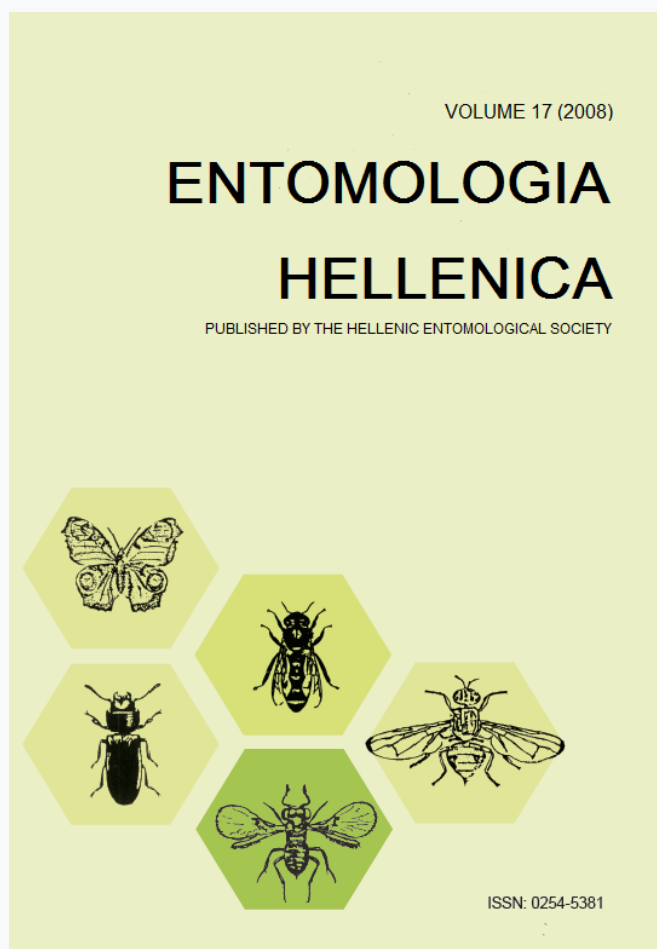


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The status of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in Greece: A case of an exotic predator that failed to establish?

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ABSTRACT

During 1994 -1999 several hundred thousands of *Harmonia axyridis* adults were released at various cultivations infested by aphids (citrus, vegetable and bean crops, maize, *etc.*) or in urban places on ornamental plants in central and southern Greece (mainly Attica and Peloponnessos region) as well as on several islands. During 1995-2007, samplings were conducted in some areas, in spring just before any new releases, in order to determine if *H. axyridis* overwintered in the field. In spring 1995 (the year that followed the first releases) as well in spring 1996-97 and 2000-07, no presence of *H. axyridis* was recorded in any of the orchards where the predator had been released. Only in spring 1998 and 1999 small colonies (<50 individuals) of overwintered *H. axyridis* adults were observed in Attica region. The above results indicate an inability of the *H. axyridis* released populations to establish in Greece, although in some areas this species became an important biocontrol agent during the season. In addition, some data collected regarding the composition of native coccinellid species pre- and post- release of *H. axyridis* could be an indication of no adverse effects imposed by *H. axyridis* on the native coccinellid fauna in Greece. Despite that, any re-initiation of releases of *H. axyridis* would not be wise because of its multiple negative effects observed in other countries of Europe and the USA. Contrary to this, the application of a survey and monitoring programme that will allow us to be prepared in case of a possible invasion of *H. axyridis* from northern Europe is suggested.

Introduction

Native to Asia, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) is considered an invasive alien ladybird in Europe and North America, where it was widely introduced as a biological control agent of aphids and coccids (Katsoyannos 1996, Pervez & Omkar 2006). In Europe, *H. axyridis* was sold by various biological control companies since 1995 in France,

Belgium and the Netherlands, and was also intentionally released in at least nine other countries. It is now regarded as established in thirteen European countries (Brown et al. 2008a). In several European countries (Belgium, Netherlands, the United Kingdom, France, the Czech Republic and Switzerland) several working groups are mapping Coccinellidae species and intensive monitoring of *H. axyridis* is taking place, while, despite releases of *H.*

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axyridis in Portugal, Spain and Greece, there is little evidence of its establishment in southern Europe (Brown et al. 2008a).

