Ecological Data on Predators of *Parlatoria pergandii* on Sour Orange Trees in Southern Greece

George J. Stathas[1]

The present study was carried out in southern Greece during 1993–1995 on sour orange trees infested with the diaspidid *Parlatoria pergandii* Comstock. The activity of the natural enemies of the scale, the composition of their population during the year as well as their relation with hymenopterous parasitoids of coccinellids, were studied. *P. pergandii* was parasitized by a hymenopterous endoparasite of the genus *Encarsia* and the extent of parasitization ranged between 5.2% and 14.1%. The observed predators were the coccinelids *Chilocorus bipustulatus* Linnaeus and *Rhyzobius lophanthae* Blaisdell and the nitidulid *Cybocephalus fodori* Endrödy-Younga. The predominant predator was *R. lophanthae* (84.3% of the larvae and 73.3% of the adults), which was active throughout the whole year in all of its developmental stages. Second most abundant was the predator *C. bipustulatus* (15.7% of the larvae and 20% of the adults) and third the predator *C. fodori* (6.7% of the adults). Larvae of *C. bipustulatus* were observed to be parasitized by the hymenopterous parasitoids *Homalotylus flaminius* Dalman (Encyrtidae) and *Tetrastichus coccinellae* Kurdjumov (Eulophidae). The parasitization percentage increased gradually from 4% in mid June to 94% around the end of September. Laboratory tests confirmed that the above mentioned parasitoids cannot infest larvae or nymphs of *R. lophanthae*.

KEY WORDS: *Chilocorus bipustulatus;* citrus; Coccinellidae; *Cybocephalus fodori;* Diaspididae; *Encarsia; Homalotylus flaminius;* parasitism; *Parlatoria pergandii; Rhyzobius lophanthae; Tetrastichus coccinellae.*

INTRODUCTION

The most important predators of Diaspididae species reported in Greece are *Chilocorus bipustulatus* Linnaeus and *Rhyzobius lophanthae* Blaisdell (Coleoptera: Coccinellidae) (3). The nitidulid predator *Cybocephalus fodori* Endrödy-Younga has also been reported in some cases as a predator of diaspidids (9). The importance of the above two coccinellid species as biological control agents against diaspidids, their large number of host species and wide abundance are reported in many other countries as well (4,7,13). There are reports on the biological and ecological characteristics of *R. lophanthae*, such as its high fecundity, the relatively low temperature thresholds of immature stages, the lack of diapause and absence of parasitoids (14,16,18). These characteristics enable its development in high populations in nature feeding on Diaspididae, and its larvae and nymphs to be active continuously throughout the year (14,16,18). *C. bipustulatus,* on the other hand, is an important natural enemy of Coccoidea as well, mainly of the family Diaspididae (11).

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[1]Laboratory of Biological Control, Benaki Phytopathological Institute, GR 145 61 Kifissia, Athens, Greece [Fax: +301-8077-506; e-mail: gstathas@x-treme.gr].

However, as reported in the literature, its populations in nature are decreasing substantially from mid summer until winter and this has been attributed to various causes, such as the scarcity of scale insects, the decline of ovogenesis due to unfavorable conditions (high temperature, low humidity), as well as its parasitization by a large number of natural enemies (4). The hymenopterous parasitoids *Homalotylus flaminius* Dalman (Encyrtidae) (4,13,16,19), *Eupelmus urozonus* Dalman (Eupelmidae), *Tetrastichus minimus* Howard (Eulophidae) (16), *Tetrastichus epilachnae* Giard (Eulophidae) (7,13,19), *Tetrastichus coccinellae* (Kurdjumov) (Eulophidae), *Tetrastichus sempronius* Erdös (Eulophidae) (7), *Phygadeuon rugulosus* Gravenhorst, *Phygadeuon kozlowi* Kokujev, *Parmortha parvula* (Gravenhorst) (Ichneumonidae) (13), the dipterous *Phalacrotophora fasciata* Fallén (Phoridae) (19), and the sac fungus *Hesperomyces virescens* Thaxter (Laboulbeniales) (2) have been recorded as natural enemies of *C. bipustulatus*. In Greece, although a decrease in the population of *C. bipustulatus* has been found during the summer period due to parasitization (9), there is no information on the species of parasitoids and the course of parasitization during the year. Finally, the predator *C. fodori* has not been reported to have natural enemies but the hymenopterous parasites *Aphanogmus* sp. (Ceraphronidae), *Zatropis* sp. (Pteromalidae), *Cerchysiella* (= *Zeteticontus*) spp. (Encyrtidae) and *Ecthroplexiella* sp. are known to parasitize larvae of *Cybocephalus* spp. (13).

