Establishment of *Harmonia axyridis* on Citrus and Some Data on Its Phenology in Greece

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In September 1993, a colony of *Harmonia axyridis* Pallas (Coleoptera:Coccinellidae) was imported from France into Greece. In 1994, insectary-reared adults were released in 11 citrus orchards in four citrus-growing areas of Greece. Between May 19 and June 8, 1994, *H. axyridis* was recovered from a total of seven localities in three of these areas. This species was established on orange, mandarin and sour orange trees heavily infested with *Toxoptera aurantii, Aphis spiraecola* and *A. gossypii*; its absence from the remaining four localities may have been the result of low prey densities. Twenty-three days after the initial releases, *H. axyridis* larvae comprised 57.9% and 83.3%, respectively, of the aphidophagous coccinellid larval populations in two localities (on Chios Island). In samples taken at Leonidion 43 days after the introduction release, both adult and larval populations of *H. axyridis* represented approximately one-third of aphidophagous coccinellid adults and larvae found, whereas the indigenous *Adalia bipunctata* comprised about one-half of the population. In cages placed outside the Athens laboratory, *H. axyridis* completed four overlapping generations annually; average longevities of 56.2, 66.8, 78.9 and 102.2 days, respectively, were recorded for the successive generations. Adults of the 3rd and 4th generations overwintered, giving rise to the following year's 1st generation. Oviposition began in April and emergence of 1st generation adults occurred in mid-May. The egg-laying activity of the females throughout the warm period of the year indicates that *H. axyridis* does not diapause in summer. From December until March, small aggregations (2–4 individuals) were observed within the cages at protected sites.

KEY WORDS: *Harmonia axyridis* Pallas; colonization; phenology; biological control; aphids; citrus; Greece.

INTRODUCTION

In Greece, aphids (Homoptera, Aphidoidea, Aphididae)–especially the black citrus aphid (*Toxoptera aurantii* Boyer de Fonscolombé), the cotton aphid (*Aphis gossypii* Glover), and the green citrus aphid (*Aphis spiraecola* Paton)–annually infest citrus to some extent, primarily in late spring–early summer and late summer–early autumn, damaging the tender new vegetation on citrus trees. Effective biological control agents are sought to suppress heavy aphid infestations on citrus (6). Among the aphidophagous coccinellids native in Greece, *Coccinella septempunctata* L. and *Hippodamia undecimnotata* (Schneider) have the largest body sizes, usually an indication

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of high voracity. However, both are characterized by a tendency to aestivate. In addition, the latter displays a preference for living on plants other than citrus. Therefore, the introduction of an exotic aphidophagous coccinellid which does not diapause in summer would complement the impact of the native predators.

The Far Eastern coccinellid *Harmonia axyridis* Pallas seems a suitable candidate. In the region of its origin, *H. axyridis* preys primarily on aphids, including *A. gossypii, A. spiraecola* and *Toxoptera* sp. (2,10), living on plants such as cotton, alfalfa, soy (4,14,15), and on fruit trees (1).

From the eastern Maritime Territory of the former Soviet Union, *H. axyridis* was introduced into Bukovina, Ukraine, in 1964 and into southeastern Kazakhstan in 1969 to control aphids on fruit trees (8,12). In the 1980s, the predator was introduced into southern France by Iperti (13) and, from there, into the Azores islands and the Algarve province of Portugal by Garcia (5) for the control of aphids on citrus.

In September 1993, a colony of some 100 adults of *H. axyridis* was imported from France into Greece by the first author. The introduction of *H. axyridis* into the field was attempted in 1994, by releasing insectary-reared adults in four citrus-growing areas of Greece. The phenology of *H. axyridis* was studied at Benaki Institute, Athens, during 1994–95.