Its effectiveness as a biological control agent has been accompanied by the adverse impact on other aphidophagous coccinellids (Adriaens et al. 2003, Koch & Galvan 2008). An expectation has recently been expressed that, because of some life-history parameters, this species may become a serious competitor of *Coccinella septempunctata* (Hodek & Michaud 2008). In addition, *H. axyridis* caused problems to fruit production (Koch 2003) and as a household nuisance it induces allergic rhino-conjunctivitis to humans (Koch et al. 2006, Majerus et al. 2006, and 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 in 1994, *H. axyridis* proved to be an effective biocontrol agent against the previously mentioned aphids in two locations (Campos-Chios and Leonidion-Arcadia). In outdoor cages *H. axyridis* overwintered at the adult stage (~30% of the adults of the 4th generation survived), and under conditions of continuous surplus of aphids it completed four overlapping generations annually and it did not diapause during summer. In the field, aphid populations are scarce in summer in Greece (Katsoyannos et al. 1997).

The purpose of the current study was to present the status of *H. axyridis* in Greece during the years 1995-1999, when the releases of the predator were continued with increased numbers in expanded areas. Additionally, data on other beneficial

coccinellid populations sampled at thirteen locations for the years 1995 to 2007, are also presented.

Materials and Methods

Harmonia axyridis rearing and releases

After the first releases (1994) of *H. axyridis* in Attica, Chios, Arcadia and Crete (Katsoyannos et al. 1997), the laboratory of Biological Control of the Benaki Phytopathological Institute (BPI) continued the rearing of the predator and its releases until spring 1999. *H. axyridis* originally obtained from France (Katsoyannos et al. 1997) was reared mainly on *Aphis fabae* (Hemiptera: Aphididae) on *Vicia faba* (Stathas et al. 2001) as well as occasionally on *Dysaphis crataegi* (Hemiptera: Aphididae) on squash (*Cucurbita pepo*, *C. maxima* and *C. moschata*) in controlled conditions at $25\pm1^{\circ}\text{C}$, $65\pm2\%$ R.H. and under a photoperiod of 16:8 (L:D). During 1995-1999 more than 100.000 insectary-reared adults of *H. axyridis* were released in various cultivations (citrus, vegetables, beans and maize) infested by aphids or in urban areas on ornamental plants, in central and southern Greece (mainly Attica and Peloponessos) and the islands: Chios, Euvoia and Crete (Table 1). Moreover, during 1997-2002 several hundreds of thousands imported *H. axyridis* individuals were released by private companies mainly in urban areas of the mainland (Attica, Peloponessos) and in the islands of Corfu, Rhodos and Crete, although no intentional releases were made by (BPI) after 1999.

Sampling method

During 1995-1999, samplings were conducted every spring just before any releases, in thirteen locations (Table 2) in order to determine if *H. axyridis* overwintered in the field. A second

sampling was conducted a month later in order to calculate the population size of the exotic predator shortly after the release.

Twelve samples were collected from each crop at every location. In arboric cultivations or in ornamental trees, branch

TABLE 1. Releases of *H. axyridis* by the laboratory of Biological Control of Benaki Phytopathological Institute, during 1995-1999.

