A study on the biology of the diaspidid scale *Parlatoria ziziphi* (Lucas) (Hemiptera: Coccoidea: Diaspididae) in Greece

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Abstract: The ecology of the diaspidid *Parlatoria ziziphi* (Lucas) infesting citrus was studied on sour oranges between January 2003 to April 2005 in Peloponnesus (southern Greece). The scale is biparental and oviparous, infesting mainly the leaves and fruits. *P. ziziphi* overwintered in all developmental stages and completed several overlapping generations each year. Generation time was estimated to last ~62 days under controlled conditions (25°C, 65% R.H. and 16h light/day) in the laboratory. Infestation density ranged from 0.2 to 6.8 live scales/cm^2 of upper leaf surface. The only wasp found to parasitize *P. ziziphi* was the aphelinid *Encarsia citrina* (Craw), and parasitism reached 22.3% during the second year of the study. Predation reached 34% and was attributed to the coccinellids *Chilocorus bipustulatus* Linnaeus and *Rhyzobius lophanthae* Blaisdell, and the nitidulid *Cybocephalus fodori* Entrödy-Younga. Larvae of *C. bipustulatus* were found to be parasitized by the encyrtid *Homalotylus flaminius* Dalman and the eulophid *Tetrastichus coccinellae* Kurdjumov, with the percentage parasitization gradually increasing from 7% in mid June to 96% towards the end of September.

Key words *Parlatoria ziziphi, citrus, Greece.*

Introduction

The black parlatoria scale, *Parlatoria ziziphi* (Lucas) (Hemiptera: Coccoidea: Diaspididae), is widely distributed in most of the tropics and subtropics and is present throughout the Mediterranean basin, apart from Israel, Albania and Croatia. Thirty-four plant species, belonging to 11 families, have been recorded as hosts (Ben-Dov, 2006), mostly (19) on *Citrus* (Rutaceae). In Greece, its presence was first noted by Koroneos (1934) on citrus (mainly on orange and mandarin trees) in Crete.

The biology and phenology of *P. ziziphi* have been studied mainly in Egypt, where it completes 2-3 generations annually: [Salama *et al*. (1985): 3 generations/year; Amin & Salem (1978), Sweilem *et al*. (1987), El-Bolok *et al*. (1987): 2 generations/year] and China, where it has 3-4 generations per year (Huang *et al*., 1988). The duration of the developmental stages of the scale under controlled conditions in the laboratory has been studied by Abdel-Fattah *et al.* (1978) and life-table data are provided by Sigwalt (1971).

The main natural enemies of *P. ziziphi* are hymenopterous parasitoids (Aphelinidae & Mymaridae), predatory beetles (Coccinellidae & Nitidulidae), acarine predators (Hemisarcoptidae & Phytoseiidae) and entomopathogenic fungi (*Aschersonia* sp.) (Ben-Dov, 2006; El-Choubassi *et al*., 2001; Momen & Hussein, 1999).

The present study looked at the life cycle, infestation density and natural enemies of *P. ziziphi* on heavily infestated sour-orange trees in the Tyros area of Peloponnesus in Southern Greece. The study involved investigations in both the field and the laboratory to help devise integrated management regimes for this pest.

Materials and methods

The study was made on 17 sour-orange trees in a citrus orchard of 5,000m^2 with orange, mandarin and lemon trees. All citrus species in the orchard were infested by *P. ziziphi* but sour-oranges had the highest populations and so were selected for the study. The trees were 25 years old, about 3m high and had 3m diameter. The trees were fertilized and irrigated. The weed control was mechanically performed. No chemicals were sprayed in the orchard during the study period. For laboratory studies of the phenology, samples consisting of about 200-300 leaves of infested sour oranges were cut from the middle shoots of the trees at a height about 2 meters from the ground and transported to the laboratory in plastic bags. The upper surface of 15 of these leaves were examined under a binocular stereoscope and the number of each instar and the number of predated, parasitized and dead scales recorded. The leaf surface (E) was estimated using the ellipse surface area equation: $E = \pi \times a \times b$, where: $\pi = 3.14$, a = min. radius, b = max. radius.

The parasitized scales were kept in the laboratory under controlled conditions (25°C, 65% R.H. and 16h light/day (artificial light) until emergence of the parasitoid adults. The identification of the parasitoid species was made by the third author, and voucher specimens are deposited at Institute of Zoology, Tbilisi, Georgia, and in Suleiman Demirel University. Isparta, Turkey in the personal collection of the third author. The predators were monitored by shaking the branches of 12 of the 17 sour-orange trees over a 1x1m white cloth. The branches were chosen at random and so the same branches could have been used on several occasions. The cloth was held horizontally about 40cm below the middle of the branches on each occasion. Sampling was conducted every 15 days between April and September and once a month for the rest of the year. The number, stage and species of each predator (larvae and adults) were recorded. The frequency of each predator species was compared using analysis of variance (ANOVA) and the population means separated by Student's "t-Test" (Landi, 1977).

