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Authors: Villanueva, Raul T., Gagné, Raymond, and Childers, Carl C.

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TWO SPECIES OF CECIDOMYIIDAE PREDACIOUS ON CITRUS RUST MITE, *PHYLLOCOPTRUTA OLEIVORA*, ON FLORIDA CITRUS

RAUL T. VILLANUEVA,[1] RAYMOND GAGNÉ[2] AND CARL C. CHILDERS[3]
[1]North Carolina State University, Mountain Horticultural Crops Research and Extension Center
455 Research Drive, Fletcher, NC 28732

[2]USDA, Systematic Entomology Laboratory, Beltsville, MD

[3]University of Florida, Citrus Research and Education Center, 700 Experiment Station Rd., Lake Alfred, FL 33850

ABSTRACT

Larvae of two undescribed species of Cecidomyiidae (Diptera) were found preying upon *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae) on Florida citrus. Identifications to genus were made from adults reared in the laboratory. The two species had distinctive larval coloration. One larval type was completely yellow and was identified as *Feltiella* n. sp., while the second larval type had an orange color with a transverse white band close to the mouthparts. The latter cecidomyiid was identified as belonging to a genus near *Lestodiplosis* in the broad sense. *Feltiella* n. sp. (n = 17) and the species near the genus *Lestodiplosis* (n = 12) consumed 33.8 ± 4.6 (mean ± SEM) and 43.0 ± 6.4 citrus rust mite eggs; 14.2 ± 1.4 and 15.0 ± 2.0 citrus rust mite nymphs, and 3.0 ± 0.4 and 5.6 ± 0.9 citrus rust mite adults/10 min., respectively. There were no significant differences ($P > 0.05$) in the consumption rates of either predator on any rust mite life stage. These data indicate that *Feltiella* n. sp. and the species near the genus *Lestodiplosis* are both efficient predators of *P. oleivora* eggs, larvae, and nymphs.

Key Words: Acari, citrus, Diptera, Eriophyidae, *Feltiella, Lestodiplosis,* predation

RESUMEN

Dos especies de larvas no descritas de Cecidomyiidae (Díptera) fueron encontradas depredando *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae) en cítricos de Florida. Las identificaciones fueron hechas en adultos criados en el laboratorio. Los dos cecidomidos tienen distintivas coloraciones larvales. Un tipo es completamente amarillo y es identificado como *Feltiella* n. sp., mientras que la otra tiene un collar blanco cerca de las partes bucales. Este cecidomido fue identificado como una especie en el genero cercano a *Lestodiplosis* en un amplio rango. *Feltiella* n. sp. (n = 17) y la especie cercana al genero *Lestodiplosis* (n = 12) consumieron 33.8 ± 4.6 (AVG ± SEM) y 43.0 ± 6.4 huevos del acaro de tostado, 14.2 ± 1.4 y 15.0 ± 2.0 ninfas del acaro del tostado, y 3.0 ± 0.4 y 5.6 ± 0.9 adultos del acaro del tostado /10 min., respectivamente. No hubo diferencias significantes entre las dos especies en los rangos de consumo de los diferentes estadios del acaro del tostado. Con los datos presentados aquí es evidente que *Feltiella* n. sp. y la especie cercana al genero *Lestodiplosis* son depredadores eficientes de huevos, larvas y ninfas de *P. oleivora*.

Translation provided by the authors.

The citrus rust mite (CRM), *Phyllocoptruta oleivora* (Ashmead), and the pink citrus rust mite *Aculops pelekassi* (Keifer) are pests on Florida citrus (Acari: Eriophyidae) (Denmark 1963; Childers & Achor 1999). Both species cause rind blemish injuries to developing and mature fruit. Other types of damage include reduced bonding force of fruit, premature fruit drop, reduced yields, and lower juice quality (Allen et al. 1994). The pink citrus rust mite also can cause leaf distortion, crinkling, and stunting of new shoot growth (C. C. Childers, unpublished). A third species, the citrus bud mite, *Aceria sheldoni* (Ewing), is frequently found on citrus but is not considered an economic pest in Florida (Childers & Achor 1999). Natural enemies of citrus rust mites include predatory phytoseiid and stigmaeid mites (Muma 1961; Muma & Selhime 1971; Peña 1992; Childers 1994). Hubbard (1883) found "a little coral-red maggot and a yellow midge larva" (Diptera: Cecidomyiidae, formerly Itonididae) feeding on CRM in Florida. Later, Muma et al. (1961) reported *Itonidini* species feeding on *P. oleivora* and the six-spotted mite, *Eotetranychus sexmaculatus* (Riley), on citrus in Lake Alfred, Florida.