MATERIALS AND METHODS

The *H. axyridis* adults imported from France were reared and multiplied on *Aphis fabae* Scopoli infesting artificially contaminated seedlings of *Vicia faba* L. (Leguminosae) and on *Dysaphis crataegi* Kaltenbach infesting artificially contaminated squash of two cultivars of *Cucurbita maxima* Duch. (Cucurbitaceae) (7), under controlled conditions (25 ± 1°C, 65 ± 5% r.h., 16/8 L/D) at Benaki Institute, Athens. *H. axyridis* releases in a total of 11 citrus orchards were made in late spring–early summer 1994, at four citrus-growing areas of Greece (Fig. 1). These areas are: Cambos, Chios Island [localities 1–4, Fig. 1]; Marathon, Attica [localities 5 and 6, Fig. 1]; Leonidion, western Peloponnesus [localities 7 and 8, Fig. 1]; and Chania, Crete [localities 9–11, Fig. 1]. Between 30 and 40 *H. axyridis* adults were released per tree, on 1–3 trees at each locality, depending on the abundance of prey and the number of predators available.

Samplings were made 3–4 weeks after each release date to check for the establishment of *H. axyridis*. At Leonidion [locality 7], three samplings were made at 2–3-week intervals after the release. The coccinellids were sampled as follows: four branches on each of seven trees infested with aphids (if available) per locality, were beaten with a rubber-covered stick over a 1-m^2 cloth screen; the numbers and species of coccinellid adults, larvae, and pupae collected, were recorded.

The associated aphid populations were monitored visually and by laboratory examination of samples taken before the release and at the time of the establishment check. The degree of aphid infestation on trees was defined as follows: very light (few individual aphids), light (a few leaves with colonies of <10 aphids), medium (a few leaves with colonies of ≥ 10 aphids), and heavy (numerous leaves and twigs with colonies of ≥ 10 aphids).

A single sample comprised four young twigs of new flush, 20–30 cm in length, one twig from each compass direction, cut from three randomly selected citrus trees obtained from each location. The larval populations of coccinellids were also sampled, since the

larvae were not usually dislodged from the leaves with aphid colonies. Sampled twigs were examined under a magnifying glass. In cases of large aphid colonies, the approximate numbers of aphids present, and the numbers and species of coccinellid larvae, were recorded.

