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Parasitoid community of *Protopulvinaria pyriformis* (Hemiptera: Coccidae) on laurel: Influence of host size, seasonal trend and aggregation on its main parasitoid

Aleixandre Beltrà [a], Antonia Soto [a], Alejandro Tena [b,*]

[a] Instituto Agroforestal del Mediterráneo, Universidad Politécnica de Valencia;Camí de Vera s/n, 46020 Valencia, Spain
[b] Unidad Asociada de Entomología UJI-IVIA-CIB CSIC, Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias, IVIA, Ctra. de Moncada a Náquera km 4.5, E-46113 Moncada, Spain

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ABSTRACT

The phenology of *Protopulvinaria pyriformis* (Cockerell) (Hemiptera: Coccidae) and its parasitoid complex were studied on laurel *Laurus nobilis* L. in urban green spaces of Eastern Spain from March 2008 to January 2010. *P. pyriformis* is bivoltine, gravid females peaked on spring and fall and the 2nd and 3rd instars were present throughout the year. The natural parasitoid community of *P. pyriformis* consisted of three primary parasitoids and four facultative hyperparasitoid species. *Metaphycus helvolus* (Compere) (Hymenoptera: Encyrtidae) accounted for 98% of the parasitoids recovered. Although *M. helvolus* parasitized all the scale instars, it attacked *P. pyriformis* mostly at the end of the spring, when scales were larger. *M. helvolus* behaved as a solitary parasitoid and its secondary sex ratio was male biased when it parasitized the 2nd instar. Its secondary sex ratio became female biased when it parasitized scales larger than 1.1 mm. *M. helvolus* behaved as a facultative gregarious parasitoid and its secondary sex ratio was always female biased (0.3 males per scale) when it parasitized the 3rd instar and the adults. The number of eggs allocated per host, as well as the number of parasitoids that emerged, increased with scale size. Mostly, broods of two to four parasitoids emerged from these scales. *M. helvolus* showed inverse host density dependence, parasitism decreased as the number of scales per leaf increased. The implications of these results on the biological control of this and other soft scales are discussed.

1. Introduction

The laurel tree *Laurus nobilis* L. (Lauraceae) is an evergreen plant of Mediterranean origin (Linné and Salvii, 1753). It is widely planted as ornamental tree and bush in urban green spaces (Tucker and DeBaggio, 2009) and also cultivated for its use in chemical and alimentary industries (Di Leo Lira et al., 2009). The integrated pest management of laurel trees in Western Europe is focused on three pests: the psyllid *Trioza alacris* Flor (Hemiptera: Psyllidae), the diaspidid *Aonidia lauri* Bouché (Hemiptera: Diaspididae) and the soft scale *Protopulvinaria pyriformis* (Cockerell) (Hemiptera: Coccidae) (Harranger, 1983; Landi and del Bene, 1994; Stathas et al., 2009).

P. pyriformis is a polyphagous soft scale of Neotropical origin (Sinacori, 1995). It is considered a pest of tropical and subtropical fruit trees as avocado and guava and also of ornamental plants in American continent, south of Africa and Mediterranean Basin (Wysoki, 1987; Llorens, 1990; Ben-Dov, 1993). In the latter area, it was firstly recorded in 1948 (Gómez-Menor, 1948) and it became a key pest of laurel and avocados (Swirski et al., 1988; Stathas et al., 2009). The scale reproduces parthenogenetically in Europe (Llorens, 1990; Sinacori, 1995) and it completes two generations per year in avocados in Spain (Llorens, 1990) and Israel (Blumberg and Blumberg, 1991) while several overlapping generations have been reported in laurel in Greece (Stathas et al., 2009). *P. pyriformis* settles on the lower part of the leaves, mainly along the veins, where they feed on the sap (Ben-Dov, 1993). Its feeding reduces plant vigor and the honeydew secreted promotes the growth of black sooty mold that interferes with photosynthesis (Ebeling, 1959). Moreover, on ornamental plants, sooty mold causes aesthetic depreciation and the honeydew disturbs the garden users. Control of *P. pyriformis* has been traditionally carried out using broad-spectrum insecticides in commercial nurseries and urban green spaces in Spain. However, laurel trees grown in commercial nurseries have been recently included in the European list of food and feed products, increasing the restrictions of the residual levels of pesticides (European Commission, 2006). Moreover, the new European directive about pesticide use establishes the reduction or even the prohibition of pesticides in a wide range of urban green areas, giving a significant priority to biological control (European Parliament and Council, 2009).