The larvae of the cosmopolitan genus *Feltiella* (Diptera: Cecidomyiidae) and all described species form a group associated exclusively with tetranychid species (Gagné 1989), and this genus belongs in the tribe Lestodiplosini that is composed

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entirely of predators or parasitoids of insects and mites (Gagné 1989, 1994, 1995). The identification of these mite-eating cecidomyiids is difficult; for example, in Europe many authors are using *Feltiella acarisuga* (Vallot) and *Therodiplosis persicae* Kieffer as synonyms (i.e., Colombo et al. 1993; Piatkowski 2000; Putte 2002). The larval stage of *F. acarisuga* (Vallot) is a well-known predator of the two-spotted spider mite, *Tetranychus urticae* Koch (Gillespie et al. 1998).

Feltiella acarisuga completes its life cycle in 8 to 10 d in Italy (Roberti 1954) and 29 d on average in Israel (Sharaf 1984). These differences in developmental times are likely dependent on temperature and/or humidity differences. The crops included in studies with *F. acarisuga* were apple (Roberti 1954), eggplant (Sharaf 1984), cucumber (Gillespie et al. 1998, 2000), strawberry (Easterbrook 1998), and plants in greenhouses (Opit et al. 1997; Enkegaard et al. 2000). *Feltiella occidentalis* (Felt) occurs on strawberry in California (Oatman et al. 1985) and *F. minuta* has been found on eggplant (Ho & Chen 1998).

There are few examples of eriophyid predation by larval cecidomyiids. Nijveldt (1969) compiled a list of cecidomyiid species and their respective eriophyid prey. The larva of a *Medetera* species (Diptera: Dolichopodidae) was reported preying on *Aculus schlechtendali* Nalepa on apple in Washington (Rathman et al. 1988). The eriophyid *Aceria litchii* Keifer is a serious pest of lychee (*Litchii chinensis* Sonnerat) in Australia and China, and the larva of *Arthrocnodax* sp. (Cecidomyiidae) was observed preying upon *A. litchii* (Waite & Gerson 1994).

The objectives of this study were to identify two cecidomyiids observed preying upon citrus rust mites in different citrus orchards in Florida, as well as to compare and quantify their consumption of different CRM stages.

Materials and Methods

Cecidomyiid Collection

Sampling for cecidomyiid larvae was conducted between June and August and again from October to the first week of December 2001 in a 'Hamlin' orange orchard in Lake Alfred, Florida. Larvae were collected from citrus leaves and fruits and transferred individually to Petri dishes with a 5-0 sable brush. Individual fruits with high numbers of CRM (>100 cm^2) were collected to prepare individual rearing arenas with an adequate food source for maintaining the two dipteran species. Some of the cecidomyiid larvae were allowed to complete their development so that adults could be obtained for identification while others were used for feeding experiments and behavioral observations. Data obtained on individual prey experiments were recorded separately.

Rearing of Cecidomyiid Larvae

Individual rectangular, transparent plastic containers (Pioneer Plastics Inc #295C, Eagan, MN) with semi-tight lids (31 cm long × 24 cm wide × 11 cm deep) served as rearing chambers for the midge larvae. A lightly moistened piece of paper towel was placed on the bottom of each container to provide increased humidity. Each CRM-infested orange arena was carefully examined and all other arthropods removed. Between six to eight oranges were then placed individually on PVC rings (3.5 cm diam. × 1 cm high) inside each plastic rearing container. Two or three cecidomyiid larvae of the same type were added to each fruit in the same container and then covered with a lid ($n \geq 20$ for yellow and orange, respectively). The containers were held in an environmental chamber at 25 ± 1°C, 60 ± 10% RH under fluorescent lights set to a photoperiod of 14:10 (L:D) h. The cecidomyiid larvae were observed daily and the oranges infested with CRM were replaced as required. As each cecidomyiid pupa formed, it was removed and isolated in a 5-cm-diam. Petri dish held under the same environmental conditions. However, pupae were difficult to find and often escaped detection so that many adults emerged in the plastic container. Pupae were found attached to fruit, on or under the paper toweling, or attached to the plastic walls of the rearing units.