District	Area	Plant	Aphid pest	Released <i>H. axyridis</i> adults				
				1995	1996	1997	1998	1999
Central Greece	I. Marathon Attica*	orange (<i>Citrus sinensis</i>)	<i>Toxoptera aurantii</i> <i>Aphis spiraecola</i> <i>A. gossypii</i>	350	640		1200	800
	Marathon Attica	lemon (<i>Citrus limon</i>)				640	700	640
	Marathon Attica	clementine (<i>Citrus reticulata</i>)		250		600	2200	800
	II. Varympopi Attica	faba beans (<i>Vicia faba</i>)	<i>Aphis fabae</i>	400	740	400	800	
	Varympopi Attica	rose (<i>Rosa</i> sp.)	<i>Macrosiphum rosae</i>	260		200	320	
	Varympopi Attica	Almond trees (<i>Pyrus amygdalus</i>)	<i>Hyalopterus pruni</i>			840		1400
	Varympopi Attica	lettuce (<i>Lactuca sativa</i>)	<i>Nasonobia ribis-nigri</i>				600	400
	III. Glyfada Attica	sour orange (<i>Citrus aurantium</i>)	<i>Aphis spiraecola</i> <i>A. gossypii</i>	600	1200	1400		
	Glyfada Attica	rose (<i>Rosa</i> sp.)	<i>Macrosiphum rosae</i>			240	420	180
	IV. Aliartos Voiotia	squash (<i>Cucurbita pepo</i>)	<i>Dysaphis crataegi</i>			1200	3000	
	Aliartos Voiotia	cucumber (<i>Cucumis sativus</i>)	<i>Myzus persicae</i>				800	2400
	Aliartos Voiotia	44lucerne (<i>Medicago sativa</i>)	<i>Acyrtosiphon pisum</i>			1200		2400
	Agrinio Aitolookarnania	squash (<i>Cucurbita moschata</i>)	<i>Dysaphis crataegi</i>		840	2000	400	
	Filothei Arta	orange (<i>Citrus sinensis</i>)	<i>Toxoptera aurantii</i> <i>Aphis spiraecola</i> <i>A. gossypii</i>		1000	450	1400	
	Filothei Arta	lemon (<i>Citrus limon</i>)					860	800
Insular Greece	V. Campos Chios*	mandarin (<i>Citrus delicioca</i>)	<i>Toxoptera aurantii</i> <i>Aphis spiraecola</i> <i>A. gossypii</i>	320	400	640	720	800
	Chania Crete*	orange (<i>Citrus sinensis</i>)				560	240	
	Fodele Crete	orange (<i>Citrus sinensis</i>)					600	260
	Karystos Euvoia	lemon (<i>Citrus limon</i>)			2000	560		1800

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

TABLE 1 (continued). Releases of *H. axyridis* by the laboratory of Biological Control of Benaki Phytopathological Institute, during 1995-1999.

District	Area	Plant	Aphid pest	Released <i>H. axyridis</i> adults				
				1995	1996	1997	1998	1999
Pelopon- nesos	VI. Galatas	clementine		360	550		1640	
	Troizinia	(<i>Citrus reticulata</i>)	<i>Toxoptera aurantii</i>					
	Kiato	lemon	<i>Aphis spiraeicola</i>	400	820	1260		
	Korinthos	(<i>Citrus limon</i>)	<i>A. gossypii</i>					
	Vrachati	orange			800	1260	1200	
	Korinthos	(<i>Citrus sinensis</i>)						
	Xylokastro	orange	<i>Aphis fabae</i>			640	1420	
	Korinthos	(<i>Citrus sinensis</i>)						
	Zeygolatío	orange	<i>Macrosiphum rosae</i>				800	800
	Korinthos	(<i>Citrus sinensis</i>)						
	VII. Zeygolatío	apricot tree	<i>Myzus persicae</i>			950	880	
	Korinthos	(<i>Prunus armeniaca</i>)						
	VIII. Leonidion	sour orange	<i>Toxoptera aurantii</i>					
	Arcadia*	(<i>Citrus aurantium</i>)	<i>Aphis spiraeicola</i>		700	800	650	1100
			<i>A. gossypii</i>					
	Leonidion Arcadia	orange	<i>Aphis spiraeicola</i>		600	1000	800	2000
		(<i>Citrus sinensis</i>)	<i>A. gossypii</i>					
	IX. Kalamata	orange	<i>Macrosiphum rosae</i>		1600	2000	3000	2000
	Messinia	(<i>Citrus sinensis</i>)						
	X. Kyparissia	orange	<i>Dysaphis crataegi</i>		720	400	560	
	Messinia	(<i>Citrus sinensis</i>)						
	XI. Skala	orange	<i>Myzus persicae</i>			480	520	
	Lakonia	(<i>Citrus sinensis</i>)						
	Elaionas	lemon	<i>Acyrtosiphon</i>		640	520	1200	800
	Achaia	(<i>Citrus limon</i>)	<i>pisum</i>					
	Elaionas	orange	<i>Dysaphis crataegi</i>		900	1600	1200	420
	Achaia	(<i>Citrus sinensis</i>)						
	XII. Akrata	orange	<i>Toxoptera aurantii</i>		800	700	1000	
	Achaia	(<i>Citrus sinensis</i>)	<i>Aphis spiraeicola</i>					
	Nea Kios	orange	<i>A. gossypii</i>		800	700	850	850
	Argolida	(<i>Citrus sinensis</i>)						
	Dalamanara	orange		260	650			440
	Argolida	(<i>Citrus sinensis</i>)						
	Nayplion	sour orange		240	580	540	640	
	Argolida	(<i>Citrus aurantium</i>)						
	XIII. Gastouni	orange		340			880	720
	Ilia	(<i>Citrus sinensis</i>)						
	Lexaina	orange	<i>Aphis spiraeicola</i>	420		700		540
	Ilia	(<i>Citrus sinensis</i>)	<i>A. gossypii</i>					

