Eur. J. Entomol. **105**: 541–544, 2008
http://www.eje.cz/scripts/viewabstract.php?abstract=1363
ISSN 1210-5759 (print), 1802-8829 (online)

The aphidophagous predator *Harmonia axyridis* (Coleoptera: Coccinellidae) in Greece, 1994–1999

DIMITRIOS C. KONTODIMAS[1], GEORGE J. STATHAS[2] and AGGELIKI F. MARTINOU[1]

[1]Benaki Phytopathological Institute, St. Delta 8, 145 61 Kifissia, Greece, e-mail: d.kontodimas@bpi.gr
[2]Technological Educational Institute of Kalamata, 24 100 Antikalamos, Greece

Key words. *Harmonia axyridis*, invasive ladybirds, native ladybirds, biocontrol agents, Greece, overwintering, *Aphis fabae*, *Toxoptera aurantii*, *Aphis gossypii*, *Aphis spiraecola*

Abstract. During the years 1994–1999, several hundreds of thousands of *H. axyridis* adults were released at various cultivations infested by aphids (citrus, vegetable and bean crops, maize etc.) and on ornamental plants in urban settings in central and southern Greece (mainly Attica and Peloponessos region) as well as on several islands. Between 1995–1999, spring sampling was conducted in some areas, just prior to releases, in order to determine if *H. axyridis* overwintered in the field. No presence of *H. axyridis* was recorded in any of the orchards where the predator had been released save in spring of 1998 and 1999 when small colonies (<50 individuals) of overwintered *H. axyridis* adults were observed in the Attica region. The above results suggest an inability of released *H. axyridis* populations to establish in Greece, although in some areas this species became an important biocontrol agent during the growing season.

INTRODUCTION

Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae) is a predator used as a biocontrol agent against a wide range of soft bodied insects (aphids and scales) (Katsoyannos, 1996, Pervez & Omkar, 2006). An expectation has recently been expressed that, because of some life-history parameters, this species may become a serious competitor of *Coccinella septempunctata* in particular habitats (Hodek & Michaud, 2008). However, its effectiveness as a biological control agent has been accompanied by an apparently adverse impact on other aphidophagous coccinellids (Adriaens et al., 2003, Koch & Galvan, 2008). In addition, *Harmonia axyridis* has caused problems in fruit production (Koch, 2003) and as a household nuisance as it can induce allergic rhino-conjunctivitis in some people (Koch et al., 2006, Majerus et al., 2006, Pervez & Omkar, 2006).

During spring 1994, approximately 620 adults of *H. axyridis* were released in four citrus growing areas of Greece (Marathon-Attica, Campos-Chios, Leonidion-Arcadia, Chania-Crete) on orange, mandarin and sour orange infested by aphids [*Toxoptera aurantii*, *Aphis spiraecola* and *A. gossypii* (Hemiptera: Aphididae)] (Katsoyannos et al., 1997). Over the next two months, *H. axyridis* proved to be an effective biocontrol agent against the afore-mentioned aphids in two locations (Campos-Chios and Leonidion-Arcadia). In outdoor cages *H. axyridis* overwintered in the adult stage (~30% of the adults of the 4th generation survived), and under conditions of a continuous surplus of aphids completed four overlapping generations annually and did not diapause during summer. In Greece, aphid populations are scarce in summer (Katsoyannos et al., 1997).

The purpose of the current study is to present the status of *H. axyridis* in Greece during the years 1995–1999, when increased numbers of the predator were released over more extensive areas.

MATERIAL AND METHODS

Harmonia axyridis rearing and releases

After the first releases of *H. axyridis* in Attica, Chios, Arcadia and Crete in 1994 (Katsoyannos et al., 1997) the rearing of the predator and release programme was continued by the laboratory of Biological Control of Benaki Phytopathological Institute. *H. axyridis* was reared on *Aphis fabae* (Hemiptera: Aphididae) on *Vicia faba* as well as on *Dysaphis crataegi* (Hemiptera: Aphididae) on squash (*Cucurbita pepo*, *C. maxima* and *C. moschata*) in controlled conditions (temperature: 25 ± 1°C; relative humidity: 65 ± 2%; photoperiod: 16L : 8D). From 1995 to 1999 more than 100.000 insectary-reared adults of *H. axyridis* were released in various cultivations (citrus, vegetables, beans, maize) infested by aphids, or in urban areas on ornamental plants, in central and southern Greece (mainly Attica and Peloponessos) and the islands of Chios, Euvoia and Crete (Table 1). Moreover, during the years 1997 through 2002, several hundreds of thousands of imported *H. axyridis* individuals were released by private companies mainly in urban areas of the mainland (Attica, Peloponessos) and on the islands of Corfu, Rhodos and Crete. Afterwards, releases of the predator were terminated due to the high market price of the predator and not due to legal prohibitions.

