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Scale Insects and Natural Enemies Associated with Conilon Coffee (*Coffea canephora*) in São Paulo State, Brazil

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Abstract: Several insect pests are related to the cultivation of conilon coffee, *Coffea canephora* (Rubiaceae), including (Hemiptera: Coccomorpha). Coccoids damage plants by sucking their sap, producing honeydew, and transmitting viruses. Parasitoids and predators are natural enemies that regulate the insect population and can be used in mealybug biological control. This study aimed to survey scale insects and natural enemies associated with *C. canephora* in the city of Jaboticabal, São Paulo State, Brazil. Two species of mealybugs from the family Coccidae and three from the family Pseudococcidae were collected in different plant structures. Natural enemies collected comprised hymenopteran parasitoids from the families Aphelinidae, Eulophidae, Encyrtidae, and Perilampidae; predator beetles of the family Coccinellidae; dipterans from the family Cecidomyiidae; and thrips of the family Aeolothripidae. This is the first report of the mealybug species *Coccus brasiliensis*, *Pseudococcus longispinus*, and *Pseudococcus cryptus*; of the parasitoids *Coccophagus rusti*, *Aprostocetus* sp., *Aenasius advena*, *Aenasius fusciventris*, *Aenasius pseudococci*, and *Perilampus* sp.; and of the predators *Cycloneda conjugata*, *Pseudoazya nana*, *Diadiplosis coccidivora*, *Diadiplosis* sp., and *Franklinothrips vespiformis*, associated with *C. canephora*. Knowledge of mealybug species and their respective natural enemies will contribute to biological control strategies in planning the integrated management of mealybugs associated with conilon coffee.

Keywords: biological control; Coccoidea; host; parasitoids; predators; prey

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1. Introduction

Coffee is an agricultural crop of great impact worldwide, mainly because it is the most consumed beverage [1] and for being, after oil, the main commercialized commodity [2,3]. *Coffee* sp. is a plant native to the African continent [3], but Brazil is the largest producer and exporter in the world [4].

The Brazilian states of Minas Gerais, Espírito Santo, São Paulo, and Bahia hold about 93% of the country's production [5]. *Coffea arabica* L. and *Coffea canephora* L. are the main cultivated species; each one has a characteristic flavor and aroma [1], with *C. canephora* being popularly known as conilon coffee and the second most commercialized after *C. arabica* [5].

Biotic factors are observed to affect *C. canephora* production, including the incidence of both nymphs and adult scale insects [6], which cause damage to coffee plants and can feed on different parts of the plant, such as floral rosettes and fruits [7], leaves, stems, and roots [8]. As a result, flower buds and new fruits drop, unhealthy fruit development occurs and hence there is lower production [7].

In addition to direct damage, mealybugs (Pseudococcidade) can indirectly inoculate toxic substances, and be vectors of diseases [9,10]. Another indirect effect is the attraction of ants to honeydew produced, which promotes the development of fungi such as sooty molds [11], which affects plant photosynthetic rate [12]. In coffee with high mealybug infestation, it can cause partial or total loss of production or even the death of the plant [10].

Apart from pests, diverse natural enemies may be present in the agricultural environment and act in the control of these coffee pests [13]. Forty-seven species of scale insects have been recorded associated with arabica coffee in Brazil, distributed in seven families [14]. Parasitoids and predators are reported as the main natural enemies of mealybugs that infest *C. arabica* [14].

However, even in the main coffee-producing regions in the world, there is little information on attacking *C. canephora* or their respective natural enemies. Knowledge of biological control agents is crucial for pest regulation and can be used in integrated pest management (IPM) [15] in coffee fields.

Thus, the objective of this study was to survey the scale insects and natural enemies associated with *C. canephora* in the city of Jaboticabal, state of São Paulo (SP), Brazil. These studies have revealed prominent biological control agents of the mealybugs that attack the coffee and recorded the first associations of scale insect species with *C. canephora*.

2. Materials and Methods

The survey was carried out monthly in seven *C. canephora* clones of the cultivar EMCAPA, from June to December 2018 and from January to May 2019. The study took place at the College of Agricultural and Veterinary Sciences, São Paulo State University (FCAV/UNESP), in Jaboticabal city, São Paulo, Brazil. Adult mealybugs were collected from rosettes, leaves, and branches of coffee trees. The insects were collected together with the parts of coffee plants where they were found.