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

beating over a 1m² canvas area was applied, while in other cultivations (vegetables, beans, maize, ornamental shrubs) the 20cm-length shoot was used as sampling unit. During 2000-2007, sampling for coccinellid species was continued in the above locations, every spring when aphid infestations occurred.

Results and Discussion

One month after the 1994 releases, *H. axyridis* was an important biocontrol agent at the Campos-Chios and the Leonidion-Arcadia locations (Katsoyannos et al. 1997). However, in the spring 1995 no presence of *H. axyridis* was recorded in any of the orchards (Table 2).

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

The observed failure in establishment of *H. axyridis* at the release locations in Greece could possibly be attributed to the maladaptation of *H. axyridis* to switching of prey between the rearing conditions prior to the release and the prey encountered in the field. *H. axyridis* was reared on *Aphis fabae* on *V. faba* seedlings, while almost all the field releases of *H. axyridis* occurred in citrus cultivations where *T. aurantii*, *A. spiraeicola* and *A. gossypii* were the aphid pests. This hypothesis is further enhanced by the fact that overwintering *H. axyridis* adults were

observed only at the location of Varympompi Attica, the only area where *H. axyridis* was released in *V. faba* fields infested by *A. fabae*. A similar case was observed by Soares et al. (2008) at Santa Maria of the Azores islands where more than one hundred thousand *H. axyridis* (third larval stage) 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).

Although the ecoclimatic indices for Greece as predicted by Poutsma et al. (2008) are appropriate for *H. axyridis* establishment in Greece, the predators seem to have failed to establish in spite of continued releases. In contrast, in the regions of USA where the climatic conditions are similar to those in Greece, *H. axyridis* established successfully (Koch and Galvan 2008). Data collected in the thirteen locations mentioned herein indicate the failure of the establishment of *H. axyridis* in Greece at least to date. Despite the fact that the efficiency of *H. axyridis* in biocontrol in Greece appears at first to overwhelm its negative secondary effects, further releases should not be organized. Especially since predictive modeling based on ecoclimatic indices shows that the climate all over Greece is suitable for the establishment of *H. axyridis* (Poutsma et al. 2008). Empirically, it is also known that the climatic conditions in northern Greece resemble mostly the conditions of mainland Europe where the predator is widely established. However, to date no surveys have been undertaken in the northern part of Greece.

According to Table 2 it seems that the native coccinellid species are not affected by *H. axyridis* however more elaborate

TABLE 2. Composition of the coccinellid populations sampled at the wider area of thirteen localities before and after *H. axyridis* releases (sums of counted adults and larvae). After 1999 no releases of *H. axyridis* were undertaken by BPI or private companies.