Such studies relating to the population dynamics of beneficial insects in nature, provide useful information for the correct planning of successful biological control applications against insect pests. Because of the economically important damage caused by diaspidids to tree crops and the importance of their predators as biological control agents, it was decided to investigate aspects of the predators' ecology which have not been studied in Greece. This was achieved by observing populations of the predators in the field and by conducting tests and measurements in the laboratory in order to study the population dynamics of predators of diaspidids in nature during the year, evaluate their action against the scales and study their natural enemies. The scale *Parlatoria pergandii* Comstock (Hemiptera: Diaspididae) on citrus, was found to be a suitable prey of *R. lophanthae* and *C. bipustulatus* which developed in sufficient numbers to enable the collection of data on their ecology.

MATERIALS AND METHODS

Field observations The field work was conducted on 30 12-year-old sour orange trees, heavily infested with *Parlatoria pergandii* Comstock (Hemiptera: Diaspididae) in a citrus field at Leonidion, in western Peloponnese, southern Greece, from January 1993 to April 1995. Samples were taken every 20 days during the warm period of the year (April–September) and monthly during the winter months. Chemical insecticides were not applied during the period of the study. A heavy trimming was done in March 1992, in order to reduce the infestation with *P. pergandii*. The infestation level of scales was evaluated from samples of 12 leaves collected from each of five randomly chosen twigs at shoulder height. These samples were brought to the laboratory, where the number and developmental stages of live armored scale individuals were recorded, as well as those dead from unknown causes, attacked by predators and parasitized. The possibility that predated nymphs had been previously parasitized, was not taken into account. The level of infestation was measured and expressed as the number of live (including males and females) scales per cm^2 of the plant surface.

Coleopterous predators were sampled by beating 12 randomly chosen branches of trees with a rubber-covered stick over a 1-m^2 cloth screen. The number of adults and larvae of these predators thus dislodged was then recorded. The beatings were carried out early in the morning (7:30–8:30 a.m.) because under warm and sunny conditions – such as exist later in the day, winged insects that fall on the collecting sheet tend to fly off from the latter very readily. After their number had been recorded, the predators were returned to the leaves on the trees with the aid of a small brush.

Meteorological data were obtained from the National Meteorological Office.

Parasitism of predator larvae The study of the larval parasitization of *C. bipustulatus* and of the possibility of larval parasitization of *R. lophanthae* by the parasitoids *Homalotylus flaminius* Dalman (Hymenoptera: Encyrtidae) and *Tetrastichus coccinellae* (Kurdjumov) (Hymenoptera: Eulophidae), was carried out with field sampling of larvae as well as in laboratory tests. Fifty larvae from each of the two parasitoids were obtained every 20 days, from June until September, by random beatings of the tree branches. The larvae were brought to Kifissia, where they were cultured separately in plastic cages (9 cm diam, 1.6 cm high) under outdoor conditions, until emergence of adults or parasitoids. The larvae were fed on nymphs of *P. pergandii* on sour oranges. In the laboratory, *R. lophanthae* and *C. bipustulatus* were cultured under controlled conditions of temperature (25±1°C), relative humidity (65±2%) and photoperiod (16:8, L:D), from hatching of larvae until the appearance of adults, in four cylindrical cages (30 cm diam, 50 cm long), as follows:

- 1st cage: 25 1st – instar larvae of *R. lophanthae* and 10 adults of *H. flaminius* (5 males, 5 females)
- 2nd cage: 25 1st – instar larvae of *R. lophanthae* and 10 adults of *T. coccinellae* (5 males, 5 females)
- 3rd cage: 25 1st – instar larvae of *C. bipustulatus* and 10 adults of *H. flaminius* (5 males, 5 females)
- 4th cage: 25 1st – instar larvae of *C. bipustulatus* and 10 adults of *T. coccinellae* (5 males, 5 females).

The larvae of the predators inside the cages were fed on nymphs of *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae), on cucurbit fruit. Inside the same cages, the parasitoids obtained from parasitized larvae of *C. bipustulatus* from Leonidio, were fed on 50% honey in water, in a 4.2 cm-diam petri dish. These tests were conducted in order to rule out the parasitization of *R. lophanthae* larvae, in case parasitized larvae did not fall on the cloth screen following the beating of the branches. Similar tests with *C. bipustulatus* larvae were used as control.

Statistical analysis In order to compare the populations of predators (*C. bipustulatus, R. lophanthae, C. fodori*), data were tested for significance using analysis of variance (ANOVA) and means were compared by the Tukey - Kramer (HSD) test (at P = 0.05), using the statistical package JMP (15).

RESULTS

A gradual reduction in the infestation level and an increase in the number of parasitized nymphs of *P. pergandii* were observed upon examination of sour orange tree leaves during