All material were packed in Kraft paper bags, identified, and transported to the Hemiptera Biosystematics Laboratory (LABHEM) of the FCAV/UNESP. After screening, 25% of the adult mealybugs were separated to assemble slides for species identification.

Female specimens were prepared in permanent slides, using the technique described by [16,17], and were identified under an optical microscope (Axio Scope A.1 -Zeiss), through morphological characteristics. Keys [18] were used to identify species of the family Coccidae, Refs. [12,16,18] for species of the family Pseudococcidae, in addition to fact sheets, keys, and the image gallery found at the scale insect database ScaleNet website [14].

The other scale insects were kept in test tubes covered with aluminum foil and sealed with plastic film. The tubes were kept in an incubator chamber B.O.D. (Biochemical Oxygen Demand) under controlled conditions (25 $\pm$ 2 °C, 12 h photoperiod, and 65 $\pm$ 5% RH) for 30 days or until obtaining natural enemies, following the method adopted by [19].

Observations were made every 24 h after emergence; parasitoids were transferred to 2 mL microtubes filled with 70% ethanol. Subsequently, the parasitoids were identified according to the possible taxon, using the taxonomic keys of [20,21] and Chalcidoidea species were identified as in [22–24].

The scale insects found in the field, along with the collected predators, were used as prey to feed immature predators until reaching maturity. The predators were identified according to the possible taxa, using the taxonomic keys of [25–30].

The natural enemies were collected by capturing their host, scale insects, or prey. At the time of collection, we recorded in which part of the plant the scale insect was collected. The specimens were deposited in the Reference Collection of Insects and Mites, except for those of the family Aphelinidae, which were deposited in the Collection of Entomophagous Insects "Oscar Monte" of the Biological Institute (Instituto Biológico, in Portuguese) in the city of Campinas, São Paulo, Brazil.

3. Results

In total, five species of scale insects were collected, *Coccus brasiliensis* Fonseca, *Coccus viridis* Green (Coccidae), *Dysmicoccus brevipes* (Cockerell), *Pseudococcus longispinus* (Targioni Tozzetti), and *Pseudococcus cryptus* (Hempel) (Pseudococcidae). This is the first report associating *C. brasiliensis*, *P. longispinus*, and *P. cryptus* with *C. canephora*.

Overall, 13 species of natural enemies were obtained, of which seven were hymenopteran parasitoids, all endoparasitoids, belonging to four families. *Coccophagus rusti* (Compere), (Aphelinidae), and *Aprostocetus* (Westwood) sp. 1, sp. 2 (Eulophidae) emerged from *C. brasiliensis. Aenasius advena* (Compere) (Encyrtidae), and *Perilampus* sp. (Perilampidae) emerged from *P. cryptus.* Moreover, the parasitoids *Aenasius fusciventris* (Girault) and *Anagyrus pseudococci* (Girault) (Encyrtidae) emerged from *P. longispinus* (Table 1). This is the first record of *C. rusti* and *Aprostocetus* sp. associated with the soft scale species *C. brasiliensis* and conilon coffee. Similarly, this is the first time *A. advena* and *Perilampus* sp. have been associated with the mealybug *P. cryptus* and conilon coffee. The parasitoids *A. fusciventris* and *A. pseudococci* were also associated with conilon coffee.

Table 1. Hymenopterous parasitoids, their hosts, and the coffee plant structure (*C. canephora*) where insects were collected.