Location	1995		1996		1997		1998		1999		2000	2001	2002	2003	2004	2005	2006	2007
	before release	after release	before release	after release	before release	after release	before release	after release	before release	after release								
I. Marathon Attica	A: 32 B: 16	A: 64 B: 40 <i>H.a.</i> : 45	A: 85 B: 33	A: 160 B: 25 <i>H.a.</i> : 15	no infestation no release		A: 25 B: 43	A: 60 B: 45 <i>H.a.</i> : 115	A: 15 B: 23	A: 20 B: 25 <i>H.a.</i> : 156	A: 37 B: 112	A: 78 B: 42	A: 18 B: 12	no infesta- tion	A: 6	no infesta- tion	A: 28 B: 4	A: 2 B: 22
II. Varympopi Attica	A: 25	A: 14 <i>H.a.</i> : 25	A: 35	A: 16 <i>H.a.</i> : 15	A: 8	A: 16 <i>H.a.</i> : 210	A: 4 B: 12 <i>H.a.</i> : 36	A: 24 B: 5 <i>H.a.</i> : 135	A: 21 B: 11 <i>H.a.</i> : 41	A: 14 <i>H.a.</i> : 55	no infesta- tion	no infesta- tion	A: 22	A: 2	no infesta- tion	no infesta- tion	A: 8 B: 5	no infesta- tion
III. Glyfada Attica	A: 11 B: 31	A: 14 B: 10 <i>H.a.</i> : 15	A: 5 B: 33	A: 7 B: 27 <i>H.a.</i> : 35	A: 26 B: 43	A: 17 B: 30 <i>H.a.</i> : 145	no infestation no release		no infestation no release		A: 12 B: 21 C: 3	A: 2 B: 11	A: 2	no infesta- tion	B: 2 C: 3	no infesta- tion	B: 4	A: 3 B: 11
IV. Aliartos Voiotia	no infestation no release		no infestation no release		A: 64 C: 5	A: 47 <i>H.a.</i> : 143	A: 131	A: 33 <i>H.a.</i> : 55	no infestation no release		A: 13	A: 2	A: 23	A: 62	no infesta- tion	no infesta- tion	no infest- a-tion	no infesta- tion
V. Campos Chios	A: 9 B: 11	A: 14 B: 2 <i>H.a.</i> : 7	A: 7 B: 7	A: 3 B: 5	A: 3 B: 13	A: 9 B: 8 <i>H.a.</i> : 5	B: 5	A: 2 B: 11 <i>H.a.</i> : 25	A: 10 B: 6	A: 3 <i>H.a.</i> : 5	A: 9 B: 11	B: 3	no infesta- tion	A: 4 B: 4	A: 4 B: 12	no infestati- on	A: 5 B: 21	A: 2
VI. Galatas Troizinia	A: 11 B: 31	A: 14 B: 10 <i>H.a.</i> : 15	A: 5 B: 33	A: 7 B: 27 <i>H.a.</i> : 35	no infestation no release		A: 26 B: 43	A: 17 B: 31, C: 2 <i>H.a.</i> : 145	no infestation no release		A: 6 B: 21 C: 5	A: 1 B: 9 C: 3	no infesta- tion	no infesta- tion	no infesta- tion	B: 2 C: 1	no infest- a-tion	no infesta- tion
VII. Zeygolatío Korinthos	no infestation no release		no infestation no release		A: 3 B: 11	A: 5 <i>H.a.</i> : 14	A: 5 B: 3	A: 6 B: 7 <i>H.a.</i> : 45	no infestation no release		no infesta- tion	no infesta- tion	A: 9 B: 5	no infesta- tion	no infesta- tion	no infesta- tion	no infest- a-tion	no infesta- tion
VIII. Leonidion Arcadia	no infestation no release		A: 4 B: 17	A: 8 B: 5 <i>H.a.</i> : 9	A: 5 B: 13 C: 1	A: 16 B: 2 <i>H.a.</i> : 23	A: 1 B: 7 C: 3	A: 7 B: 1, C: 7 <i>H.a.</i> : 33	A: 13 B: 21 C: 5	A: 17 B: 10 <i>H.a.</i> : 13	A: 2 B: 5 C: 1	A: 1 B: 2 C: 3	B: 1 C: 2	A: 7 B: 2 C: 4	no infesta- tion	no infesta- tion	A: 11 B: 5 C: 5	A: 3 B: 1 C: 2

data collection on coccinellid species, composition and abundances that would be based on a more extensive sampling regime would have enabled us to ascertain this case more accurately. In addition, a more intensive sampling scheme could have provided some evidence on the effects of *H. axyridis* on other natural enemies of soft-bodied insects such as predators and parasitoids while to date investigating the effect of *H. axyridis* on other native to Greece natural enemies has been neglected.