Sampling method

During 1995–1999, samplings were conducted every spring just before releases were made, in thirteen areas (I, II, III, IV, VII, XI, XV, XVII, XVIII, XIX, XX, XXII, and XXVI) in order to determine if any *H. axyridis* had overwintered in the field. A second sampling was conducted a month later in order to estimate the population size of the exotic predator shortly after the release. Twelve samples were collected from each location on each sampling date. In arboric cultivations and ornamental trees, branch beating over a 1 m^2 canvas area was applied, whereas in other cultivations (vegetables, beans, maize, ornamental shrubs) a 20 cm-length shoot of the plant was used as a sampling unit.

TABLE 1. Releases and records of *H. axyridis* during 1995–1999 in Greece.

Area	Plant	Aphid pest	Released *H. axyridis* adults					Recorded *H. axyridis* larvae/adults						
			1995	1996	1997	1998	1999	1995 after release	1996 after release	1997 after release	1998 before release	1998 after release	1999 before release	1999 after release
I.*	orange, lemon, clementine	*Toxoptera aurantii, Aphis spiraecola, A. gossypii*	600	640	1240	4100	2240	29/16	9/6			83/32		112/44
II.	faba beans, rose, almond trees, lettuce	*Aphis fabae, Macrosiphum rosae, Hyalopterus pruni, Nasonovia ribis-nigri*	660	740	1440	1720	1800	18/7	9/6	145/65	0/36	89/46	0/41	27/28
III.	sour orange, rose	*A. spiraecola, A. gossypii, M. rosae*	600	1200	1400	420	180	11/4	20/15	93/52				
IV.	squash, cucumber, lucerne	*Dysaphis crataegi, Myzus persicae, Acyrthosiphon pisum*			2400	3800	4800			79/64		23/32		
V.	moschato squash	*D. crataegi*		840	2000	400								
VI.	orange	*T. aurantii, A. spiraecola, A. gossypii*		1000	450	2260	800							
VII.*	mandarin	*T. aurantii, A. spiraecola, A. gossypii*	320	400	640	720	800	4/3		2/3		15/10		1/4
VIII.*	orange				560	240								
IX.	orange					600	260							
X.	lemon	*A. spiraecola, A. gossypii*		2000	560		1800							
XI.	clementine		360	550		1640		7/8	21/14			88/57		
XII.	lemon	*T. aurantii, A. spiraecola, A. gossypii*	400	820	1260									
XIII.	orange			800	1260	1200								
XIV.	orange				640	1420								
XV.	orange					800	800			6/8		24/21		
XVI.	apricot tree	*Myzus persicae*			950	880								
XVII.*	sour orange, orange			1300	1800	1450	3100		4/5	9/14		20/13		9/
XVIII.	orange			1600	2000	3000	2000		8/3	6/6		99/40		25/20
XIX.	orange			720	400	560			5/7	2		2/1		
XX.	orange				480	520				2/2		4/6		
XXI.	lemon, orange	*T. aurantii, A. spiraecola, A. gossypii*		1540	2120	2400	1220							
XXII.	orange			800	700	1000			10/4	8/4		3/6		
XXIII.	orange			800	700	850	850							
XXIV.	orange		260	650			440							
XXV.	sour orange		240	580	540	640								
XXVI.	orange		340			880	720	6/1				13/16		30/29
XXVII.	orange		420		700		540							
XXVIII.	maize	*Rhopalosiphum padi*				840								

LOCALITIES: Central Greece: I. – Marathon Attica, II. – Varympompi Attica, III. – Glyfada Attica, IV. – Aliartos Voiotia, V. – Agrinio Aitoloakarnania, VI. – Filothei Arta. Insular Greece: VII. – Campos Chios, VIII. – Chania Crete, IX. – Fodele Crete, X. – Karystos Euvoia. Peloponnesus: XI. – Galatas Troizinia, XII. – Kiato Korinthos, XII. – Vrachati Korinthos, XIV. – Xylokastro Korinthos, XV. – Zeygolatio Korinthos, XVI. – Zeygolatio Korinthos, XVII. – Leonidion Arcadia, XVIII. – Kalamata Messinia, XIX. – Kyparissia Messinia, XX. – Skala Lakonia, XXI. – Elaionas Achaia, XXII. – Akrata Achaia, XXIII. – Nea Kios Argolida, XXIV. – Dalamanara Argolida, XXV. – Nayplion Argolida, XXVI. – Gastouni Ilia, XXVII. – Lexaina Ilia, XXVIII. – Stavrodromi Ilia.