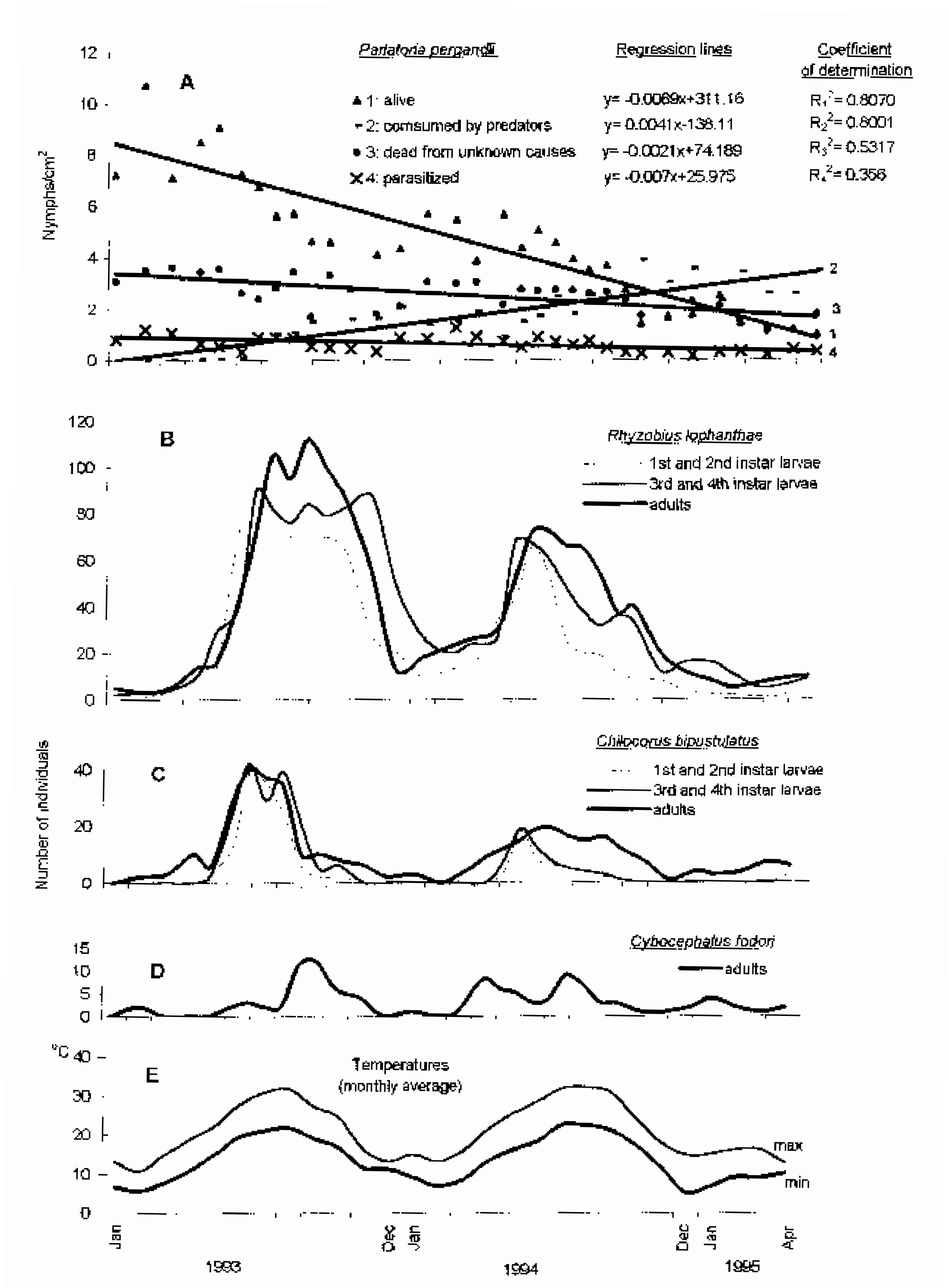

Fig. 1. Composition of the *Parlatoria pergandii* population (A) and the numbers of its predators (B,C,D) on sour orange trees from January 1993 until April 1995, and average monthly temperatures (E), in southern Greece.