Parasitoid		Mealybug		Plant Structure
Family	**Genus or Species**	**Family**	**Genus or Species**	
Aphelinidae	*Coccophagus rusti* *	Coccidae	*Coccus brasiliensis* *	Fruits and leaves
Encyrtidae	*Aenasius advena* *	Pseudococcidae	*Pseudococcus cryptus* *	Fruits and stem
Encyrtidae	*Aenasius fusciventris* *	Pseudococcidae	*Pseudococcus longispinus* *	Fruits
Encyrtidae	*Anagyrus pseudococci* *	Pseudococcidae	*Pseudococcus longispinus*	Stem
Eulophidae	*Aprostocetus* sp. 1 *	Coccidae	*Coccus brasiliensis*	Fruits and leaves
Eulophidae	*Aprostocetus* sp. 2 *	Coccidae	*Coccus brasiliensis*	Fruits and leaves
Perilampidae	*Perilampus* sp. *	Pseudococcidae	*Pseudococcus cryptus*	Fruits and stem

* New records associated with *C. canephora.*

In addition to the parasitoids, six species of predators were collected and distributed into three orders: Coleoptera, Diptera, and Thysanoptera. All collected coleopterans belong to the family Coccinellidae, *Azya luteipes* (Mulsant), *Pseudoazya* cf. *nana* (Marshall), and *Cycloneda conjugata* (Mulsant) (Table 2).

Table 2. Predators, scale insects, and the structure of the coffee plant (*C. canephora*) where prey was collected.

Predator		Mealybug		Plant Structure
Order/Family	**Genus or Species**	**Family**	**Genus or Species**	
Coleoptera/Coccinellidae	*Azya luteipes*	Coccidae	*Coccus viridis* *	Fruits and leaves
Coleoptera/Coccinellidae	*Cycloneda conjugate* *	Coccidae	*Coccus brasiliensis*	Fruits
Coleoptera/Coccinellidae	*Pseudoazya* cf. *nana* *	Coccidae	*Coccus brasiliensis* *Coccus viridis*	Fruits, leaves and branch
Diptera/Cecidomyiidae	*Diadiplosis coccidivora* * *Diadiplosis* sp. 1 *	Coccidae	*Coccus brasiliensis*	Fruits
Thysanoptera/Aelothripidae	*Franklinothrips vespiformis* *	Coccidae	*Coccus brasiliensis*	Fruits

* New records associated with *C. canephora.*

Two species of dipterans were obtained, *Diadiplosis coccidivora* (Felt) and *Diadiplosis* sp. 1 (Cecidomyiidae), and one species of thrips *Franklinothrips vespiformis* (Crawford) (Thysanoptera: Aelothripidae). All predators were associated with the mealybug species *C. brasiliensis*, except for *A. luteipes*, which was associated with the species *C. viridis*. Moreover, besides being associated with *C. brasiliensis*, *Pseudoazya* cf. *nana* was also associated with *C. viridis* (Table 2). Among the predators collected, it is the first report of *C. conjugata*, *Pseudoazya* cf. *nana*, *D. coccidivora*, *Diadiplosis* sp., and *F. vespiformis* associated with the

soft scale *C. brasiliensis* and conilon coffee. In addition, this was the first association of *Pseudoazya* cf. *nana* with *C. viridis* in *C. canephora*.

4. Discussion

Coffea canephora plants can harbor a great diversity of arthropods. In this work, scale insect species were reported for the first time associated with conilon coffee. Phytophagous species that feed on coffee plant, such as scale insects, are well known. These insects are one of the main coffee pests, mainly because they reduce crop yields as a result of the injuries caused by their attack [31,32]. Allied with this, their respective natural control agents, such as parasitoids and predators, should be investigated, highlighting the complex trophic interactions present in conilon coffee plants. The results of this research emphasize the need to understand natural enemies to be used in biological pest control [33,34].

Many species of the genus *Coccus* have been found in the Neotropical region [14]. Among the species associated with coffee plants, *C. brasiliensis* has only been reported associated with *C. arabica* in the region of Botucatu, state of São Paulo [17]. However, even for *C. arabica*, there is little information on the behavior of *C. brasiliensis* on the plant, its geographical distribution, or its natural enemies. Thus, this is the first report that shows the parasitoids and predators associated with the soft scale *C. brasiliensis* in conilon coffee trees.

However, the natural enemies found in this study have already been reported as parasitoids and predators of other arthropod species. The species *C. rusti* has already been reported as a parasitoid of other soft scales, such as *Saissetia oleae* (Coccidae) in olive trees [35] and *C. viridis* in coffee trees [36]. The hosts of *Aprostocetus* are extremely variable and often associated with gall-inducing insects, such as the dipteran Cecidomyiidae [23]. *C. conjugata* has been reported as a predator of *Psylla* sp. (Hemiptera: Psyllidae) [37]. *Pseudoazya* cf. *nana* has been associated with *Triozoida limbata* (Enderlein) (Hemiptera: Psyllidae) [38], and *D. coccidivora* has been identified as a predator of mealybugs in coffee systems [39]. Finally, *F. vespiformis* is a generalist predator thrips that feeds on a wide variety of insects and mites [27].