Cecidomyiid Predation on Citrus Rust Mites

The predatory behavior of cecidomyiids was observed on individual Hamlin oranges heavily infested with all stages of CRM. Individual oranges were placed on PVC rings (3.5 cm diam. × 1 cm high) as described earlier. The presence of high densities of CRM was confirmed on each fruit by using a dissecting stereomicroscope (>100 cm^2). Since it was not possible to estimate the age of the larvae used in the experiments, larvae of similar lengths (1.6-1.8 mm) were selected for each assay. A single cecidomyiid larva was placed on a fruit within the center field of view by using a stereomicroscope and monitored for 10 min. rotating the fruit to maintain a constant focus on the maggot's movements. The number of eggs, combined larvae and nymphs, and adult CRM stages consumed per larva during each 10-min. interval were tallied separately for each cecidomyiid species. In total, 17 yellow and 12 orange cecidomyiid larvae were observed. Data were analyzed with a single factor analysis of variance (Zar 1984). Sub-samples of eriophyid mite populations were collected from fruits into 80% ethanol and then later slide-mounted in a modified Hoyer's medium and identified to species (Baker et al. 1996). Multiple slides were prepared with each containing 10 or more rust mite motile stages.

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RESULTS

Cecidomyiid Collection and Rearing

Two distinct types of larvae were observed. One was yellow in color (Fig. 1a) and the other was orange with a white collar behind the mouthparts (Fig. 1b).

Success in rearing cecidomyiids to the adult stage was obtained with larvae collected during November and December, but those collected during July and August did not complete development (Fig. 2). Adult males and females were reared from yellow larvae whereas only two females were reared from orange larvae. The two distinct types of larvae were identified as belonging to two different genera. The yellow larvae were an undescribed species of *Feltiella* and the females obtained from the orange larvae were identified as a species near the genus *Lestodiplosis* (in the broad sense). However, they do not fit well in the genus *Lestodiplosis*, suggesting that this species may represent a new genus.

Predation by Cecidomyiids on Citrus Rust Mites

All eriophyids sub-sampled from each 10-min observation interval were identified as the citrus rust mite, *Phyllocoptruta oleivora*. The number of eggs, combined larvae and nymphs, and adult citrus rust mites consumed during 10-min observations by both species are shown in Fig. 3. There were no significant differences ($P > 0.05$) between the rust mite stages consumed by either *Feltiella* n. sp. or the second dipteran species. *Feltiella* n. sp. and the second species consumed 33.8 ± 4.6 (mean ± SEM) and 43.0 ± 6.4 ($F = 1.99$; 1,27 *df*; $P = 0.16$) CRM eggs; 14.2 ± 1.4 and 15.0 ± 2.0 CRM larvae and nymphs ($F = 1.41$; 1,27 *df*; $P = 0.24$), and 3.0 ± 0.4 and 5.6 ± 0.9 CRM adults ($F = 0.26$; 1,27 *df*; $P = 0.60$), respectively.

DISCUSSION

Previous reports of cecidomyiid species feeding on CRM were based largely on anecdotal information. To date, there is no reliable description of CRM feeding by cecidomyiid predators in Florida or other citrus growing areas of the world. McMurtry (1977) and Perring & McMurtry (1996) cited Muma et al. (1961), who only mentioned a predatory midge he recovered from *P. oleivora* colonies. There was no empirical quantification or scientific description of cecidomyiid feeding behavior. Furthermore, all citations refer to the original descriptions by Hubbard (1883) who apparently described the two types of larvae (one yellowish and the other orange with a white collar) reported here. Adults of *Feltiella* n. sp. (yellow larva) and a species near the genus *Lestodiplosis* (orange larva with a white collar) were successfully collected, reared, and identified to genus in this study. Voucher specimens are deposited with the USDA, Systematic Entomology Laboratory, Beltsville, MD.

