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ANTS (HYMENOPTERA: FORMICIDAE) ASSOCIATED WITH *DIAPHORINA CITRI* (HEMIPTERA: LIVIIDAE) AND THEIR ROLE IN ITS BIOLOGICAL CONTROL

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Abstract

After the arrival of the Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) in Florida, several studies mentioned the presence of ants where *D. citri* was present, but there was no clarification of their specific interaction with the psyllid. The goal of this study was to elucidate the role of ants in the biological control of *D. citri* by observing ant behavior and by determining if ant presence, modified by exclusion manipulations, affected parasitism of *D. citri* by *Tamarixia radiata* (Waterston, 1922) (Hymenoptera: Eulophidae), an introduced parasitoid of the psyllid, when the insect was infesting orange jasmine, *Murraya paniculata* (L.) Jack (Sapindales: Rutaceae) and Persian lime, *Citrus latifolia* Tanaka (Sapindales: Rutaceae). During a preliminary survey on *M. paniculata* in Homestead, Florida, we observed 2 ant species in association with *D. citri*, i.e., the big headed ant, *Pheidole megacephala* Fabricius, and the rover ant, *Brachymyrmex obscurior* Forel. In 2 ant exclusion experiments, using a 2-cm-wide barrier of Tanglefoot®, *P. megacephala* was the only ant species found in *M. paniculata* while *P. megacephala*, *B. patagonicus* and *Solenopsis invicta* Buren were observed in *C. latifolia*. The number of *P. megacephala* found in the unprotected flushes in *M. paniculata* fluctuated between 0.15 and 0.5 per flush while in *C. latifolia* the number of ants, pooled across species, varied between 1.44 and 6.61. In *M. paniculata* flushes from Tanglefoot-treated plants, 20.36% of the nymphs were parasitized by *T. radiata* compared to 0.39% parasitism in untreated control flushes where ants had not been excluded. Fifty-eight percent of the psyllid nymphs were parasitized in the *C. latifolia* Tanglefoot® ant-exclusion flushes compared with 8.57% parasitism in the non-exclusion control. An additional experiment using the ant bait Extinguish Plus® (Hydramethylnon 0.365%+ S-Methoprene 0.250%) applied to the soil surrounding the trunk showed that the use of a granular bait can help to reduce ant populations and consequently increase the percentage parasitism of the Asian citrus psyllid.

Key Words: Asian citrus psyllid, ants, natural enemies

Resumen

Varios estudios han mencionado la presencia de hormigas relacionada con la presencia de *Diaphorina citri* en Florida, sin embargo, en estos estudios no se ha clarificado la asociacion entre las hormigas y el psílido. El objetivo de este estudio fue el determinar el papel que tienen las hormigas en relacion con el control biologico de *D. citri* por medio de observaciones de comportamiento de hormigas y determinando si la presencia de estas afecta el parasitismo causado por *Tamarixia radiata*. Primero, durante un muestreo realizado en Homestead, Florida, se determinó que *Pheidole megacephala* Fabricius y *Brachymyrmex obscurior* Forel estaban en asociacion con *D. citri*. Mas tarde, mediante dos experimentos donde se utilizó como barrera excluyente 2 cm de Tanglefoot®, tanto en *Murraya paniculata* como en *Citrus latifolia*, se encontro que el tratamiento donde no habia barrera excluyente en *M. paniculata* se encontraba presente la hormiga *P. megacephala* , mientras que en *C. latifolia* se encontraban las hormigas *P. megacephala, B. patagonicus* y *Solenopsis invicta.* En los brotes de *M. paniculata*,que estaban impregnados con Tanglefoot se encontro que un 20.36% de D. citri parasitadas por *T. radiata*, comparado con un 0.39% de parasitismo cuando las hormigas no estaban excluidas.

El 58% de las ninfas del psílido fueron parasitadas en el tratamiento de exclusion de hormigas en *C. latifolia* comparado con 8.57% cuando las hormigas no estaban excluidas. En un experimento adicional en el cual se utilizó el cebo 'Extinguish Plus® (Hydramethylnon 0.365%+S-Methoprene 0.250%(Wellmark International , Schaumburg, IL) (5 g/arbol) aplicado al suelo, se encontró que el cebo granulado reduce las poblaciones de hormigas y en consecuencia incrementa el porcentaje de parasitismo del psílido.