Coccus viridis is known as the green coffee scale [40]. Its adults are green with U-shaped black spots on their dorsal surface, measure between 2.5 and 3.3 mm, and have an oval and flattened shape. The insect is ovoviviparous and reproduces by thelytokous parthenogenesis, having three nymphal instars and an adult instar [41]. Its occurrence has been recorded on different continents [32] and affects many plant species of economic importance [17,40].

In *C. canephora*, *C. viridis* attacks leaves, sprouts, and fruits [8,14] and can be present throughout the entire coffee development, causing problems [42]. By feeding, green leafhoppers inject toxins into the vascular tissue of plants, causing hypertrophy of exchange cells and collapse of phloem cells [43]. The attack reduces stem diameter, leaf area, root weight, and harvest yield [40]. Like many scale insect species, *C. viridis* is easily adaptable to new areas and has a high reproductive capacity [44].

Due to honeydew production, ants actively protect colonies of *C. viridis* against predators and parasitoids and hence the mealybug population grows [45]. Thus, high populations of *C. viridis* may interfere with the biological control of these mealybugs [46]. Some natural enemies of *C. viridis* have been already described [47,48]. The Coccinellidae found in *A. luteipes*, associated with *C. viridis*, was also reported by [49], preying on this soft scale species. However, ours was the first report of the coccinellid *Pseudoazya* cf. *nana*, complementing the knowledge of natural enemies of *C. viridis* in the studied region.

Dysmicoccus brevipes, known as pink pineapple mealybug [50], has widespread occurrence in all parts of the world [51]. It is associated with more than 140 plant genera [52], including *C. canephora* [8]. Its reproduction is sexual or by thelytokous parthenogenesis [53], with adult females measuring about 3 mm long and their bodies covered by a powdery secretion of white wax. It presents rosy coloration, oval shape, and 17 pairs of wax-producing glands that produce short wax filaments, with the four posterior pairs being higher at the end of the abdomen [9].

Dysmicoccus brevipes has a short development time; females present a paurometabolous development, with three instars followed by the adult stage. On the other hand, males have two free instars at the end of the second and build a cocoon of waxy filaments. Inside the cocoon, males undergo pre-pupal, pupal, and adult stages, followed by their emergence after sexual maturity [54]. It has high survival rates and is considered a pest of economic importance for some crops such as pineapples [55] and grapevines [53]. Moreover, it is a vector of diseases such as the mealybug wilt of pineapple [56].

In this study, *D. brevipes* was found feeding on sap and inflorescence of coffee plants, corroborating the same habits reported by [57]. [58] mentioned the symbiotic relationship between *D. brevipes* and ants, especially the bigheaded ant *Pheidole megacephala* (Fabricius) (Hymenoptera: Formicidae). This ant species is responsible for spreading mealybugs and protecting them against natural enemies, removing excess honeydew produced. Although we did not find natural enemies associated with *D. brevipes*, several insects are known to regulate populations of *D. brevipes* [59]. These include the encyrtids *Anagyrus ananatis* Gahan, and *Euryrhopalus propinquus* Kerrich, and the predators *Lobodiplosis pseudococci* (Felt) (Cecidomidae), and *Nephus bilucernarius* Mulsant (Coccinelidae) [58].

The longtailed mealybug (*P. longispinus)* has been reported in many countries. It is an oviparous species with four instars in females and five in males [60]. Its adults have some long wax filaments around their bodies, with the last two being longer, resembling long tails [61]. It has been reported in pineapple plants [50], ornamental plants [62], and grapevines [63]. In coffee plants, this insect has been sporadically reported. It has been recorded sucking the sap of coffee fruit. Moreover, *P. longispinus* causes lesions, drying, and fruit drop [64].