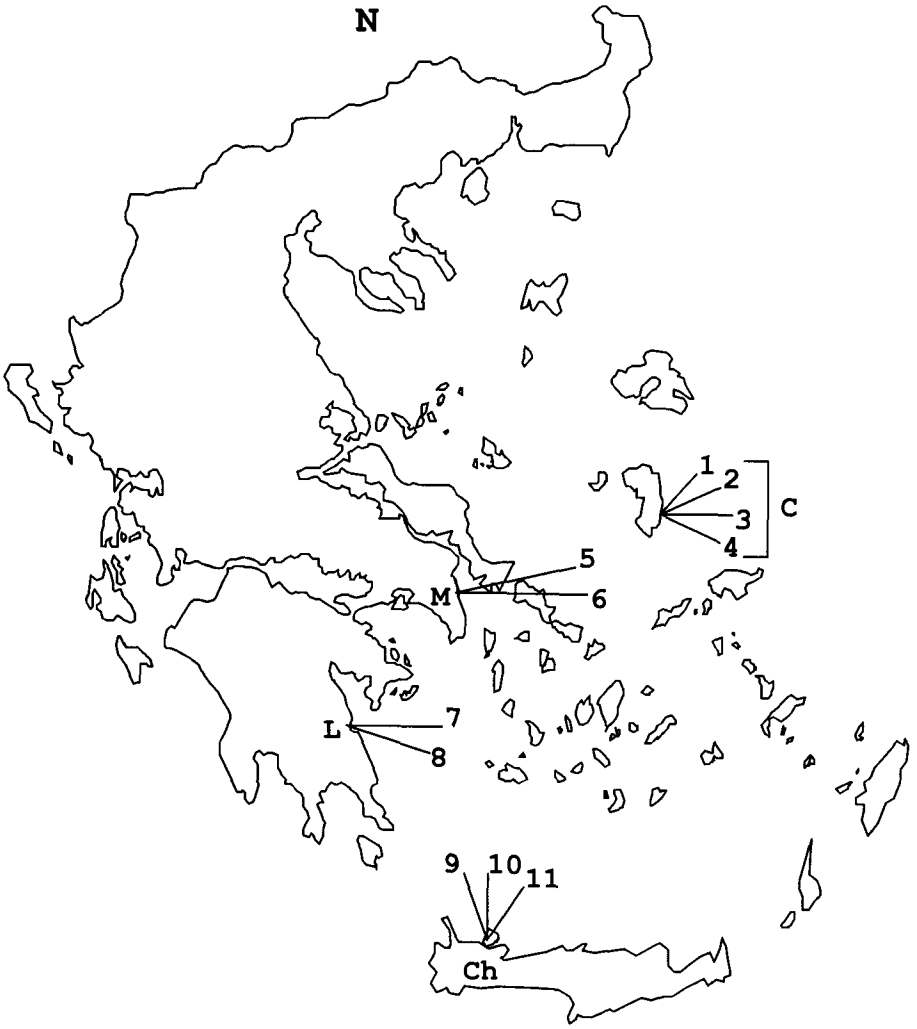

Fig. 1. Distribution of the introduction-release sites for *Harmonia axyridis* on citrus in Greece. The numbers of the localities correspond to those given in Tables 1 and 2. C: Cambos, Chios island; M: Marathon, Attica; L: Leonidion, western Peloponnesus; Ch: Chania, Crete.

The phenology of *H. axyridis* was studied by placing 40 adult insects which had been reared on *V. faba* seedlings artificially infested with *A. fabae*, in cylindrical (50 cm length × 30 cm diameter) Plexiglas cages outside the Benaki Institute laboratory. Successive generations were separated from one another by moving each newly hatched larval population into a new cage. Only the first 40 adults to emerge were kept. The numbers of living adults

and eggs in each cage were recorded twice weekly. Meteorological data were obtained daily, from the Benaki Institute.

RESULTS AND DISCUSSION

Establishment of H. axyridis

The results of the establishment check of *H. axyridis* after its introduction-releases on citrus are presented in Table 1. *H. axyridis* was recovered from seven of the 11 release sites sampled 3–6 weeks post-release within three of the citrus-growing areas (Cambos-Chios, Leonidion and Chania-Crete). Both larvae and adults were recovered in five of the seven localities, whereas only adults were recovered in the two others (Chania-Crete). Within these environmental parameters, *H. axyridis* survived on orange [locality 1], mandarin [2, 3, 4, 7] and sour orange [9, 10] trees. This agrees with results obtained in the Azores and the Algarve (5), suggesting that several citrus species and cultivars provide a suitable habitat for *H. axyridis*. *H. axyridis* fed on the three most important aphids infesting citrus: *T. aurantii* [localities 1, 2, 3, 4], *A. spiraecola* [7] and *A. gossypii* [4, 9, 10]. In localities where trees were heavily infested with aphids [1, 2, 7], *H. axyridis* reproduced immediately. This may be related to the high trophic requirements of this large-bodied coccinellid (13). Conversely, insufficiency of prey may explain the absence of *H. axyridis* in the establishment checks at Marathon [localities 5, 6], Leonidion [8] and Chania-Crete [11]. Its recovery in such small numbers at other localities [4, 9, 10], where medium aphid infestations on the release date became very light by the check date, may be explained similarly.

The composition of the coccinellid populations sampled on citrus heavily infested with aphids at three localities [1, 2, 3] in Cambos-Chios and at one locality [7] in Leonidion is presented in Table 2. The few *H. axyridis* adults recovered at Cambos-Chios were probably some of the adults released 23 days earlier. The *H. axyridis* larval population was dominant among the aphidophagous coccinellid species present at localities 1 and 2 and compared favorably with the most common native ladybeetle, *Scymnus rubromaculatus* (Goeze), at locality 3. In the 42 days following its release at Leonidion [locality 7], both the adult and larval populations of *H. axyridis* reached second place in order of importance, after the native *Adalia bipunctata* L. *H. axyridis* represented approximately one-third of the total numbers of coccinellid adults and larvae found. This is explained partly by the high fecundity of *H. axyridis* females (9), and partly by the predator's tendency to settle on citrus with an abundance of prey.