The study of the parasitism of the coccinellid larvae was undertaken in 2004 only, when 25 larvae of both *Chilocorus bipustulatus* Linnaeus and *Rhyzobius lophanthae* Blaisdell were collected every 15 days between June and September by beating the branches as described above. The larvae were cultured separately in Petri-dishes (9 cm in diameter, 1.6cm high, with vent) in the laboratory under the above controlled conditions until the adult beetle or parasitoid emerged. The beetle larvae were fed on *P. ziziphi* on sour-oranges leaves cut from the infested trees at every sampling date and kept in the fridge (at ~5°C) for 15 days. The leaves were changed every 2-3 days.

Development of *P. ziziphi* was studied in the laboratory on five artificially infested sour-orange saplings. Each experimental sapling was fastened to a branch within the canopy of an infested sour-orange tree, so as to ensure contact between the leaves of the saplings and those of the sour-orange trees; they were left there for 3 days to allow settlement of the crawlers. The infested saplings were then transferred to the laboratory and kept under the above controlled conditions. A total of 264 settled 1st-instar nymphs were individually marked by encirclement with 2 concentric rings of entomological glue (Tanglefoot). Each marked nymph was checked weekly to observe their development until the end of their reproductive life (i.e. when F_1 crawlers were no longer captured on the inner of the two glue rings). The post-oviposition period of marked individuals could not be measured because removal of the scale cover would have resulted in the scale's death. The post-oviposition period was therefore studied using 30 non-marked ovipositing females (easily distinguished by the small, characteristic elliptic caudal appendage which appears externally when oviposition starts). These were almost the same age as the marked individuals and reared on the same saplings. The percentage of live insects was recorded weekly until all were dead. The dead scales were recognised because they become brown and dehydrated.

Results and discussion

P. ziziphi is biparental and oviparous and infests mainly the upper-leaf surfaces and the fruits. All developmental stages were present throughout the year, indicating that the diaspidid completed several overlapping generations annually in Greece. Throughout the present study, the number of crawlers was very low during January and February, followed by a sharp increase in April. This decrease may be attributed to the low temperatures during the winter. Male nymphs increased during June and July 2003, and again in April 2004 and 2005. The settled 1st-instar instar nymphs, 2nd-instar nymphs and adults were found on leaves throughout the year and their population showed little variation during the period of the study.

The total number of live scales per cm^2 of leaf surface on sour-orange leaves ranged from 0.2 (June 2004) to 6.8 individuals (October 2003) (Fig. 1a). The number of live, predated and parasitized scales also showed no regular fluctuations (Fig. 1b), although there appeared to be a slight increase in the number predated during the summer of 2004, with a maximum of 34% at the end of September. However, these data refer only to partially destroyed scales and do not include totally consumed individuals such as crawlers that (obviously) cannot be estimated. The frequency of damaged scales is indicative of the action of the predatory beetles *Chilocorus bipustulatus* Linnaeus, *Rhyzobius lophanthae* Blaisdell (Coleoptera: Coccinellidae) and *Cybocephalus fodori* Entrödy-Younga (Coleoptera: Nitidulidae). These predators are common and are known to be natural enemies of diaspidid scales infesting citrus in Greece (Katsoyannos, 1996). They were collected by beating as described above, although the larval and adult populations were quite low from October through to March (Fig. 2).

The only predatory larvae that were observed during the winter were those of *R. lophanthae. R. lophanthae* was numerous and the total number of larvae and adults observed during the study was significantly greater than that of both *C. bipustulatus* ($t= 6.7, P<0.05$) and *C. fodori* ($t= 9.1, P<0.05$). Furthermore, the number of *C. bipustulatus* was significantly greater than that of *C. fodori* ($t= 4.5, P<0.05$). This dominance of *R. lophanthae* might be because this ladybird is active throughout the year and does not enter diapause (Stathas, 2000; 2001). These three predatory species, particularly *C. bipustulatus* and *R. lophanthae*, are widely distributed throughout the world (Hodek & Honk, 1996).

In North Mediterranean countries, they are considered to be the most important predators of armored scale insects (Katsoyannos, 1996) which are believed to be the beetles' essential food (Hodek & Honk, 1996; Katsoyannos, 1996).

