντας, Κ.Σ. Λιαπής και Ν.Β. Ζιώγας

Περίληψη Στην παρούσα μελέτη διερευνήθηκε η ικανότητα των επιφυτικών ζυμών *Rhodotorula glutinis* και *Rhodotorula rubra* στην αποδόμηση του εντομοκτόνου diazinon σε τεχνητό θρεπτικό υλικό και καρπούς τομάτας. Όπως παρατηρήθηκε, οι ρυθμοί βιοαποδόμησης του diazinon *in vitro* ήταν ανάλογοι της αύξησης του μολύσματος των ζυμών και της συγκέντρωσης του εντομοκτόνου και επηρεάστηκαν από το χρόνο και τη θερμοκρασία επώασης των καλλιεργειών των ζυμών. Η προσθήκη γλυκόζης στο θρεπτικό υλικό καθυστέρησε τη βιοαποδομητική δράση της *Rh. glutinis* ενώ αντίθετα ενίσχυσε τη δράση της *Rh. rubra*. Η ανίχνευση και ο ποσοτικός προσδιορισμός του diazinon και του μεταβολίτη του 2-isopropyl-6-methyl-4-pyrimidinol (IMP) πραγματοποιήθηκε με επιτυχία στο χρωματογραφικό σύστημα LC-MS/MS. Η εφαρμογή ενζυμικών δοκιμών υπέδειξε την πιθανή ανάμιξη των τρανσφερασών της γλουταθειόνης στην αποδόμηση του diazinon, γεγονός το οποίο ενισχύθηκε έπειτα από την προσθήκη των συνεργιστικών παραγόντων triphenyl phosphate, diethyl maleate και piperonyl butoxide στα πειράματα βιοαποδόμησης. Η μείωση των υπολειμμάτων του diazinon σε καρπούς τομάτας επιβεβαίωσαν τα αντίστοιχα ευρήματα στις καλλιέργειες των ζυμών. Με βάση τα αποτελέσματα της μελέτης οι ζύμες *Rh. glutinis* και *Rh. rubra* θα μπορούσαν πιθανώς να χρησιμοποιηθούν με επιτυχία για την απομάκρυνση ή την αποτοξικοποίηση του diazinon στην τομάτα.

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SHORT COMMUNICATION

First record of *Sphegigaster* Spinola (Hymenoptera: Pteromalidae) in Greece

G.K. Partsinevelos[1], D.C. Kontodimas[2], A. Michaelakis[2] and P.G. Milonas[1]

Summary The genus *Sphegigaster* Spinola (Hymenoptera: Pteromalidae) is recorded for the first time in Greece. Adult endoparasitoids emerged from parasitized pupae of the asparagus miner *Hexomyza simplex* (Loew) (Diptera: Agromyzidae) which had been collected in Orestiada region in 2003.

Additional keywords: asparagus, Chalcidoidea, Diptera, distribution, parasitoid

The asparagus miner *Hexomyza simplex* (Loew) (Diptera: Agromyzidae) is an important pest of asparagus crop. Damage begins as feeding larvae destroy the sub epidermal cells of the cortex and continue by burrowing mines in the small stems and the young shoots until they complete larval development. Moreover, larval feeding causes also qualitative degradation and deformation of the shoots, acquiring a bitter taste, while becoming more susceptible to infestations by pathogens such as *Fusarium oxysporum* Schlecht (Hypocreales: Nectriaceae) and *Fusarium proliferatum* Matsushima, Nirenberg (Hypocreales: Nectriaceae) (Higley and Pedigo, 1984; Morrison *et al*., 2011).

In Greece, *H. simplex* was first recorded in the region of Orestiada in 2002 and two years later, in 2004, in the region of Kavala, Giannitsa and Aitoloakarnania (Anagnou *et al*., 2003; Anagnou *et al*., 2004). During a monitoring survey of *H. simplex* in Orestiada region in 2003 – 2004, regular stem samplings from asparagus fields and records of captures in yellow glue traps, were taken fortnightly. The samples were placed into plastic bags, transferred and examined at the Department of Entomology and Agricultural Zoology in Benaki Phytopathological Institute. Pupae of *H. simplex* were dissected from the infested stems and maintained in the laboratory at 25°C, 12:12 L:D h, until adult emergence. A number of pupae were observed to be parasitized. Specimens of parasitoids emerged were kept in Eppendorf tubes and four of them were sent for identification to the Natural History Museum of London. The specimens belonged to two different species of the genus *Sphegigaster* Spinola (Hymenoptera: Pteromalidae). Identification was done by Dr John Noyes. The specimens have been deposited in the Natural History Museum of London and in the Benaki Phytopathological Institute.

This is the first record of the genus *Sphegigaster* in Greece. Moreover, it is the first report of *Sphegigaster* associated with *H. simplex* (Noyes, 2012). There are a few records of *Sphegigaster* associated with *Hexomyza* species but not for *H. simplex* (Noyes, 2012). The genus *Sphegigaster* contains endoparasitoid wasps. It is a virtually cosmopolitan genus, represented worldwide by about 80 described species with many yet to be discovered. Due to lack of studies there are not sufficient data for the biology, ecology and morphology of this genus (Noyes, 2012 per-

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sonal communication). Thirty species of this genus have been recorded in the Palaearctic region, China (Huang, 1990), Italy (Burgio *et al*., 2000), France (Vago, 1993), United Kingdom (Graham, 1969; Herting, 1978; Thompson, 1958), Romania, Ireland, Spain (Mitroiu, 2004), Iran (Mitroiou *et al*., 2011), Turkey (Cikman *et al*., 2006; Uygun *et al*., 1995), Bulgaria (Georgiev, 2004) and Sweden (Hedqvist, 2003).