Phenology

In 1994–95, *H. axyridis* completed four annual generations in cages outside the Athens laboratory (Fig. 2). Two to three of these generations overlapped, since some adults lived up to 7 months. Adults of the 1st and 2nd generations, with average longevities of 56.2 and 66.8 days, respectively, died before winter. Adults of the 3rd and 4th generations, with average longevities of 78.9 and 102.2 days, respectively, overwintered. Fourth generation females gave rise to the following year's 1st generation. The completion of four annual generations by *H. axyridis* reared in outdoor cages represents an optimum obtained by keeping for reproduction only the earliest eggs and/or adults of each generation and providing aphids in abundance at times when they would usually be scarce in the field.

Oviposition began in April and emergence of 1st generation adults occurred in mid-May. Second generation adults appeared in late June, 3rd generation adults in late August, and 4th generation adults emerged in early October. The egg-laying activity of the females was high throughout the warm period of the year (Fig. 2). These results agree with studies in Japan (11) indicating that *H. axyridis* does not diapause in summer. After October, the egg-laying activity of the 3rd and 4th generation females decreased until December, when it ceased. The late-produced eggs did not survive the winter. The 4th generation females oviposited again the following spring (Fig. 2).

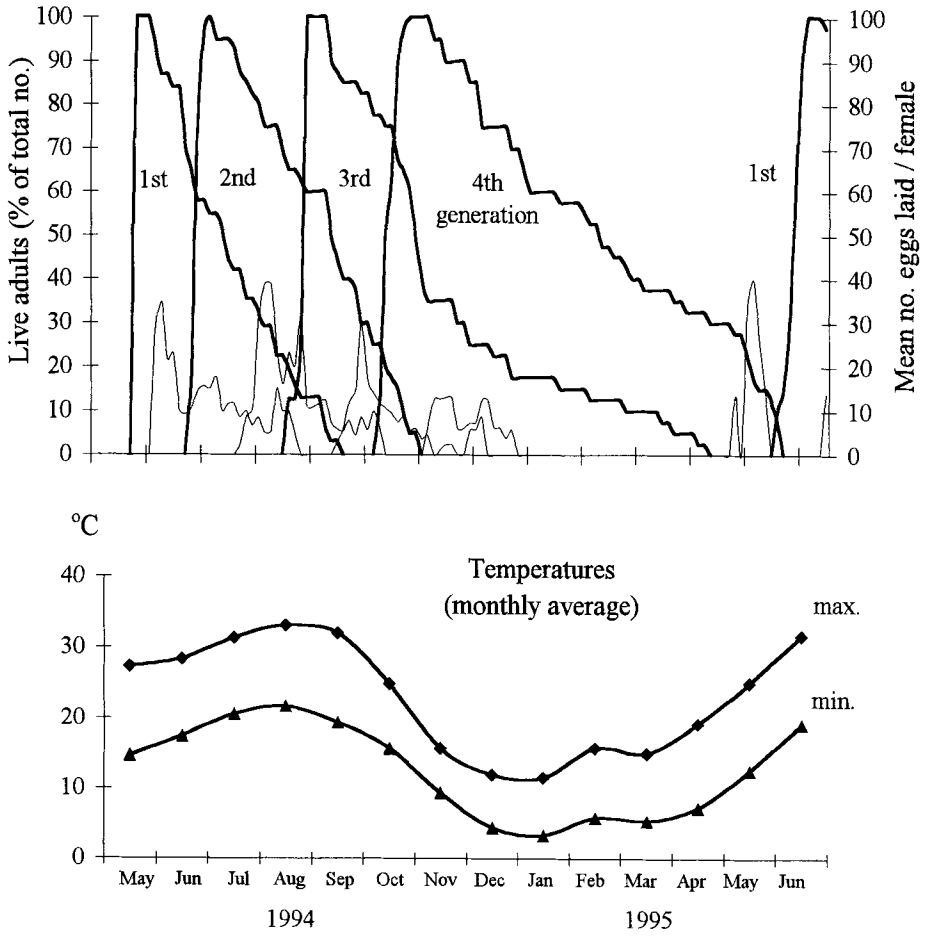

Fig. 2. Emergence and longevity of four generations of *Harmonia axyridis* adults (heavy line), and egg-laying activity (recorded twice weekly; thin line) of the females, reared in cages outside the laboratory; and outdoor temperatures at the site (Benaki Institute, Athens).

