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(*Short communication*)

WASMANNIA AUROPUNCTATA (ROGER) (HYMENOPTERA: FORMICIDAE), A SMALL BUT VORACIOUS PREDATOR OF *DIAPHORINA CITRI* KUWAYAMA (HEMIPTERA: LIVIIDAE)

WASMANNIA AUROPUNCTATA (ROGER) (HYMENOPTERA: FORMICIDAE), PEQUEÑO PERO VORAZ DEPREDADOR DE *DIAPHORINA CITRI* KUWAYAMA (HEMIPTERA: LIVIIDAE)

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ABSTRACT. The effect of predation by the little fire ant *Wasmannia auropuntacta* (Roger) (Hymenoptera: Formicidae) on Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) kept under experimental conditions for the breeding of the parasitoid *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) was evaluated. An experiment was carried out using cages with capacity for 9 plants of *Murraya paniculata* (L.) Jack (Rutaceae) infested with an average of 600 nymphs of *D. citri* and 100 adults of *T. radiata* per cage. Three cages were exposed to ants and one had no ant exposure (Control). In each cage, the numbers of healthy and parasitized nymphs and the numbers of adults of *D. citri* and *T. radiata* were counted every 2 days for 15 days. Exposure to predation by *W. auropunctata* significantly affected the number of nymphs that reached the adult stage as well as the number of nymphs parasitized by *T. radiata* (Fisher's exact test). In the ant-exposed cages, about 3% of the nymphs survived to the adult stage in contrast to 18% in the control cage. Likewise, the action of the parasitoid was affected, with 24% of nymphs parasitized in the ant-exposed cages in contrast to 66% in the control cage. *Wasmannia auropunctata* has a great potential as a natural enemy of this psyllid pest in citrus nurseries. The ant also consumes *T. radiata* by consuming parasitized nymphs of *D. citri* and this predation may negatively impact the control of *D. citri* by the parasitoid.

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RESUMEN. Se evaluó el efecto de depredación de *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae) sobre ninfas de *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) mantenidas en condiciones experimentales para la cría de *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae). Se realizó un ensayo empleando jaulas con capacidad para 9 plantas de *Murraya paniculata* (L.) Jack (Rutaceae) infestadas con un promedio de 600 ninfas de *D. citri* y 100 adultos de *T. radiata* por jaula. Tres jaulas fueron expuestas a las hormigas y una sin exposición (Control). En cada jaula se contabilizó el número de ninfas sanas y parasitadas y el número de adultos de *D. citri* y *T. radiata*, cada 2 días durante 15 días. La exposición a la depredación por *W. auropunctata* afectó de forma significativa el número de ninfas que alcanzaron el estado adulto y el número de ninfas parasitadas por *T. radiata* (prueba exacta de Fisher). En las jaulas expuestas, cerca del 3% de las ninfas sobrevivieron al estado adulto en contraste con 18% en la jaula control. De igual manera se vio afectada la acción del parasitoide, con un 24% de ninfas parasitadas en las jaulas expuestas en contraste con 66% en la jaula control. *Wasmannia auropunctata* tiene gran potencial como controlador de esta plaga en viveros de cítricos. La hormiga también consume *T. radiata* de forma indirecta al consumir ninfas de *D. citri* parasitadas.

Many ant species have been reported as predators of Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (e.g., Michaud, 2004), although predation by ants has not been directly observed. In an ant exclusion experiment conducted in Florida (USA), Navarrete *et al*. (2013) reported that the presence of the ants *Pheidole megacephala* Fabricius, 1793, *Brachymyrmex patagonicus* Mayr, 1868, and *Solenopsis invicta* Buren, 1972, correlated positively with high rates of parasitization by the wasp *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae). In that study, Navarrete *et al*. (2013) did not observe any aggressive behavior of the ants towards *D. citri* nymphs, and the number of nymphs of *D. citri* showed no statistical difference between ant-tended and not ant-tended nymphs, suggesting that ants do not feed on *D. citri*. In a glass house where *D. citri* is mass reared together with its natural enemy *T. radiata*, the little fire ant, *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae), was observed feeding on nymphs (Fig. 1a) and just emerged adults (Fig. 1b) of *D. citri* and causing serious losses to the breeding stock. This is one of the few studies where predation of *D. citri* by ants has been verified.