Sphegigaster species are known as parasitoids of agromyziid flies, usually of stemmining, where they lay their eggs into the pupal stages of their hosts. The general appearance of head, mesosoma, coxae is metallic green or blue. Size ranges from 1.8 to 2 mm (Heydon and LaBerge, 1988).

Literature Cited

Anagnou-Veroniki, M., Kontodimas, D.C., Chadou, Th., Tzortzopoulos, Ch. and Katsileros, A. 2003. *Hexomyza simplex* (Loew), Diptera, Agromyzidae, A new pest of Asparagus in Greece. *Agriculture-Livestock,* 4-April 2003: 22-24. (*in Greek*).

Anagnou-Veroniki, M., Kontodimas, D.C., Chadou, Th. and Tzortzopoulos, Ch. 2004. First record in Greece of the asparagus insect pest *Hexomyza simplex* (Loew): Diptera, Agromyzidae. *Annales de l' Institut Phytopathologique Benaki*, N.S., 20(1): 52-56.

Burgio, G., Lanzoni, A. and Baur, H. 2000. New records of species *Sphegigaster* Spinola (Hymenoptera: Pteromalidae) from Italy. *Boll. Ist. Ent. "G. Grandi" Univ. Bologna*, 54: 55-58.

Çikman, E., Beyarslan, A. and Civelek, H.S. 2006. Parasitoids of leafminers (Diptera: Agromyzidae) from southeast Turkey with three new records. *Turk. J. Zool*., 30: 167-173.

Georgiev, G. 2004. Two new chalcidoidea (Hymenoptera) parasitoids of the poplar twiggall fly, *Hexomyza schineri* (Gir.) (Diptera: Agromyzidae) in Bulgaria. *Silva Balcanica,* 5: 57-60.

Graham, M. 1969. *The Pteromalidae of North-Western Europe* (Hymenoptera: Chalcidoidea). Bulletin of The British Museum. London, 909 p.

Hedqvist, K.-J. 2003. Katalog över svenska Chalcidoidea. *Entomologisk Tidskrift,* 124 (1-2):117.

Heydon, S.L. and LaBerge, W.E. 1988. A review of North American Species of *Sphegigaster* North of Mexico and the Biology of their Hosts (Hymenoptera, Pteromalidae). *Journal of the Kansas Entomological Society,* 61: 258-277.

Herting, B. 1978. Neuroptera, Diptera, Siphonaptera. *A catalogue of parasites and predators of terrestrial arthropods*. *Section A. Host or Prey/Enemy*. Commonwealth Agricultural Bureaux, Commonwealth Institute of Biological Control, 5: 85-86

Higley, L.G. and Pedigo, L.P. 1984. Seedcorn maggot (Diptera: Anthomyiidae) Population Biology and Aestivation in Central Iowa. *Environmental Entomology,* 13: 1436-1442.

Huang, Da-Wei 1990. On the Chinese species of the genus *Sphegigaster* Spinola (Hymenoptera: Pteromalidae: Miscogasterinae). *Sinozoologica,* 7: 267-283.

Mitroiu, M.D. 2004. Pteromalidae (Hymenoptera: Chalcidoidea) new to Romania. *Analele Ştiinţifice ale Universităţii „Al.I.Cuza" Iaşi, s. Biologie animală,* p.86-88.

Mitroiu, M.-D., Abolhassanzadeh, F. and Madjdzadeh, S.M. 2011. New records of Pteromalidae (Hymenoptera: Chalcidoidea) from Iran, with description of a new species. *North-Western Journal of Zoology*, 7: 243-249.

Morrison W.R., Tuell J.K., Hausbeck M.K. and Szendrei Z. 2011. Constraints on asparagus production: The association of *Ophiomyia simplex* (Diptera: Agromyzidae) and *Fusarium* spp. *Crop Science*, 51: 1414-1423.

Noyes, J.S. 2012 Universal Chalcidoidea Database. World Wide Web electronic publication. http://www.nhm.ac.uk/chalcidoids. Accessed 18/11/2012.

Thompson, W.R. 1958. *A catalogue of the parasites and predators of insect pests*. *Section 2. Host parasite catalogue*, Part 5. Commonwealth Agricultural Bureaux, Commonwealth Institute of Biological Control, Ottawa, Ontario, Canada. 622 p.

Uygun, N., Polatoz, Z. and Baspinar, H. 1995. Faunistic studies on Agromyzidae (Diptera) in the south east Mediterranean rigion of Turkey. *Türkiye Entomoloji Dergisi,* 19: 123-136.

Vago, J.-L. 1993. *Eurydinota leptomera* Forster et autres chalcidiens pteromalides recoltes dans le Pas de Calais (Hymenoptera Chalcidoidea Pteromalidae). *Bulletin de la Société Entomologique du Nord de la France*, 267: 3-8.

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ΣΥΝΤΟΜΗ ΑΝΑΚΟΙΝΩΣΗ

Πρώτη καταγραφή του *Sphegigaster* Spinola (Hymenoptera: Pteromalidae) στην Ελλάδα

Γ.Κ. Παρτσινέβελος, Δ.Χ. Κοντοδήμας, Α. Μιχαηλάκης και Π.Γ. Μυλωνάς

Στην παρούσα εργασία αναφέρεται για πρώτη φορά στην Ελλάδα η παρουσία παρασιτοειδών εντόμων του γένους *Sphegigaster* Spinola (Hymenoptera: Pteromalidae) στην Ορεστιάδα το 2003. Βρέθηκαν δύο άγνωστα είδη του γένους *Sphegigaster* να παρασιτούν νύμφες του υπονομευτή του σπαραγγιού *Hexomyza simplex* (Loew) (Diptera: Agromyzidae) σε φυτά σπαραγγιού.

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Contents

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