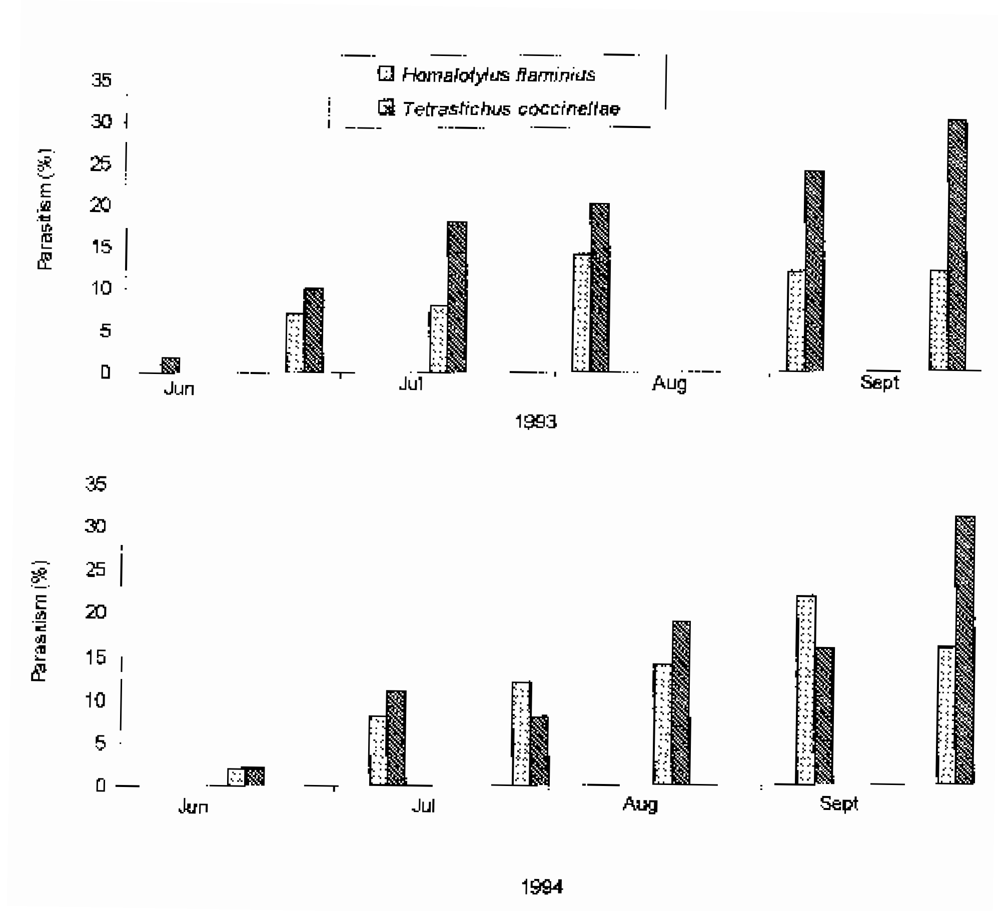

Fig. 2. Parasitism of *Chilocorus bipustulatus* larvae by *Homalotylus flaminius* and *Tetrastichus coccinellae* during the summer of 1993 and of 1994 in southern Greece.

the period of the study (Fig. 1A). The number of dead nymphs from unknown causes as well as of those parasitized, did not show great changes with time, as evident from the small slope of their trendlines (Fig. 1A). The percentage of parasitized nymphs of *P. pergandii* ranged during the period of the study from 5.2 to 14.1. The parasitoid observed was an *Encarsia* sp. (Hymenoptera: Aphelinidae) endoparasite. As found following the beatings of the tree branches, the predators of the scale were the coccinellids *R. lophanthae* and *C. bipustulatus* and the nitidulid *C. fodori* (Figs. 1B, C, D). Larvae of all instars and adults of *R. lophanthae* were observed throughout the year, whereas larvae of *C. bipustulatus* were seen only during the summer months (April–October). Branch beatings did not turn up larvae of *C. fodori*, but in laboratory examination single larvae of the predator were occasionally seen under the scale covers of *P. pergandii* during the summer months. Statistical analysis uncovered significant differences among the three species of predators (F= 36.17, d.f.= 2 and 96, $P<0.0001$). Means comparison showed that the number of

R. lophanthae larvae and adults observed during the period of the study was significantly higher than the number of individuals of *C. bipustulatus* and *C. fodori*, whereas respective numbers of *C. bipustulatus* and *C. fodori* did not differ significantly. Of a total of 2586 larvae of *R. lophanthae* and *C. bipustulatus* observed during the period of the study, 84.3% were larvae of *R. lophanthae* and 15.7% were larvae of *C. bipustulatus.* Of a total of 1839 adults of *R. lophanthae*, *C. bipustulatus* and *C. fodori*, 73.3% were *R. lophanthae*, 20% *C. bipustulatus* and 6.7% *C. fodori.* Observations of the larvae of predators collected from sour orange trees and cultured inside cages under outdoor conditions showed that the larvae of *R. lophanthae* were not parasitized and all of them developed into adults. In contrast, larvae of *C. bipustulatus* were parasitized by the hymenopterous parasites *T. coccinellae* and *H. flaminius* (Fig. 2). Parasitization increased during the summer in the 2 years of the study. The percentage of parasitization of larvae of *C. bipustulatus* by the parasitoid *T. coccinellae* was higher in all the observations in 1993 and in four of six observations in 1994 (except for those on July 30 and Sept. 11). However, the above differences in parasitization between the two parasitoids were not significant (F= 2.69, d.f.= 1 and 22, $P = 0.12$). None of the larvae of *R. lophanthae* collected during the winter was mummified. Similarly, none of the individuals (larvae and/or pupae) of *R. lophanthae* cultured in the laboratory with the parasitoid *H. flaminius* or with *T. coccinellae* under controlled conditions inside separate cages was parasitized, and all developed into adults. In contrast, all individuals of *C. bipustulatus* cultured with each of the above two parasitoids inside separate cages were parasitized and none of them developed into adults. In field observations of the population of predators, no exterior signs of infection by pathogenic fungi were found.