PLANTS: Orange – *Citrus sinensis*, lemon – *C. limon*, clementine – *C. reticulata*, sour orange: *C. aurantium*, mandarin: *C. delicioca*, faba beans: *Vicia faba*, rose: *Rosa* sp., almond trees: *Pyrus amygdalus*, lettuce: *Lactuca sativa*, squash: *Cucurbita pepo*, moschato squash: *C. moschata*, cucumber: *Cucumis sativus*, lucerne: *Medicago sativa*, apricot tree: *Prunus armeniaca*, maize: *Zea mays*.

* Release-locations of *H. axyridis* adults in 1994 (Katsoyannos et al., 1997).

RESULTS AND DISCUSSION

A month after the 1994 releases, *H. axyridis* became an important biocontrol agent at the Campos-Chios and the Leonidion-Arcadia locations (Katsoyannos et al., 1997). In spring 1995, however, the presence of *H. axyridis* was not recorded in any orchards.

In the following years, despite continued massive releases of *H. axyridis*, only in spring 1998 and spring 1999 were small colonies (< 50 individuals) of overwintered *H. axyridis* adults found in the Attica region, although in some of the locations the released predator became abundant during the season (Table 1). The above result shows that although *H. axyridis* has some ability to overwinter in Greece, this has occurred only rarely and in very low numbers of adults.

Although the ecoclimatic indices for Greece have been predicted by Poutsma et al. (2008) as appropriate for *H. axyridis* establishment, the predator seems so far to have failed to establish despite continued releases. However, absence of evidence does not necessarily mean evidence of absence. In regions of the Western Hemisphere (USA, Mexico) where the climatic conditions are similar to those in Greece, *H. axyridis* was not established during the intentional releases but later in time and in a long distance from any of the initial release sites (Koch et al., 2006; Koch & Galvan, 2008).

It is noticeable that in Greece the overwintering *H. axyridis* adults were observed only at the location of Varympompi Attica, the only area where the exotic predator was released in *Vicia faba* fields infested by *A. fabae*, the prey that was also used for the laboratory rearing. In contrast, in the other places (almost all the field releases of *H. axyridis* occurred in citrus cultivations where *Toxoptera aurantii*, *Aphis spiraecola* and *A. gossypii* were the aphid pests) no overwintering *H. axyridis* individuals were recorded. However given the known polyphagous habits of the predator (Lucas et al., 1997, 2002; Michaud, 2001, 2002, 2004) as well as the evidence from our study that the predator became an important biocontrol agent during the season, the failure of the establishment of the exotic predator could not be attributed to prey identity.

A similar case of a failure in establishment of *H. axyridis* was observed by Soares et al. (2008) at Santa Maria of the Azores islands where more than one hundred thousand third instar larvae were released into several citrus orchards. Although after the releases there were records of individuals that dispersed and fed on citrus, apple trees and bean plants, the exotic predator failed to establish (Soares et al., 2008). According to this study, the released populations failed to establish due to ecological factors such as the maladaptation to the local conditions and functional diversity saturation. It is likely that these factors also apply for the failure of *H. axyridis*' establishment in Greece.

In any case, given the current adverse impact of *H. axyridis* in other European countries and North-America, (Koch, 2003; Koch et al., 2006; Roy et al., 2006; Babendreier, 2007; Eschen et al., 2007; Brown et al., 2008a, b; Koch & Galvan, 2008) it would not be wise to continue field releases of this insect. This has been recently concluded by scientists working on risk assessment of biological control agents (van Lenteren et al., 2008; Roy & Wajnberg, 2008). Finally, scientists should be alerted as *H. axyridis* has shown a lag phase in its establishment (Soares et al., 2008) and in the future *H. axyridis* might resurface elsewhere in Greece.

REFERENCES

Adriaens T., Branquart E. & Maes D. 2003: The multicoloured Asian ladybird Harmonia axyridis Pallas (Coleoptera: Coccinellidae), a threat for native aphid predators in Belgium? *Belg. J. Zool.* **133**: 195–196.

Babendreier D. 2007: Pros and cons of biological control. In Nentwig W. (ed.) *Biological Invasions*. Springer, Berlin, pp. 403–418.