Figure 1. *Wasmannia auropunctata*, a predator of *Diaphorina citri*; **a**) a worker of *W. auropuntata* feeding on a nymph of *D. citri*, **b**) two workers of *W. auropuntata* feeding on a newly-emerged adult of *D. citri*. Photos by L. Colorado.

Location: The breeding stock of *D. citri* is located in a glass house of the Corporación Colombiana de Investigación Agropecuaria [Colombian Corporation for Agricultural Research] (Agrosavia), Palmira Research Center, in the municipality of Palmira, department of Valle del Cauca, Colombia, 03°31′17″N, 76°18′25″W, ca. 1,000 m asl. The experiment was conducted during April 11-22, 2016. After an initial heavy attack of *W. auropunctata* on a mass-rearing facility of *D. citri*, follow-up observations were made in order to verify the reduction of psyllid individuals from the breeding cages by this predatory ant.

Experimental unit. Four cages (70 cm × 70 cm × 70 cm) with a capacity of 9 plants of *Murraya paniculata* (L.) Jack (Rutaceae), each infested with an average of 600 nymphs of *D. citri* were used in the experiment. Three of the four cages were exposed to the ants and one served as a control (without exposure to *W. auropunctata*). The number of nymphs (healthy and parasitized) and adults of *D. citri* and adults of *T. radiata* were counted every 2 days for 15 days. Healthy and parasitized nymphs that disappeared in the following count were assumed to have been eaten by the ants.

Data Analysis. Fisher´s exact test was run using PAST 3.16 (Hammer *et al*., 2001), to analyze contingency tables in order to assess the association between the presence of *W. auropunctata* and the survival of *D. citri* to adult stage, the number of *D. citri* nymphs parasitized by *T. radiata*, and the survival of *T. radiata* to adult stage.

During the observation period, all the cages showed a decrease in the number of healthy nymphs and adults of *D. citri* (Fig. 2). The cages that were exposed to *W. auropunctata* had a lower survival of *D. citri* individuals with a mean of 2.78% +/- 2.08% compared to the control cage (no exposure to *W. auropunctata*) 18.3% (Fig. 3). The *D. citri* population in the control cage decreased until the 8^{th} day and remained around 20% of the initial population until the end of the experiment. The cages that were exposed to *W. auropunctata* showed a steady decrease in *D. citri* numbers, with an average of 1% of the original population remaining at the end of the experiment (Fig. 2).

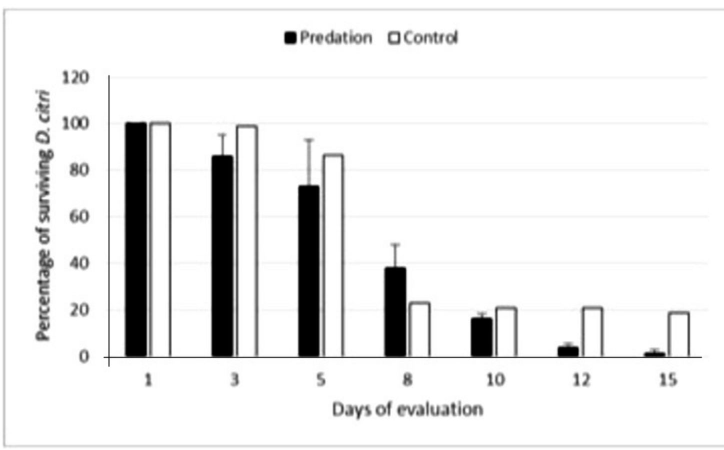

Figure 2. Percentage of surviving individuals of *D. citri* (nymphs and adults) in the experimental cages as recorded from day 1 to 15. The psyllids in the cages in the predation treatment were exposed to *Wasmannia auropunctata*. The percentage of surviving individuals was calculated as follows $((hn + Dc)/hn_1)*100$; hn: number of healthy *D. citri* nymphs, Dc: number of *D. citri* adults, hn_0: Initial number of healthy *D. citri* nymphs. Error bars represent the standard deviation, not calculated for control treatment (n=1).

Fisher´s exact test confirmed that exposure to predation by *W. auropunctata* was significantly associated with the number of *D. citri* nymphs that were able to reach the adult stage (DF= 1, χ^2 = 179.35, p=6.7057 E-41). In other words, cages exposed to *W. auropunctata* had a lower survival percentage of *D. citri*. The same pattern was observed with the survival of *T. radiata* to adult stage (Fig. 3); and a significant association also was detected between the two variables (DF= 1, χ2 = 279.16, p = 1.1451 E-62).