DISCUSSION

The population of *P. pergandii* decreased gradually, as shown in Figure 1A (line 1, slope: -0.0089). The number of dead from unknown causes and the number of parasitized nymphs of the diaspidid decreased slightly (line 3, slope: -0.0021, and line 4, slope: -0.007, respectively), whereas the number of predated nymphs rose (line 2, slope: +0.0041). This is indicative of the contribution of the predators to the reduction in the scale infestation. The activity of *R. lophanthae* and *C. bipustulatus* against *P. pergandii* has also been reported by Abbassi in Morocco (1). Applebaum *et al.* (2) reported from Israel that *C. bipustulatus* was found to prey on *P. pergandii*, but it is possible that this scale does not provide adequate nutrition. There are no respective references for *C. fodori*. Populations of the three predators, high numbers of predated nymphs of *P. pergandii* and the absence of other predators of diaspidids indicate that the above mentioned entomophagous insects fed on *P. pergandii*. This was confirmed for *C. bipustulatus* and *R. lophanthae* in the laboratory, since larvae of the two coccinellids – which were carried to the insectary for the study of parasitism – were reared on *P. pergandii* nymphs on sour orange leaves.

The beating of branches, which was used as a means to collect predators, is not equally effective for all insect species, because the percentage of individuals which fall down onto the cloth screen depends on several factors, such as the body size of the insect (5). However, useful information can be obtained for each insect species. The numbers of predators decreased during the winter period (Figs. 1B, C, D), possibly due to low temperatures (Fig. 1E). The more intense activity of *R. lophanthae* and *C. bipustulatus* against *P. pergandii* during the summer, in comparison with the winter, was noted also by Abbassi (1). In the present study, larvae of *R. lophanthae* were found in nature even during winter. In contrast, larvae of *C. bipustulatus* were present only during summer. This phenomenon may be explained by the lower thermal development thresholds of immature stages of *R. lophanthae* (7.6–9.3°C) (18) compared with those of *C. bipustulatus* (10.6°C) (4). The existence of larvae of *R. lophanthae* in nature during winter has been observed in Morocco (16) and Georgia (former USSR) (14) as well.

An increase in the parasitization percentage of *C. bipustulatus* larvae from summer until autumn found in the present study (Fig. 2), has been observed elsewhere. Popova (12) reported that in orchards by the Black Sea, the percentage of parasitized larvae of *C. bipustulatus* increased from 72 to 90 in summer. Murashevskaya (10) stated that the parasitoids *T. coccinellae* and *H. flaminius* destroyed up to 80% of larvae of *C. bipustulatus* and *Chilocorus renipustulatus* (Scriba) in autumn, in northern Caucasus. Smirnoff (17) found that 95% of a colony of *C. bipustulatus* was destroyed by *H. flaminius* in northern Africa. Hodek and Honěk (8) also reported that parasitization by *Homalotylus* may be as high as 90–95% on *C. bipustulatus* along the Black Sea coast. Parasitization of *C. bipustulatus* (Fig. 2) by *T. coccinellae* was observed in higher (not significant) percentages than that of *H. flaminius* in ten of the 12 observations that were conducted during the 2 years of this study. This may be explained in part by the different voltinism of the two parasitoid species. Murashevskaya (10) reported that *T. coccinellae* completes seven generations per year in northern Caucasus both on *C. bipustulatus* and on *C. renipustulatus,* whereas *H. flaminius* in the same area and on the same hosts completes only five or six generations. In addition, Murashevskaya (10) noted that only one or two individuals of *H. flaminius* completed their development in a single *C. bipustulatus* larva, as compared with 7–15 of *T. coccinellae*. Similarly, Domenichini (6) reported one to three parasites of *H. flaminius* in a single larva of *C. bipustulatus*, and Hodek (7) found in genus *Chilocorus* usually six to eight parasites of *Tetrastichus* but sometimes as many as 18. The inability of parasitoids to attack *R. lophanthae* which was noted in nature and confirmed in the laboratory during the present study, has been reported by others as well (14,16).

