Feeding Behavior of *Cryptolaemus montrouzieri* on Mealybugs Parasitized by *Anagyrus pseudococci*

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The citrus mealybug *Planococcus citri* (Risso) and the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) are two worldwide polyphagous pests of citrus, vineyards and ornamental plants in greenhouses. Biological control of these pests may rely on the combined release of parasites and predators, which can be affected by intraguild predation (IGP). This study investigated the feeding behavior of different stages of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) on mealybugs parasitized by *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae) 2, 4, 6, 8 and 10 days. The study was conducted in a climate-controlled room at 28±1°C, 16L:8D, and 65±10% r.h. The highest consumption values for all stages of *C. montrouzieri* occurred with 2- and 4-day parasitized mealybugs, whereas the predator did not feed on either species of mealybug parasitized for longer periods, due to the onset of mummification.
KEY WORDS: Citrus mealybug; intraguild predation; *Planococcus citri; Planococcus ficus;* vine mealybug.

INTRODUCTION

The citrus mealybug *Planococcus citri* (Risso) is a polyphagous citrus pest found in tropical and subtropical regions. The vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) is another cosmopolitan species and pest of grapes and figs as well as ornamental plants in greenhouses (3,17,18). Both mealybug species feed on the roots, trunk, cordon, canes, leaves and fruit of host plants, damaging them either directly, by depleting their sap, or indirectly, as a result of sooty moulds that grow on the honeydew excreted by the insects as they feed. Moderately high mealybug population densities can lead to leaf drop and weakened plants, resulting in reductions in crop quality and yield. Furthermore, as vectors of viral diseases (1,9), mealybugs, even at low densities, can have negative economic consequences for growers. A lack of effective control of *P. citri* has been shown to result in up to 80–90% losses of yield in citrus plants (39). Control of *P. citri* and *P. ficus* with insecticides is difficult because of the mealybugs' ability to hide in bark crevices and other inaccessible places and to secrete thick layers of protective wax (18). Biological control by parasitoids and predators represents the most important method of controlling these species, not only because chemical control is not effective enough, but because it is environmentally undesirable as well (4,5).

Mass rearing and release of parasitoids and predators are among the basic elements of biological control of mealybugs. At the same time, protection of endemic natural

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enemies should be considered in the success of any biological control program. *Anagyrus pseudococci,* a solitary, koinobiont and endoparasitoid species, is one of the most important and well-known parasitoids of *P. citri* and *P. ficus* (29). As a generalist parasitoid, *A. pseudococci* attacks several other mealybug species as well (19). It prefers 3^{rd} instar nymphs and virgin adult female mealybugs, but it is also capable of parasitizing 1^{st} and 2^{nd} instar nymphs (17,36). Due to its extensive range of hosts and geography, *A. pseudococci* has often been used for biological control of mealybugs (17,23,35,36). Not only is it one of the most commonly used commercial parasitoids (2), it is also a basic parasitoid of citrus and vine mealybugs in Israel (5), Italy (12), Argentina (37) and Turkey (19,38). Another important biological control agent of *P. citri* and *P. ficus* is the predator *Cryptolaemus montrouzieri* (13,15,16). Native to Australia, *C. montrouzieri* is unable to overwinter in subtropical regions (40); therefore, its use as a biological control agent in most non-tropical countries is dependent on mass rearing and augmentative releases. *C. montrouzieri* has been mass-reared at the Adana and Antalya Plant Protection Research Institutes since 1970 and has been used throughout Turkey to control *P. citri* (40).

Biological control of mealybugs can be carried out through the release of either a single parasitoid or predator species, or through the combination of two or more agents released together. While the concurrent release of several natural enemies can be more effective than the release of a single agent (10,13,21,26), it can, conversely, lower the success of a control program as a result of competition between natural enemies (7,30,32), including those that are endemic as well as those that are released for control. For example, the simultaneous release of a predator and a parasitoid can negatively affect a population of native parasitoids, as the predator feeds on the parasitoid feeding within the host. This killing and eating among potential competitors (2) is known as intraguild predation (IGP) and is a common occurrence in ecological systems (31). Feeding on another predator or immature parasitoid in or on prey allows predators to eliminate potential competitors as well as to supplement their nutrition. IGP generally depends upon the size and feeding specificity of the protagonists and occurs mainly with generalist predators that attack prey of a smaller size (31). The mobility of the parasitized prey also influences the results of IGP, as in the case of mealybugs, where the reduced mobility of the parasitized prey makes them more prone to predation (27). This study examined the mechanism of IGP between the two main antagonists of two important mealybug species with the aim of increasing the effectiveness of biological control efforts.