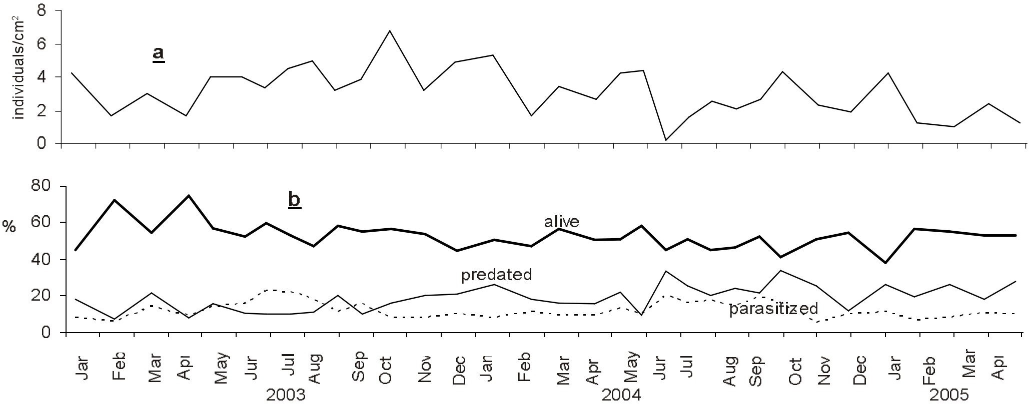

Figure 1 a: Total number of *P. ziziphi* individuals/cm^2 of leaf of sour orange from January 2003 to April 2005; **b**: Composition of individuals of *P. ziziphi* found on the leaves.

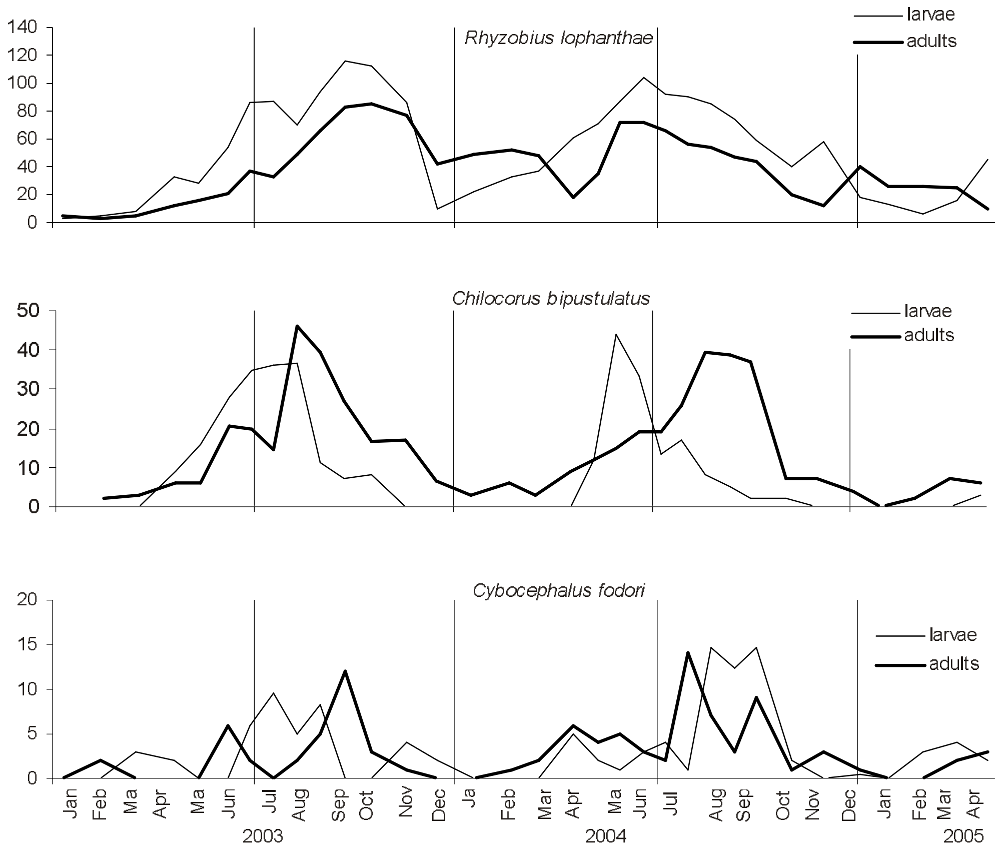

Figure 2. The frequency of three predators on sour orange infested by *Parlatoria ziziphi* from January 2003 to April 2005.