Biological control of *P. pyriformis* has been studied in detail in avocados in Israel (Blumberg, 1991; Blumberg et al., 1993) and

* Corresponding author. Fax: +34 96 342 41 51.
E-mail address: atena@ivia.es (A. Tena).

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South Africa (Robertson and De Villiers, 1986) and just recently in laurel in Greece, where its control by its natural enemies is unsatisfactory (Stathas et al., 2009). Several parasitoids of genus *Metaphycus* have been identified as the main parasitoids of *P. pyriformis*: *Metaphycus stanleyi* Compere and *Metaphycus swirskii* Annecke & Mynhardt in avocados in Israel (Blumberg, 1991; Blumberg et al., 1993); these two parasitoids and, also, *Metaphycus galbus* Annecke in this crop in South Africa (Robertson and De Villiers, 1986); and, finally, Stathas et al. (2009) only encountered *Metaphycus helvolus* in laurel in Greece. In Almeria (southern Spain), Blumberg et al. (1993) collected *M. stanleyi* and *M. galbus* on avocados in an occasional sample. However, the parasitoid community of *P. pyriformis* in laurel has never been studied in Spain and, more importantly, the behavioral ecology of *M. helvolus*, the main parasitoid when the scale is settled on laurel (Stathas et al., 2009), is unknown. Species of genus *Metaphycus* are primary endoparasitoids of soft scales and have some characteristics that make them potential effective natural enemies of *P. pyriformis*. Most of them are generalists and can alternate different hosts. Moreover, they commonly develop as facultative gregarious parasitoids and their secondary sex ratio, proportion of emerged males, is frequently female biased. In addition, some of them kill young scales when feeding on their haemolymph (Lampson et al., 1996; Bernal et al., 1998; Kapranas et al., 2008; Tena and Garcia-Marí, 2008; Kapranas et al., 2009; Tena and Garcia-Marí, 2009). However, the lifespan of adults depends highly on the presence of sugar resources (Bernal et al., 1999; Kapranas and Luck, 2008). Moreover, the encapsulation of *Metaphycus* eggs by *P. pyriformis* is considered an important cause of the unsatisfactory biological control of this pest in Israel and Greece (Blumberg, 1991; Blumberg et al., 1993; Stathas et al., 2009).

The recent European directive about sustainable use of pesticides in urban green spaces and the satisfactory biological control of other soft scales by parasitoids of genus *Metaphycus* lead us to initiate a study to elucidate the relationship between *P. pyriformis* and its parasitoids in Spain in order to improve its biological control. More specifically, we sought to determine the parasitoid complex of *P. pyriformis* in laurel and the interaction between *P. pyriformis* and its main parasitoid, focusing on parasitism rates, the influence of scale size on clutch size (number of eggs allocated per host), brood size (progeny emerged per host) and sex ratio of these parasitoids and the effect of host distribution on parasitism. With these results and the seasonal trend of *P. pyriformis* described herein we suggest strategies to improve the biological control this pest.