The overwintering habits of this species in the Far East were recorded by Fan and Yang (3). In Greece, from December until March, small aggregations (usually 2–4 individuals)

TABLE 1. Introduction and establishment of *Harmonia axyridis* on citrus in Greece in 1994

Locality No. (see Fig. 1)	Plantation	Prey	Relative degree of aphid infestation at release	*H. axyridis* introduction release		Aphid infestation at establishment check	*H. axyridis* at establishment check			
				Date	No. of Adults		Date	Number of		
								Larvae	Pupae	Adults
1	orange	*Toxoptera aurantii*	heavy	April 26	60	medium	May 19	11	0	2
2	mandarin	*T. aurantii*	heavy	April 26	60	medium	May 19	10	0	2
3	mandarin	*T. aurantii*	medium	April 26	60	light	May 19	5	0	1
4	mandarin	*T. aurantii* and *Aphis gossypii*	medium	April 26	60	very light	May 19	1	0	1
5	mandarin	*Aphis spiraecola*	medium	May 11	30	very light	May 30	0	0	0
6	lemon	*A. spiraecola*	light	May 24	60	very light	June 10	0	0	0
7	mandarin	*A. spiraecola*	heavy	April 18	120	medium	May 10	9	0	20
						light	May 30	1	9	32
						very light	June 20	0	0	0
8	orange	*A. spiraecola* and *A. gossypii*	medium	April 18	80	very light	May 10	0	0	0
9	sour orange	*A. gossypii*	medium	May 5	30	very light	June 8	0	0	1
10	sour orange	*A. gossypii*	medium	May 5	30	very light	June 8	0	0	2
11	sour orange	*A. gossypii*	medium	May 5	30	very light	June 8	0	0	0

TABLE 2. Composition of the coccinellid populations sampled at four localities after releases of *Harmonia axyridis* on citrus heavily infested with aphids (1994)

Locality No. (see Fig. 1)	Coccinellid adults										Coccinellid larvae and pupae									
	Total No.	%									Total No.	%								
		H. axyridis	Coccinellini				Scymnini			Chilocorini		*H. axyridis*	Coccinellini				Scymnini			Chilocorini
			a	b	c	d	e	f	g	h			a	b	c	d	e	f	g	h
1[z]	26	7.7	0	3.8	0	34.6	0	0	30.8	23.1	19	57.9	0	0	0	0	0	10.5	26.3	5.3
2[z]	11	18.2	0	0	0	45.4	0	0	18.2	18.2	12	83.3	0	0	0	0	0	8.3	8.3	0
3[z]	15	6.7	0	0	0	0	6.7	13.3	73.3	0	9	33.3	0	0	0	22.2	0	11.1	33.3	0
7[y]	110	29.1	0.9	47.3	15.4	2.7	1.8	2.7	0	0	31	32.2	0	51.6	6.4	0	6.4	3.2	0	0

[z] Sampled on May 19.
[y] Sampled on May 30.
[a] *Coccinella septempunctata* L.
[b] *Adalia bipunctata* L.
[c] *Adalia decempunctata* L.
[d] *Synharmonia conglobata* L.
[e] *Scymnus (Pullus) subvillosus* (Goeze).
[f] *Scymnus apetzi* Mulsant.
[g] *Scymnus rubromaculatus* (Goeze).
[h] *Exochomus quadripustulatus* L.

were observed in protected sites, *e.g.* at the edges of the muslin covering the ends of the cylindrical Plexiglas cages, under *V. faba* leaves, or under enclosed plastic boxes containing the aphid-contaminated *V. faba* seedlings. No mating, oviposition or larvae were observed during this period. Therefore, it may be concluded that *H. axyridis* adults are quiescent during the winter months.

Harmonia axyridis appears to possess those biological and ecological traits that would make it an effective biological control agent against heavy infestations of citrus aphids in Greece. Wherever there was an abundance of prey in this study, the predator became established easily in the introductory releases. The phenological study showed continuous reproductive activity throughout the summer months, a finding yet to be confirmed for females in the field.

Augmentative releases, as needed, of insectary-reared *H. axyridis* against local outbreaks of aphids on citrus, may be the most suitable way of using this exotic predator for complementing the action of the native complex of aphidophagous coccinellids, especially during the summer months.

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