MATERIALS AND METHODS

Mealybug source A *P. citri* colony was obtained from a long-established rearing colony at the Antalya Provincial Agricultural Directorate, and a *P. ficus* colony was obtained from a vineyard in Ankara Province. Both mealybug species were reared on potatoes in plastic cages ($44 \times 47 \times 67$ cm) covered by muslin (40 mesh/cm^2) in a climate-controlled room at 28 ± 1°C, 16L:8D and 65 ± 10% r.h. One ovisac from each mealybug culture was placed on a potato in a small plastic cage ($5 \times 5 \times 3$ cm) for use in the experiment.

Parasitoid source A stock culture of *A. pseudococci* obtained from *P. ficus* mealybugs collected from a vineyard in Ankara. The parasitoids were reared on *P. citri* and *P. ficus* grown on sprouted potatoes in plastic cages as described above for rearing of mealybugs. *A. pseudococci* visually sexed (males are black and $\sim$1.0 mm in length, whereas females are grayish brown and $\sim$1.5–2.0 mm), and 15 pairs were transferred into a small plastic cage

(5×5×3 cm) containing one potato infested with 3^{rd} instar nymphs and virgin females of each species of mealybug. The mealybugs were collected on a daily basis using a fine brush in order to obtain parasitized specimens of different ages for the experiments.

Predator source A stock culture of *C. montrouzieri* was from the Adana Plant Protection Research Institute. The predators were reared on *P. citri* and *P. ficus* grown on sprouted potatoes in plastic cages as described above for rearing of mealybugs. Thirty pairs of adult *C. montrouzieri* were transferred into a large plastic cage containing sprouted potatoes infested with mealybugs reared weekly from an ovisac, and presented to the predators.

Experimental procedure This experiment was planned to identify variations in *C. montrouzieri* predation of *P. citri* and *P. ficus* by predator stage and age of mealybug parasitized by *A. pseudococci*. In order to obtain mealybug specimens parasitized for 2, 4, 6, 8 and 10 days, 15 pairs of adult *A. pseudococci* were released into a small plastic cage containing one sprouted potato infested with 3^{rd} instar nymphs and virgin adult females of both mealybug species (*P. citri,* aged 18 days; *P. ficus,* aged 21 days). Parasitoids were removed 24 h after their introduction. Parasitized mealybugs were gathered using a fine brush and offered to the *C. montrouzieri* in quantities pre-determined according to predator stage to ensure that the available prey exceeded the amounts that the predators could consume, as follows: 1^{st} instar larvae (L_1), three parasitized mealybugs; 2^{nd} instar larvae (L_2), five parasitized mealybugs; 3^{rd} instar larvae (L_3), seven parasitized mealybugs; 4^{th} instar larvae (L_4), adult females, adult males: 15 parasitized mealybugs. All predators were placed individually in plastic petri dishes (55×12 mm) and starved for 24 h prior to feeding to equalize conditions for each predator's development stage. *P. citri* or *P. ficus* mealybugs parasitized for either 2, 4, 6, 8 or 10 days were then added to the petri dishes, and the number of parasitized mealybugs consumed by each predator after 24 h was recorded. The same procedure was followed using non-parasitized mealybugs as a control. The experimental procedure was replicated 20 times for each combination of parasitized mealybug ages and predator stages.

Mean consumption values for L_1, L_2, L_3, L_4, adult females and adult males (adult sex determined by the color of the forelegs: females are dark brown, whereas males are yellowish) of *C. montouzieri* by mealybug species and duration of parasitization (0, 2, 4, 6, 8 and 10 days) were subjected to analysis of variance followed by Duncan's Multiple Range Test ($P \leq 0.05$) using the Statistica software package (StatSoft Inc., Tulsa, OK, USA).

RESULTS

Tables 1 and 2 show the consumption of *P. citri* and *P. ficus* mealybugs by *C. montrouzieri* to the predator's stage and the parasitoid's age. First instar *C. montrouzieri* consumption of both *P. citri* and *P. ficus* was highest when the predator was offered 4-day parasitized mealybugs (*P. citri:* 1.27±1.12; *P. ficus:* 1.05±0.05). Consumption values for 4-day parasitized *P. citri* and *P. ficus* were significantly higher than those for 6-, 8- and 10-day parasitized mealybugs of both species, whereas differences in consumption values for 4-day and 2-day parasitized and non-parasitized (control) mealybugs were not significant.

Second instar *C. montrouzieri* consumption of both *P. citri* and *P. ficus* was also highest when the predator was offered 4-day parasitized mealybugs (*P. citri:* 2.00±0.17; *P. ficus:* 2.25±0.20). Consumption values for 4-day parasitized *P. citri* and *P. ficus* were significantly higher than those for 6-, 8- and 10-day parasitized mealybugs as well as non-

TABLE 1. Consumption by *Cryptolaemus montrouzieri* of *Planococcus citri* parasitized by *Anagyrus pseudococci* (means±S.E.)