Yothers & Mason (1930) reported that these midges appeared on occasion but only when high numbers of CRM were present. This was observed with *Feltiella minuta* (Felt) when it increased on *Tetranychus kanzawai* Kishida (Ho & Chen 1998). Both species examined in this study fed on *P. oleivora* eggs, larvae, and nymphs of CRM and completed their development to adults on this diet. If we extrapolate the average number of all *P. oleivora* stages consumed in 10 min to 4 min, then 1.2 adults, 5.7 nymphs and larvae, and 13.5 eggs could be consumed in 4 min by a single midge larva. This rate of consumption surpasses the predatory capacity of *Iphiseiodes quadripilis* (Banks), one of the most abundant phytoseiids on Florida citrus (Villanueva & Childers 2005), feeding on *A. pelekassi,* (Villanueva 2002) in which a female starved for 24 h fed on 1.8 ± 0.5 *A. pelekassi* in 4 min. A similar comparison was shown between *F. minuta* and *Amblyseius womersleyi* Schicha on eggplant (Ho & Chen 1998).

Laboratory observations of the two cecidomyiid predators revealed that they search for CRM eggs by continuously moving the anterior part of their bodies to the left and to the right while moving forward and changing direction. This appeared very similar to the 'questing' behavior described for syrphid larvae seeking aphids (Bargen et al. 1998). Once an egg is detected during this sweeping movement, it is rapidly consumed while the larva continues onward. Consumption of CRM eggs by either cecidomyiid species was difficult to observe due to the small size and transparent color of the egg (about 30-40 μm). The presence of *P. oleivora* eggs is essential for larval development of both cecidomyiids for two reasons. First, larvae of both species were frequently found on the bottom of the plastic containers completely separated from the fruit. However, the arenas the cecidomyiid larvae had abandoned were still infested with motile stages of CRM but lacked CRM eggs that were previously consumed. When a new fruit with abundant CRM eggs was provided, the cecidomyiid larvae would immediately begin feeding and remained on the fruit. Second, on many occasions, both species of larvae encountered adult CRM but usually they were not consumed. Other times, the larva would raise the adult CRM off the substrate with their mouthparts and appear to cast the adult aside. On occasion, when the attacks on CRM adults were successful, the larval mouthparts were directed to the ventrolateral part of the mite's body, just behind the second pair of legs. This was not observed when the attacks were directed to the immature rust mite stages. Larval and nymphal CRM stages appeared to be more vulnerable and