Brown P.M.J., Adriaens T., Bathon H., Cuppen J., Goldarazena A., Hägg T., Kenis M., Klausnitzer B.E.M., Kovar I., Loomans A.J.M., Majerus M.E.N., Nedvěd O., Pedersen J., Rabitsch W., Roy H.E., Ternois V., Zakharov I.A. & Roy D.B. 2008a: Harmonia axyridis in Europe: spread and distribution of a non-native coccinellid. *BioControl* **53**: 5–21.

Brown P.M.J., Roy H.E., Rothery P., Roy D.B., Ware R.L. & Majerus M.E.N. 2008b: Harmonia axyridis in Great Britain: analysis of the spread and distribution of a non-native coccinellid. *BioControl* **53**: 55–67.

Eschen R., Babendreier D., Nauer S., Bigler F. & Kenis M. 2007: Surveys for ladybirds (Coleoptera: Coccinellidae) in Switzerland and confirmation of the presence of the invasive ladybird species, Harmonia axyridis Pallas. *Mit. Schweiz. Entomol. Ges.* **80**: 7–14.

Hodek I. & Michaud J.P. 2008: Why is Coccinella septempunctata so successful? *Eur. J. Entomol.* **105**: 1–12.

Katsoyannos P. 1996: *Integrated Insect Pest Management for Citrus in Northern Mediterranean Countries.* Benaki Phytopathological Institute, Athens, 110 pp.

Katsoyannos P., Kontodimas D.C., Stathas G.J. & Tsartsalis C.T. 1997: The establishment of Harmonia axyridis (Coleoptera: Coccinellidae) on citrus and some data on its phenology in Greece. *Phytoparasitica* **25**(3): 183–191.

Koch R.L. 2003: The multicolored Asian lady beetle, Harmonia axyridis: A review of its biology, uses in biological control, and non-target impacts. *J. Insect Sci.* **3**(32): 16.

Koch R.L. &. Galvan T.L. 2008: Bad side of a good beetle: the North American experience with Harmonia axyridis. *BioControl* **53**: 23–35.

Koch R.L., Venette R.C. & Hutchison W.D. 2006: Invasions by Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae) in the Western Hemisphere: implications for South America. *Neotrop. Entomol.* **35**: 421–434.

Lenteren van J.C., Loomans A.J.M., Babendreier D. & Bigler F. 2008: Harmonia axyridis: an environmental risk assessment for Northwest Europe. *BioControl* **53**: 37–54

Lucas E., Coderre D. & Vincent C. 1997: Voracity and feeding preferences of two aphidophagous coccinellids on Aphis citricola and Tetranychus urticae. *Entomol. Exp. Appl.* **85**: 151–159.

Lucas E., Gagné I. & Coderre D. 2002: Impact of the arrival of Harmonia axyridis on adults of Coccinella septempunctata and Coleomegilla maculata (Coleoptera: Coccinellidae). *Eur. J. Entomol.* **99**: 457–463.

Majerus M., Strawson V. & Roy H. 2006: The potential impacts of the arrival of the harlequin ladybird, Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae), in Britain. *Ecol. Entomol.* **31**: 207–215.

Michaud J.P. 2001: Numerical response of Olla v-nigrum (Coleoptera: Coccinellidae) to infestations of Asian citrus psyllid, (Hemiptera: Psyllidae) in Florida. *Fla Entomol.* **84**: 608–612.

Michaud J.P. 2002: Biological control of Asian citrus psyllid, Diaphorina citri (Hemiptera: Psyllidae) in Florida: a preliminary report. *Entomol. News* **113**: 216–222.

Michaud J.P. 2004: Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. *Biol. Control* **29**: 260–269.

Pervez A. & Omkar 2006: Ecology and biological control application of multicoloured asian ladybird, Harmonia axyridis: a review. *Biocontr. Sci. Technol.* **16**: 111–128.

Poutsma J., Loomans A.J.M., Aukema B. & Heijerman T. 2008: Predicting the potential geographical distribution of the harlequin ladybird, Harmonia axyridis, using the CLIMEX model. *BioControl* **53**: 103–125.

Roy H.E., Brown P.M.J. & Majerus M.E.N. 2006: Harmonia axyridis: A successful biocontrol agent or an invasive threat? In Eilenberg J. & Hokkanen H. (eds): *An Ecological and Societal Approach to Biological Control*. Kluwer, Dordrecht, 295–309.

Roy H. & Wajnberg E. 2008: From biological control to invasion: the ladybird Harmonia axyridis as a model species. *BioControl* **53**: 1–4.

Soares A.O., Borges I., Borges P.A.V., Labrie G. & Lucas E. 2008: Harmonia axyridis: What will stop the invader? *BioControl* **53**: 127–145.

Received September 24, 2007; revised and accepted January 18, 2008