Development stage of *P. citri*	n	Control	n	2-day parasitized	n	4-day parasitized	n	6-day parasitized	n	8-day parasitized	n	10-day parasitized
L_1	20	1.05±0.09 a[z]	19	1.16±1.12 a	15	1.27±1.12 a	20	0.05±0.05 b	20	0.00±0.00 b	10	0.00±0.00 b
L_2	25	1.40±0.12 b	19	1.89±0.17 a	15	2.00±0.17 a	15	0.20±0.11 c	17	0.00±0.00 c	10	0.00±0.00 c
L_3	20	4.15±0.23 a	24	4.71±0.21 a	20	4.50±0.35 a	18	0.39±0.14 b	20	0.10±0.07 b	11	0.00±0.00 b
L_4	20	9.10±0.49 b	17	9.88±0.57 b	20	12.00±0.32 a	20	0.50±0.17 c	18	0.39±0.10 c	10	0.10±0.10 c
♂	20	7.05±0.44 b	20	8.90±0.45a	20	6.75±0.35 b	19	0.84±0.19 c	20	0.20±0.12 c	12	0.00±0.00 c
♀	20	7.86±0.63 b	20	9.80±0.61 a	20	10.86±0.50 a	16	0.50±0.22 c	26	0.54±0.15 c	12	0.42±0.15 c

[z] Within rows, means followed by the same letter do not differ statistically ($P \leq 0.05$).

TABLE 2. Consumption by *Cryptolaemus montrouzieri* of *Planococcus ficus* parasitized by *Anagyrus pseudococci* (means±S.E.)

Development stage of *P. ficus*	n	Control	n	2-day parasitized	n	4-day parasitized	n	6-day parasitized	n	8-day parasitized	n	10-day parasitized
L_1	20	1.05±0.05 a[z]	20	1.05±0.05 a	20	1.10±0.07 a	10	0.00±0.00 b	10	0.00±0.00 b	10	0.00±0.00 b
L_2	20	1.66±0.15 b	20	2.05±0.21 a	20	2.25±0.20 a	10	0.10±0.10 c	10	0.00±0.00 c	10	0.00±0.00 c
L_3	20	4.20±0.37 a	20	4.60±0.22 a	20	4.56±021 a	20	0.10±0.07 b	10	0.00±0.00 b	10	0.00±0.00 b
L_4	20	11.35±0.65 a	20	11.50±0.46 a	20	12.20±0.31 a	20	0.15±0.08 b	20	0.05±0.05 b	10	0.00±0.00 b
♂	20	8.55±0.47 ab	20	9.00±0.27 a	20	8.05±0.37 b	20	0.25±0.12 c	20	0.15±0.08 c	10	0.00±0.00 c
♀	20	9.15±0.47b	20	11.70±0.42 a	20	9.45±0.52b	20	0.25±0.12c	20	0.20±0.09c	10	0.00±0.00c

[z] Within rows, means followed by the same letter do not differ statistically ($P \leq 0.05$).

parasitized (control) mealybugs of both species, whereas differences between 4-day and 2-day parasitized mealybugs were not significant ($F_{(5,95)}$=51.307, *P*=0.000 for *P. citri*; $F_{(5,84)}$= 31.826, *P*=0.000 for *P. ficus*).

Third instar larval *C. montrouzieri* mealybug consumption was highest with 2-day parasitized mealybugs (*P. citri:* 4.71±0.21; *P. ficus:* 4.60±0.22) followed by 4-day parasitized mealybugs (*P. citri:* 4.50±0.35; *P. ficus:* 4.56±0.21). Consumption values for 4-day parasitized *P. citri* and *P. ficus* were significantly higher than those for 6-, 8- and 10-day parasitized mealybugs as well as non-parasitized (control) mealybugs of both species, whereas differences in consumption values for 4-day and 2-day parasitized mealybugs were not significant ($F_{(5,107)}$=109.41, *P*=0.000 for *P. citri*; $F_{(5,94)}$=98.849, *P*=0.000 for *P. ficus*).

Fourth instar larval *C. montrouzieri* mealybug consumption was highest with 4-day parasitized mealybugs (*P. citri:* 12.00±0.32; *P. ficus:* 12.20±0.31). Consumption values for 4-day and 2-day parasitized *P. citri* mealybugs as well as for non-parasitized (control) *P. citri* mealybugs were significantly higher than those for 6-, 8- and 10-day parasitized *P. citri* mealybugs ($F_{(5,99)}$=221.64, *P*=0.000). Consumption values for 4-day and 2-day parasitized *P. ficus* mealybugs were significantly higher than those for 6-, 8- and 10-day parasitized *P. ficus* mealybugs as well as for non-parasitized (control) mealybugs ($F_{(5,104)}$=269.31, *P*=0.000).