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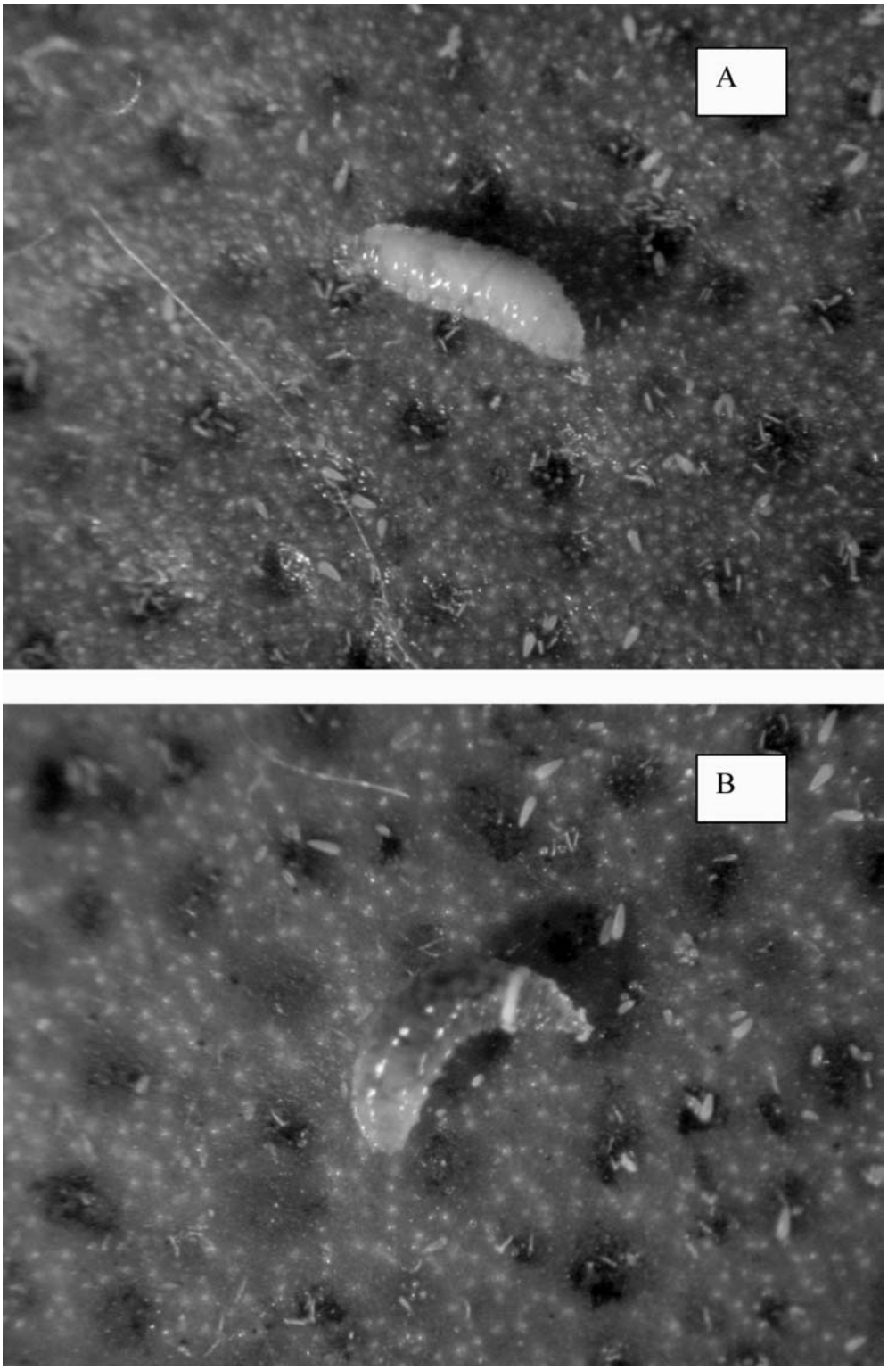


Fig. 1. Two morphologically distinct larval types of two new species of cecidomyiids surrounded by their citrus rust mite *Phyllocoptruta oleivora* prey. (A) *Feltiella* n. sp. and (B) a species near the genus *Lestodiplosis*. Note the color and ring around the anterior end of the larva.

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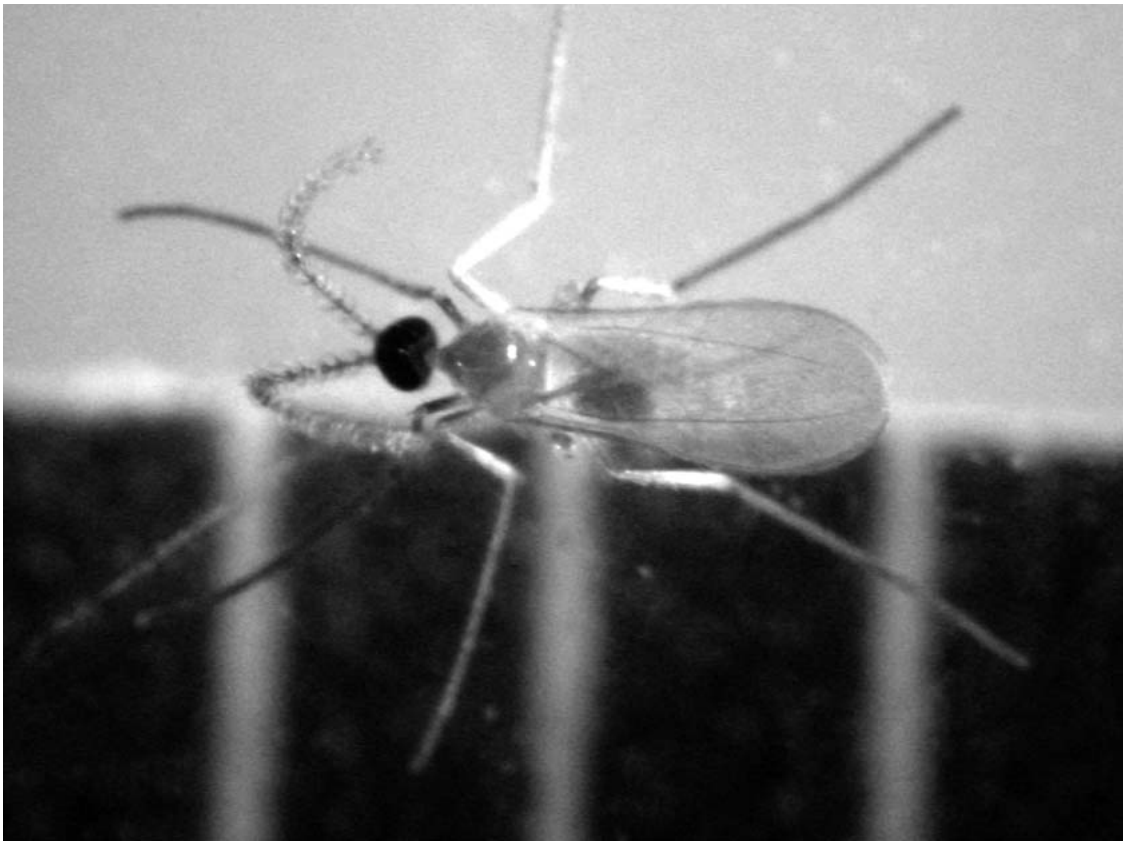