Palabras Clave: Psílido Asiático de los cítricos, hormigas, enemigos naturales

Diaphorina citri Kuwayama (Hemiptera: Liviidae) is a serious threat to the citrus industry in Florida, not only because it extracts the sap of citrus plants and causes leaf distortion and sooty mold, but, most importantly, because it is associated with a bacterium that appears to be the causal agent of probably the worst disease affecting citrus in the world, citrus greening or huanglongbing disease (Halbert & Manjunath 2004; Tsai 2008). *Diaphorina citri* invaded Florida in 1998 (Halbert et al. 2003) and citrus greening was reported within 8 yr of the arrival of the vector (Bové 2006). A classical biological program was implemented with the importation, rearing and release of 2 specific parasitoids of *D. citri*, the ectoparasitoid, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), and the endoparasitoid, *Diaphorencyrtus aligharhensis* (Shafee, Alam and Agarwal) (Hymenoptera: Encyrtidae) (Hoy et al. 2001). Several surveys conducted after the release of the biological control agents showed that only *T. radiata* was established, but that it caused low mortality of *D. citri* (Qureshi et al. 2009; Chong et al. 2010). These results were surprising when compared with the "good performance" of *T. radiata* in islands like Reunion, Guadeloupe, and Puerto Rico (Aubert et al. 1996; Etienne et al. 2001; Pluke et al. 2008).

Several theories have been proposed for the lack of success of *D. citri* parasitoids in Florida. Some of them relate the intensive use of insecticides affecting negatively *T. radiata* (Hall & Nguyen 2010), while others consider the genetic variability of the released parasitoid (Barr et al. 2009). Another reason may be the interaction with other natural enemies present in the system. For example, experimental data from central and southwest Florida support this point of view, suggesting that coccinellids are intraguild predators responsible for the poor performance of the parasitoid (Michaud 2004; Qureshi & Stansly 2009). Current research in the southern tip of the Florida peninsula suggests that *Zelus longipes* (L.) (Hemiptera: Reduviidae) might be an intraguild predator of *T. radiata* (Navarrete et al., unpubl.).

Important, but poorly studied players in the complex of organisms involved in the *D. citri* greening system are ants (Hymenoptera: Formicidae), which are well known for establishing mutualistic relationships with some hemipterans, either by tending their young or by using the hemipterans' (i.e., soft scales, aphids) sugary excretions (i.e., honeydew) as a carbohydrate source. As a reward, the hemipterans receive ant protection against natural enemies (Way 1963; Way & Khoo 1992). This relationship can be obligatory when the ant and the honeydew-producing hemipteran have coevolved together and are interdependent, or facultative when the association is not indispensable for the survival of the hemipteran. The latter relationship is more common in nature than the obligatory mutualism (Delabie 2001). The mutualism between ants and hemipterans is frequent in aphids, mealybugs, and membracids and most of the published literature about this phenomenon addresses members of these families (Delabie & Fernandez 2003). Ant mutualism with psyllids have been less documented; one example is the relationship of the hawthorn psyllids *Cacopsylla peregrina* Forster, *C. melanoneura* Forster, and *C. crataegi* (Schrank) with the ants *Lasius niger* (L.) and *Formica pratensis* Retzius that causes an increase in parasitism due to the expelling of hyperparasitoids (Novak 1994). A second example is the mutualistic relationship of the ants *Pheidole megacephala* F. and *Crematogaster striatula* Emery, with the psyllid *Diaphorina enderleini* Klimaszewski in Africa. Workers of these ants build shelters in order to protect the immatures of the psyllid against natural enemies and environmental adversities (Alene et al. 2011). Several species of ants have been reported associated with *D. citri* in Florida. Michaud (2004) found *Brachymyrmex obscurior* Forel, *Camponotus floridanus* (Buckley), *Crematogaster ashmeadi* (Mayr), *Dorymyrmex reginicula* (Trager), *Monomorium floricola* Jerdon, *Paratrechina bourbonica* (Forel), *Pseudomyrmex gracilis* (Fabricius), *Solenopsis invicta* Buren and *Dorymyrmex bureni* (Trager) collecting honeydew from *D. citri* nymphs, and also he observed *Do. bureni* carrying *D. citri* nymphs on several occasions. *Camponotus floridanus* and *Pheidole* spp. were observed carrying nymphs in *D. citri* infested flushes of *M. paniculata* in south Florida (Peña et al. 2008). In the same area, Chong et al. (2010) found *Crematogaster* spp., *Pheidole* spp., *Pseudomyrmex* spp. and *Solenopsis* spp. associated with *D. citri* in *M. paniculata*.