Adult male *C. montrouzieri* mealybug consumption was highest with 2-day parasitized mealybugs for both species (*P. citri*: 8.90±0.45; *P. ficus:* 9.00±0.27). The consumption values observed for 2-day parasitized *P. citri* were significantly different from values observed for other ages of parasitized *P. citri* as well as for non-parasitized (control) *P. citri*, whereas the consumption values for 2-day parasitized *P. ficus* were significantly different from values observed for other ages of parasitized mealybugs, but not significantly different from values observed for non-parasitized (control) ($F_{(5,95)}$=51.307, *P*=0.000 for *P. citri*; $F_{(5,84)}$=31.826, *P*=0.000 for *P. ficus*).

Adult female *C. montouzieri* consumption of *P. citri* mealybugs was highest for 4-day parasitized *P. citri* mealybugs (10.86±0.50), whereas consumption of *P. ficus* mealybugs was highest for 2-day parasitized *P. ficus* mealybugs (11.70±0.42). Consumption values for 4-day and 2-day parasitized *P. citri* mealybugs as well as for non-parasitized (control) *P. citri* mealybugs were significantly higher than those for 6-, 8- and 10-day parasitized *P. citri* mealybugs ($F_{(5,108)}$=123.85, *P*=0.000). Consumption values for 4-day and 2-day parasitized *P. ficus* mealybugs were also significantly higher than those for 6-, 8- and 10-day parasitized *P. ficus* mealybugs as well as for non-parasitized (control) mealybugs ($F_{(5,104)}$=218.73, *P*=0.000).

Irrespective of development stage, none of the *C. montrouzieri* fed on parasitized mealybugs following the onset of mummification.

DISCUSSION

All stages of *C. montrouzieri* were observed to feed on both parasitized and non-parasitized *P. citri* and *P. ficus* in this study. Fourth instar *C. montrouzieri* consumed the greatest quantities of both mealybug species when compared with other stages of the predator. These findings corroborate those of Telli and Yiğit (34), who reported that 4^{th} instar *C. montrouzieri* consumed more *P. citri* larvae than other stages of the predator. In general, 4^{th} instar coccinellids are known to consume greater quantities of prey than other stages (22).

In the present study, prey consumption values for all stages of *C. montrouzieri* were affected by the extent of mealybug parasitization, with all stages of the predator feeding on more 2- and 4-day parasitized mealybugs than 6-, 8- and 10-day parasitized mealybugs. Moreover, in most cases, consumption values were higher for 2- and 4-day parasitized mealybugs than for non-parasitized mealybugs for both mealybug species. Şengonca and Yanuwiadi (33) also reported that 4^{th} instar and adult female *C. montrouzieri* consumption of *Leptomastix dactylopii* (Hymenoptera: Encyrtidae)-citrus mealybugs was highest with 2- and 4-day parasitized mealybugs.

It is likely that *C. montrouzieri* stopped feeding on mealybugs parasitized for more than 6 days because of the onset of mummification, after which the predator is unable to break through the hardened mealybug cuticle. Previous research has shown that (i) *P. citri* mobility decreases after 5 days of parasitism, (ii) the mealybug cuticle begins to harden after 6-7 days of parasitism (28), and (iii) predators cannot feed on mealybugs following mummification.

Key factors determining the net effects of host predation include the conditions of the prey host, whether it is parasitized or not, and the age of the parasitized host (6,20,24,25). Our study found that the capacity of all stages of *C. montrouzieri* to feed on 2- and 4-day parasitized *P. citri* and *P. ficus* mealybugs was generally higher than the capacity to feed on non-parasitized mealybugs. We suggest that the consumption habits of the predator are affected by the mobility of the parasitized mealybugs, which slows significantly after 2 days of parasitism. Preference for parasitized *vs* non-parasitized hosts can vary greatly depending on the species of both parasitoid and predator. Predators may attack only non-parasitized hosts, only parasitized hosts, or may show a partial preference for non-parasitized hosts (8,11,14,32).

This study should help to provide some understanding of IGP between *C. montrouzieri*, a generalist predator, and *A. pseudococci*, a specialist parasitoid, on *P. citri* and *P. ficus* mealybugs. Considering that escape from predators constitutes one of the mealybug's most effective defense strategies, in this case the results of IGP are partially determined by the presence of parasitoid larvae within the prey, whose resultant decreased mobility thus increases its vulnerability to capture (27).

Our findings showed that *C. montouzieri* consumption of parasitized mealybugs tends to exceed that of non-parasitized mealybugs, but that consumption of parasitized mealybugs becomes impossible after the onset of mummification. This represents a significant phenomenon in the mealybug – predator – parasitoid complex, and one that must be considered when releasing predators and parasitoids in a biological control program. In this regard, we suggest that predator release should take place a minimum of 6 days following parasitoid release in order to minimize parasitoid losses by predation.

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