The number of *D. citri* nymphs parasitized by *T. radiata* was also affected by the exposure to the little fire ant (Fig. 4). The proportion of parasitized nymphs in the control cage (65.8%) was almost three times higher than in the ant-exposed cages (23.9% +/-12.9%). A significant association was found between the two variables (DF= 1, $\chi 2$ = 203.7, p =3.2549 E-46).

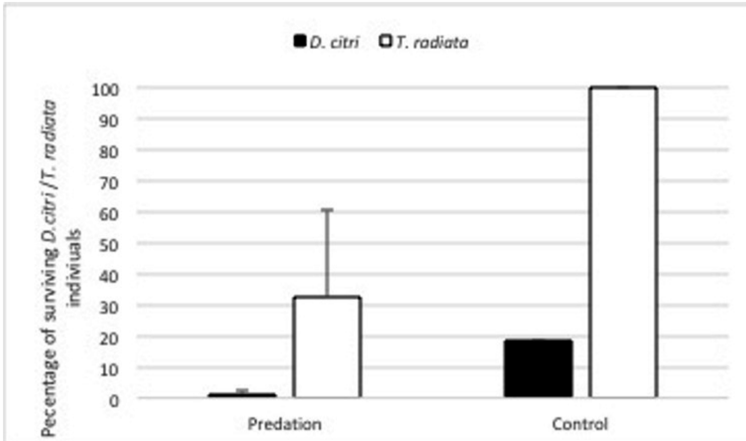

Figure 3. Average survival of *D. citri* and *T. radiata* in cages exposed to *W. auropunctata* (predation) and control cages (not exposed). Error bars represent the standard deviation, not calculated for control treatment (n=1).

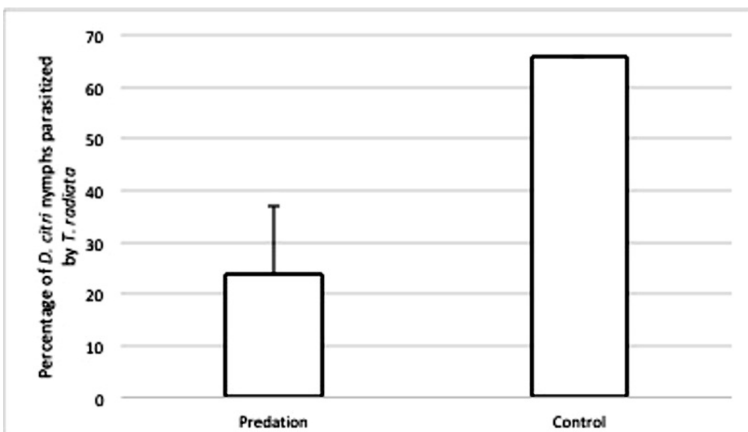

Figure 4. Average percentage of *D. citri* nymphs parasitized by *T. radiata* in cages exposed to *W. auropunctata* (predation) and control cages (not exposed). Error bars represent the standard deviation, not calculated for control treatment (n=1).

Although the little fire ant is an effective predator of *D. citri* nymphs, it also has a negative effect on the psyllid's parasitoid *T. radiata*, both directly by consuming parasitized *D. citri* nymphs and indirectly by preventing the wasps from parasitizing *D. citri*, however, the exact mechanism by which *W. auropunctata* interferes with the parasitoid *T. radiata* cannot be inferred from these results. Nevertheless, the negative effect of predation by these ants on *T. radiata* should be considered in biological control programs of *D. citri* by the parasitoid wasp.

Kondo *et al*. (2017) presented a list of 101 species of arthropods (arachnids and insects), distributed in nine orders and 26 families worldwide, reported to be natural enemies of *D. citri*. The majority of the natural enemies of *D. citri* recorded in the literature are ladybirds (Coleoptera: Coccinellidae) with 39 species (38.6%), followed by the lacewings (Neuroptera: Chrysopidae) with 13 species (12.9%) and syrphid flies with 8 species (7.9%). With the addition of *W. auropunctata* the number of arthropod natural enemies of *D. citri* worldwide is increased to 102 species. Among the many species of ants recorded as natural enemies of *D. citri*, there is only some evidence of predation by the species, *Dorymyrmex bureni* (Trager, 1988) and *Pseudomyrmex gracilis* (Fabricius, 1804) (Michaud, 2004). With this record, the number of confirmed predatory ants of *D. citri* is increased to three species.

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