2. Material and methods

2.1. Survey site and sampling protocol

Three urban green spaces that contained trees and bushes of laurel infested by *P. pyriformis* were sampled from March 2008 until January 2010. The urban green spaces were located in Valencia (Blasco Ibañez Garden [39.477662N; 0.361471W] and Tendetes Park [39.482231N; 0.389121W]) and Castellón de la Plana (Paseo Ribalta Park [39.988619N; 0.043965W]). These sites were sampled twice a month during the periods of fast scale growth (May–August) and monthly during the rest of the year. Blasco Ibañez Garden contained 20 trees (between 3 and 30 years old) and 10 bush-lines of laurel trees. Tendetes Park (Valencia) contained 12 laurel trees (5–10 years old) spread in a green area of 1 ha. Paseo Ribalta Park (Castellón de la Plana) contained 16 laurel trees (8–30 years old) spread within a green area of 2 ha. Insecticides were not applied during the sampling period to these urban green spaces. Additionally, other eight areas, located along Eastern Spain, were sampled once between spring and summer of 2008 to widen the parasitoid complex. The data of these eight sites was used only to determine the parasitoid complex.

Eight scale-infested shoots (1–2 years old) were collected from four different trees or bushes on each sampling site and date. Shoots were cut from the peripheral 40 cm of each plant between 0.3 and 2 m above the ground. We only selected twigs within trees or bushes that were infested with *P. pyriformis* because the prior aim of this study was to determine its main parasitoids and their relationship. Samples were placed in plastic bags and immediately transported to the laboratory for examination. Once there, plastic bags were opened and the shoots were maintained in a plant growth chamber at 10 °C and 50% R.H. Samples were processed according to the procedures described below on the next 24 h.

2.2. Scale phenology

Two leaves per shoot (16 leaves in total) with more than ten scales each were randomly selected and their lower side examined with a stereoscope provided with an ocular micrometer in the eyepiece. *P. pyriformis* settles down only on the lower side of the leaf when it feeds on laurel. For each leaf, we determined the developmental instar of 10 randomly selected scales and we also measured them. They were characterized as 2nd and 3rd nymphal instar following the description of Ray and Williams (1982), and the adult stage classified as young or gravid adults according to the presence of eggs. The length of these scales was measured to the nearest 0.01 mm. The total number of live scales present on each leaf was also recorded. First instar nymphs were not measured and were counted separately from the other instars because they cannot be parasitized.

2.3. Parasitoid survey, parasitism rates and parasitoid-scale relation

All the scales present in the leaves were directly checked for parasitism signs (eggs, larvae and pupae of parasitoids). The scale's transparent cuticle of the immature *P. pyriformis* coupled with the *Metaphycus*'s egg stalk that protrude the scale dorsum allowed us to locate parasitized scales (Kapranas et al., 2009; Tena et al., 2009). Parasitism rates from March to December 2008 and 2009 were calculated as the proportion of the total number of parasitized scales in relation to the total number of scales susceptible of parasitism (2nd and 3rd instar and adults). We pooled the data of the 16 sampled leaves and calculated the parasitism rate for each instar only when there were at least 15 scales of that instar Leaves with parasitized scales were placed in a plastic ventilated cage at 23 ± 5 °C under outside natural photoperiod, with a wet cotton piece inside to maintain the leaf humidity. Scales were observed twice a week to check the development of the parasitoids. We counted the number of eggs allocated by parasitoids of genus *Metaphycus* in each scale. The eggs can be recognized by the presence of the protruding stalk through the scale dorsum (Kapranas et al., 2009; Tena et al., 2009). Once the parasitoid pupated, its host scale was gently separated from the plant with a wet camel's hair brush and introduced individually in a 3.0 by 0.8-cm glass vial. The vials were stopped with a cotton plug and conserved at the same conditions described above and checked twice a week for parasitoid emergence. Upon emergence, each vial was placed in a freezer to kill adult parasitoids. Then, they were identified using different Chalcidoidea keys (Guerrieri and Noyes, 2000) and the number and sex of parasitoids that emerged per scale was recorded. These data were used to determine the clutch and brood size and the secondary sex ratio of the parasitoids.

2.4. Statistical analysis

Clutch size, brood size and secondary sex ratio were evaluated using general linear modeling techniques (Wilson and Hardy,