Data obtained in the present study cover some ecological characteristics of predators of *P. pergandii* and could be useful in the planning of IPM programs, since *R. lophanthae* and *C. bipustulatus*, and less so *C. fodori*, are common predators of several species of Diaspididae in many areas all over the world (4,7,13).

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REFERENCES

1. Abbassi, M. (1975) Notes bio-écologiques sur *Parlatoria pergandei* Comstock [*sic*] (Homopt. Coccidae) au Maroc. *Fruits* 30:179-184.
2. Applebaum, S.W., Kfir, R., Gerson, U. and Tadmor, U. (1971) Studies on the summer decline of *Chilocorus bipustulatus* in citrus groves of Israel. *Entomophaga* 16:433-444.
3. Argyriou, L.C. and Katsoyannos, P. (1977) Coccinellidae species found in the olive-groves of Greece. *Ann. Inst. Phytopathol. Benaki* (N.S.) 11:331-345.
4. Bodenheimer, F.S. (1951) Citrus Entomology in the Middle East. W. Junk, the Hague, the Netherlands.
5. Dent, D.R. and Walton, M.P. (1997) Methods in Ecological and Agricultural Entomology. University Press, Cambridge, UK.

6. Domenichini, G. (1956) Contributo alla conoscenza dei parassiti e iperparassiti dei Coleoptera Coccinellidae. *Boll. Zool. Agrar. Bachic. Milano* 22:215-246.
7. Hodek, I. (1973) Biology of Coccinellidae. W. Junk, the Hague, the Netherlands.
8. Hodek, I. and Honěk, A. (1996) Ecology of Coccinellidae. Kluwer Academic Publishers, Dordrecht, the Netherlands.
9. Katsoyannos, P. (1984) Notes on life history and field efficiency of *Cybocephalus fodori* predator of *Quadraspidiotus perniciosus* in northern Greece. *Entomol. Hell.* 2:35-40.
10. Murashevskaya, Z.S. (1969) Species of *Chilocorus. Zashch. Rast.* 14:36-38.
11. Podoler, H. and Henen, J. (1983) A comparative study of the effects of constant temperatures on development time and survival of two coccinellid beetles of the genus *Chilocorus. Phytoparasitica* 11:167-176.
12. Popova, A.I. (1971) *Chilocorus* in the control of the California scale. *Zashch. Rast.* 16:42-43.
13. Rosen, D. (1990) Armored Scale Insects: Their Biology, Natural Enemies and Control. Elsevier, Amsterdam, the Netherlands.
14. Rubstov, I.A. (1952) *Lindorus* - an effective predator of Diaspine scales. *Entomol. Obozr.* 32:96-106.
15. SAS (1989) JMP – A Guide to Statistical and Data Analysis. Version 3. SAS Institute Inc., Cary, NC, USA.
16. Smirnoff, W. (1950) Sur la biologie au Maroc de *Rhyzobius (Lindorus) lophanthae* Blaisd. (Col.Coccinallidae). *Rev. Pathol. Veg. Entomol. Agric. Fr.* 29:190-194.
17. Smirnoff, W.A. (1957) La cochenille du palmier - dattier (*Parlatoria blanchardi* Targ.) en Afrique du Nord. Comportement, importance économique, prédateurs, et lutte biologique. *Entomophaga* 2:1-98.
18. Stathas, G.J. (2000) The effect of temperature on the development of the predator *Rhyzobius lophanthae* Blaisdell (Coleoptera: Coccinellidae) and its phenology in Greece. *BioControl* 45:439-451.
19. Yinon, U. (1969) The natural enemies of the armored scale lady-beetle *Chilocorus bipustulatus* (Col.: Coccinellidae). *Entomophaga* 14:321-328.