A few *Clitostethus arcuatus* (Rosi), *Rodolia cardinalis* (Mulsant) and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) were also collected on the beating trays, but they were not counted because it is believed that *C. arcuatus* was feeding mainly on some small colonies of *Aleurothrixus floccosus* (Maskell) (Hemiptera: Aleyrodidae); *R. cardinalis* was feeding mainly on *Icerya pyrchasi* Maskell (Hemiptera: Margarodidae), and *C. septempunctata* was feeding on aphids, all of which occasionally appeared on the trees. The literature also suggests that some acarina species are natural enemies of diaspidids, but their role was not investigated in the present study.

The proportion of live scales collected on the leaves every 15 days was remarkably constant throughout the study, mainly between 50 and 60%, with a peak of about 75% in April 2003, and with a low of about 40% in Jan. 2005 (Fig. 2b). At the same time, percentage parasitism and predation also remained reasonably constant, the former oscillating around about 15% and the latter appearing to rise gradually from perhaps 15% at the start of the year to about 25% by the end of the observations (Fig. 2b). These data suggest that the scale populations were in some sort of balanced equilibrium with their predators and parasitoids.

Parasitism of Chilocorus bipustulatus

The effectiveness of *C. bipustulatus* was almost certainly reduced by significant parasitism during the summer (only studied in 2004). The larvae of *C. bipustulatus* were parasitized by two hymenopterous parasitoids: *Homalotylus flaminius* Dalman (Encyrtidae) and *Tetrastichus coccinellae* Kurdjumov (Eulophidae). There was no significant difference in the frequency of these two parasitoids ($t = 0.3$, $P<0.05$) (Fig. 3). The combined parasitism was 7% in mid-June but increased rapidly to 96% by the end of September (Fig. 4). This increasing parasitization of *C. bipustulatus* during the summer has been recorded previously on the Black Sea coast by Rubstov (*in* Hodek & Honk, 1996), who quotes a parasitization level of 90-95% by *Homalotylus* and 90% or more by *Tetrastichus*. In the present study, only the overall parasitization level of the larvae was recorded. The activity of the parasitized larvae was not studied, but Iperti (*in* Hodek & Honk, 1996) states that *Homalotylus* oviposits in coccinellid larvae mostly during ecdysis while the host is attached to the substrate, and that the parasitized larva then attaches itself to the substrate again several days later, but, instead of moulting, it dies and the cuticle becomes hard and dark. As far as the parasitization by *Tetrastichus* is concerned, Iperti & Klausnitzer (*in* Hodek & Honk, 1996) refer to other studies in which the female parasitoid usually lay the eggs in the 3rd- and 4th-instar larvae, although they can also oviposit in the pupae and in younger larvae (Filatova, *in* Hodek & Honk, 1996). The parasitized 4th-instar larva then pupates but soon dies and becomes darker (Hodek & Honk, 1996). Parasitism was not recorded on *R. lophanthae* larvae and all larvae of *R. lophanthae* collected from the orchard and reared in laboratory pupated and developed into adults. The inability of *H. flaminius* and *T. coccinellae* to parasitize *R. lophantahe* has been confirmed in the laboratory in a previous experiment in Greece (Stathas, 2001). The lack of parasitism of *R. lophanthae* has also been noted in other countries, such as Morocco (Smirnoff, 1950) and the Crimea (Rubstov, 1952). Although the parasitism of the nitidulid *C. fodori* was not studied in the laboratory, no larva of the predator was found mummified whilst beating the infested trees.

The only wasp found parasitising *P. ziziphi* was *Encarsia citrina* (Craw) (Hymenoptera: Aphelinidae). The percentage parasitism remained reasonably constant at around 10% throughout the year but was perhaps greatest during the summer, with a peak of 22% in June 2003 (Fig. 1b).

Laboratory observations on the phenology of P. ziziphi

The development of *P. ziziphi* on the infested saplings in the laboratory is presented in Fig. 4. The time from the initial infestation by crawlers (F_0) to the next hatching (F_1) was estimated at 61 days, and the interval between the appearance of settled 1st-instar nymphs of the F_0 and F_1 generations was 63 days. Ovipositing females were present for 71 days. Mortality was measured 19% for settled 1st-instar nymphs, 14.5% for 2nd-instar nymphs and 4.5% for ovipositing females. Abdel-Fattah *et al*. (1978) reported that *P. ziziphi* completed a generation on mandarins in 84-93 days at 22.2-25.7°C and 67-71% R.H. The difference between Abdel-Fattah et al.'s results and those of the current study are probably due to the different temperature and host, as McClure (*in* Rosen, 1990) indicated that differences of temperature, moisture and host plant can cause considerable differences in the voltinism, diapause, fecundity and reproduction (i.e., even changing from ovoviviparity to oviparity) of diaspidids. Our results give a generation time of about 62 days on sour-orange in the laboratory. Even though we only followed the experimental population through one complete generation, we had crawlers, nymphs and adults present simultaneously on the experimental saplings for much of the time. It is clear that there are several overlapping generations each year.