Fig. 2. Adult *Feltiella* n. sp., a predator of the citrus rust mite *Phyllocoptruta oleivora* reared in the laboratory. The vertical bars beneath the insect are in millimeters.

were usually eaten when grasped on any part of their bodies.

The relative abundance of these cecidomyiids was not recorded in this study because the priority was to identify the species involved and quantify their predation on CRM stages. Most larvae collected in the field were more abundant on fruit than on leaves during October and November. The predominance of midge larvae on fruit surfaces was consistent with the higher densities of CRM present on fruits than on leaves.

Observations in both laboratory and field showed that larvae of both cecidomyiid species were capable of jumping or springing off a plant substrate when disturbed. Larvae were observed to raise the middle part of their bodies into an inverted U-shape while keeping both the head and terminal ends attached to the substrate. The larva would then rapidly release its hold on the substrate and leap or spring from the plant surface using tension on the sternal spatula or breastbone to provide the springing action. Cecidomyiid larvae falling to the ground would likely desiccate and die. From observations in the laboratory rearing arenas, it appears that this movement is directional. When the fruit in the rearing containers had a few CRM eggs, the larva would

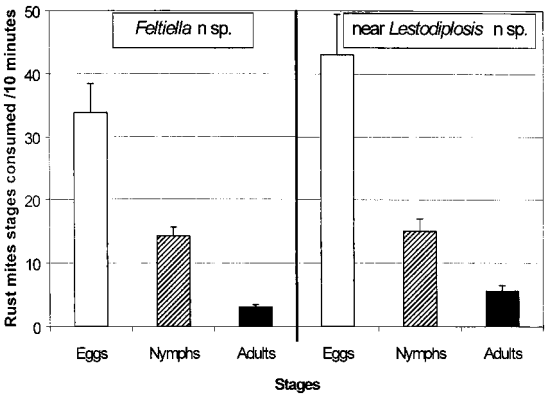


Fig. 3. Mean numbers ± SEM of different citrus rust mite (*Phyllocoptruta oleivora*) stages consumed by larvae of *Feltiella* n. sp. and a species near the genus *Lestodiplosis*.

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abandon the fruit and be found on an adjoining new fruit that had abundant rust mite eggs.

The dispersal behavior of these midges and their unnoticed predation on CRM eggs likely contributed to earlier failed attempts to rear these species. Similar situations were encountered by Yothers & Mason (1930) who wrote: "These larvae are very small and extremely delicate, and all attempts to rear them to maturity have failed". Few accurate studies on the biology of predacious cecidomyiids and their effects on prey mite populations are available. This is the case for cecidomyiids preying on Eriophyidae in general, and also on different species of Tetranychidae (Chazeau 1985).

In this study, we demonstrated that these two undescribed cecidomyiids are capable of feeding and reproducing on an exclusive diet of CRM. Both species appeared to have highly specialized abilities eating CRM eggs and immature stages.

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