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 with field releases of this insect. This has recently been concluded also by scientists working on risk assessment of biological control agents (van Lenteren et al. 2008, Roy & Wajnberg 2008).

It is predicted that the spread and increase of *H. axyridis* within Europe will continue and that it will become one of the most widely distributed coccinellids of the continent (Brown et al. 2008). We believe that it is necessary to develop and apply a survey and monitoring ladybird scheme similar to those currently applied in European countries and the USA in order to obtain a database on the distribution and abundance of all coccinellid predators and especially *H. axyridis*. This way it will be possible to prevent the establishment of this exotic predator. There is a great necessity to increase public awareness on issues such as *H. axyridis* and invasive species and for this reason the collaboration between entomologists, government officials, farmers, conservation organisations and park managers is essential.

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

Δεδομένα σχετικά με το *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) στην Ελλάδα. Μια περίπτωση εξωτικού αρπακτικού που απέτυχε να εγκατασταθεί;

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ΠΕΡΙΛΗΨΗ

Κατά τη διάρκεια 1994-1999 εκατοντάδες χιλιάδες ακμαία του *Harmonia axyridis* εξαπολύθηκαν σε διάφορες καλλιέργειες προσβεβλημένες από αφίδες (εσπεριδοειδή, φασόλια, καλαμπόκι κ.α.) καθώς και σε διάφορα καλλωπιστικά φυτά σε αστικό περιβάλλον, στην κεντρική και νότια Ελλάδα (κυρίως στην Αττική και στην Πελοπόννησο) καθώς και σε διάφορα νησιά. Από το 1995-2007 πραγματοποιήθηκαν δειγματοληψίες σε διάφορες περιοχές, κάθε άνοιξη πριν από τις νέες εξαπολύσεις, με σκοπό να διαπιστωθεί εάν το *H. axyridis* κατόρθωσε να διαχειμάσει. Την άνοιξη του 1995 (δηλαδή την άνοιξη μετά τις πρώτες εξαπολύσεις) καθώς και την άνοιξη των ετών 1996-1997 και 2000-2007, δεν καταγράφηκε το *H. axyridis* σε καμία από τις περιοχές όπου είχε εξαπολυθεί. Μόνο την άνοιξη των ετών 1998 και 1999 μικρές αποικίες (<50 άτομα) βρέθηκαν να διαχειμάζουν σε περιοχή της Αττικής. Τα παραπάνω αποτελέσματα δείχνουν ότι οι πληθυσμοί του *H. axyridis* που εξαπολύθηκαν δεν κατόρθωσαν να εγκατασταθούν στην Ελλάδα, παρόλο που το εξωτικό αυτό αρπακτικό αποτέλεσε σημαντικό παράγοντα καταπολέμησης στις περιοχές που εξαπολύθηκε. Επίσης δεδομένα που καταγράφηκαν σχετικά με τους πληθυσμούς των ιθαγενών ειδών Coccinellidae που υπήρχαν πριν και μετά τις εξαπολύσεις του *H. axyridis*, αποτελούν ένδειξη για μη αρνητικές επιπτώσεις του *H. axyridis* στην ιθαγενή κοινότητα των αφιδοφάγων Coccinellidae της Ελλάδας. Παρόλα αυτά, δεδομένων των αρνητικών επιπτώσεων που έχει προκαλέσει σε χώρες της Ευρώπης και της Αμερικής δεν συνίσταται με κανέναν τρόπο η επανέναρξη των εξαπολύσεων του *H. axyridis* στον ελληνικό χώρο. Αντιθέτως, πρέπει να οργανωθεί ένα πρόγραμμα παρακολούθησης και καταγραφής Coccinellidae και *H. axyridis*, ούτως ώστε να καταγραφεί εγκαίρως τυχόν εισβολή του εξωτικού αρπακτικού από την βόρεια Ευρώπη.