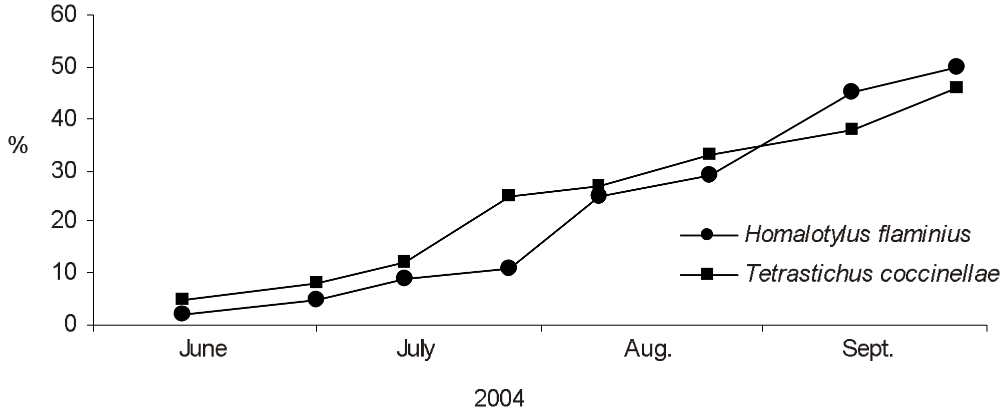

Figure 3. Percentage parasitism of *Chilocorus bipustulatus* larvae by *Homalotylus flaminius* and *Tetrastichus coccinellae* during June-September 2004 in Greece.

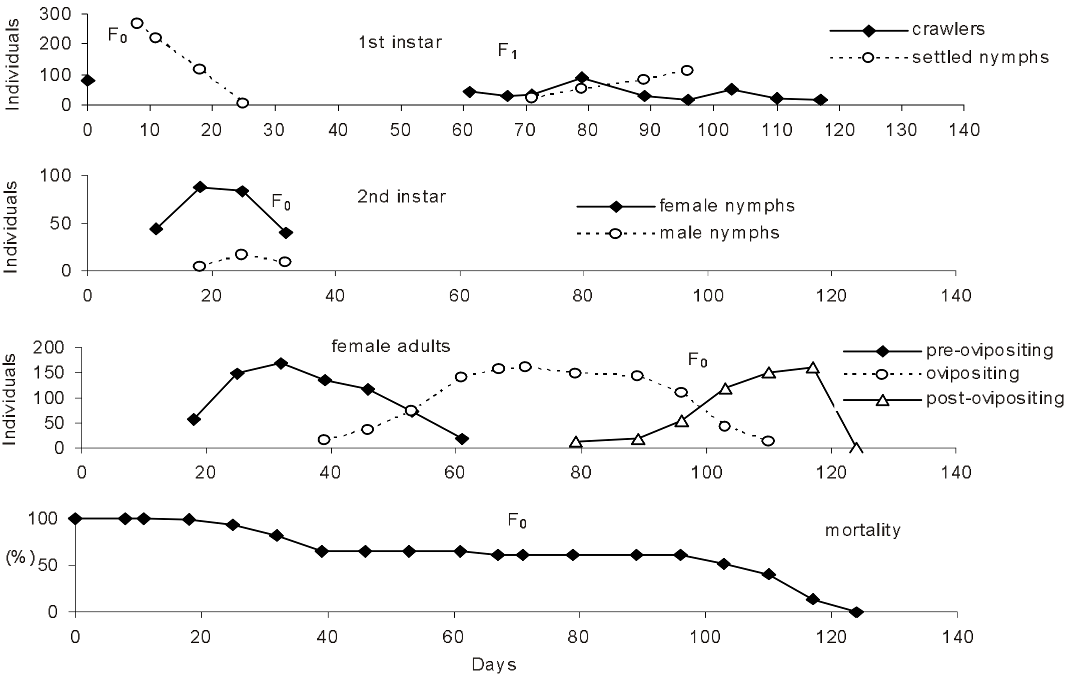

Figure 4. Development of *Parlatoria ziziphi* on sour orange trees under controlled conditions (25°C, 65%R.H., 16h light/day) in the laboratory. Where F_0 = the initial population and F_1 = 2nd generation.

Acknowledgments

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