Sympherobius fallax Navas (Neuroptera: Hemerobiidae) larvae are predators of *P. longispinus* [61]. These mealybugs can secrete ostiolar fluids that harden on the mouth apparatus of predators. In addition, they can push the lacewing away with their caudal filaments. Moreover, *A. fusciventris* and *A. pseudococci* are already known as parasitoids of the longtailed mealybug, but these are associated with crops other than coffee [65,66].

Furthermore, *P. cryptus* (synonym *P. citriculus* Green), known as the cryptic mealybug, is a well-known mealybug, mainly because it is an important pest in citrus [66]. *Pseudococcus cryptus* is an extreme polyphagous pest with more than 80 documented genera including *Coffea* and can be found in all parts of the world. According to [14], two species of coffee trees (*C. arabica* and *Coffea liberica)* are associated with this mealybug; however, *C. canephora* is not yet associated. The cryptic mealybug has ovoviviparous reproduction, as well as three nymphal stages and the adult stage [67]. However, these developmental stages are hardly discriminated from each other, as nymphal stages undergo few changes [68].

Unlike the other scale insects found in our study, *P. cryptus* prefers to feed on roots but can attack all other structures of the plant [69]. Although several natural enemies have been reported associated with *P. cryptus* [39,66], this is the first finding of the genus *Perilampus* and the species *A. advena*. According to [20], little is known about the hosts and species of *Perilampus.* Often they are associated with agricultural pests, related as hyperparasitoids of the family Ichneumonidae and Tachinidae dipterans. *A. advena* has been recorded as a parasitoid of the mealybug *Ferrisia virgata* (Cockerell) (Hemiptera: Pseudococcidae) in several countries, in addition to being reported parasitizing *P. longispinus* [70].

Despite all the above, mealybugs are difficult to control, mainly by chemical methods. This stems mainly from their protective wax coating [59] or cryptic habits [39], which prevents insecticides from reaching them. Therefore, these insects are among the most economically important pests for agriculture [66]. Hence, the species and natural enemies of mealybugs associated with conilon coffee should be known for the planning of MIP in coffee plantations.

Demand for healthy foods and concern about the environment has led to the adoption of suitable control measures. Among them, there is a challenge in how to use natural enemies to control mealybugs. Thus, due to a lack of studies, these natural enemies are

unavailable in a commercial scope [66]. In this context, our findings provide elementary knowledge to plan control strategies for mealybugs associated with conilon coffee.

5. Conclusions

Parasitoids and predators are organisms responsible for the biological regulation of countless pest insects. The scale insects *Coccus brasiliensis*, *Coccus viridis* (Hemiptera: Coccidae), *Dysmicoccus brevipes*, *Pseudococcus longispinus*, and *Pseudococcus cryptus* (Hemiptera: Pseudococcidae) were recorded in this work. Natural enemies collected included *Coccophagus rusti* (Hymenoptera: Aphelinidae), *Aprostocetus* sp. 1, sp. 2 (Hymenoptera: Eulophidae), *Aenasius advena*, *Aenasius fusciventris*, *Anagyrus pseudococci* (Hymenoptera: Encyrtidae), *Perilampus* sp. (Hymenoptera: Perilampidae), *Azya luteipes*, *Pseudoazya* cf. *nana*, *Cycloneda conjugata* (Coleoptera: Coccinellidae), *Diadiplosis coccidivora*, *Diadiplosis* sp. (Diptera: Cecidomyiidae), and *Franklinothrips vespiformis* (Thysanoptera: Aelothripidae). All natural enemies were associated with the scale insects. However, discovering natural enemies that can be found in coffee plants is of prominent importance for the maintenance of crops through biological pest control.

Author Contributions: I.L.S. and H.H.d.P. developed the idea for the study, the research question, and the experimental design. H.H.d.P. conducted the experiments. I.L.S., M.A.d.S., V.A.C. and A.P.G.d.S.W. identified the Parasitoids. H.H.d.P. and A.L.B.G.P. identified the mealybugs. I.L.S. and H.H.d.P. analyzed the data and interpreted the results. V.A.C. took the picture of the graphical abstract. I.L.S. and H.H.d.P. wrote and revised the manuscript based on comments and suggestions from N.M.M. and A.L.B.G.P. All authors have read and agreed to the published version of the manuscript.

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