The objective of this study was to elucidate the role of ants in the biological control of *D. citri* by observing ant behavior and by determining if ant presence affects parasitism and psyllid densities in orange jasmine, *M. paniculata* and Persian lime, *C. latifolia*.

Materials and Methods

All of the experiments were conducted at the University of Florida, Tropical Research and Education Center, Homestead, Florida (N 25° 30' 30.56" W 80° 30' 08.22"; 3 m asl).

Identification and Ant Behavior in Relation to *D. citri* on *M. paniculata*

Surveys were conducted on 11-12 Oct and 8-9 Nov 2011 with observations made every 2 h during a 24-h period, beginning at 9 AM and ending at 7 AM on the next day. A 30-m long orange jasmine hedge was selected. Three 2.1-m long plots distanced 2 m from each other were chosen. Five *D. citri* infested flushes were randomly chosen on each plot, then, ant number and behavior (i.e., tending nymphs, feeding on the nymphs' excretion, walking and/or interacting with other natural enemies of ants) were recorded for a 2-min period per flush. A headlamp (Super Bright 53 LED Flashlight Torch) was used during night observations. Thereafter, each flush containing ants and *D. citri* nymphs was excised from the hedge, the flush was placed into a 50-mL vial, and brought to the laboratory for further examination under a microscope. The number of *D. citri* nymphs per flush was recorded; ants were sorted and one of us (MD) identified the ants and a reference collection was deposited at the Archbold Biological Station, Venus, Florida. A one-way analysis of variance (PROC GLM) was made comparing the number of ants present in the infested flushes per evaluation time. A regression analysis (PROC REG) was performed between the number of ants and the number of *D. citri* nymphs per flush (SAS Institute, Inc. 2001).

Ant Exclusion Experiments on *M. paniculata* and *C. latifolia*

These experiments were conducted in a 30-m long *M. paniculata* hedge and in 20 randomly selected *C. latifolia* trees located at the Tropical Research and Education Center. Both hosts were hedged in order to promote flushing. *M. paniculata* was hedged on 20 Dec 2011 and *C. latifolia* was hedged on 10 Mar 2012. When leaf flushes reached 2 to 3 cm in length, 36 flushes naturally infested with *D. citri* eggs were randomly chosen per site and ant exclusion was performed by smearing a 2-cm wide barrier of Tanglefoot® (Tanglefoot Company, Grand Rapids, Michigan) at the base of half of the flushes while half of the flushes had no Tanglefoot®. The number of ants was counted, and the presence of predators and parasitoids of *D.* citri was observed for 2-min on each of the selected flushes; the observations were made from 4:00 PM to 6:00 PM. The evaluations were performed every 2 days during a 30-day period; ant specimens were identified by MD. Thirty days after treatment, when most of the *D. citri* nymphs infesting the flushes were in the fifth instar, all 36 shoots were tagged, cut and brought to the laboratory where they were inspected under the microscope with the objective to count the number of parasitized and unparasitized nymphs to calculate percent parasitism. Differences in percentages of parasitism by *T. radiata* in both treatments were compared using unpaired *t*-test (PROCTTEST, SAS Institute, Inc. 2001).

Effect of Ant Control on *D. citri* Parasitism.

Two experiments were conducted in a 4-yr old *C. latifolia* grove. First the whole grove was hedged in order to promote flushing on 16 May 2012 and 18 trees were randomly chosen in the grove. Nine trees were treated with Extinguish Plus® granular bait, Hydramethylnon 0.365%+ S-Methoprene 0.250% (Wellmark International, Schaumburg, Illinois) (5 g/tree) applied to the soil surrounding the trunk, and 9 trees were left untreated. A buffer tree was left between the treated trees. Five flushes within each tree containing *D. citri* eggs were selected and tagged and the number and species of ants present, as well as presence of other natural enemies, was observed for 2 min per flush. The evaluations were repeated every 4 days starting on 23 May and ending on 12 Jun 2012. When most of the *D. citri* nymphs reached the fifth instar, the flushes were cut and brought to the laboratory in order to determine the percentage of parasitism by *T. radiata*. The differences between populations of ants and *D. citri* nymphs and percentages of parasitism by *T. radiata* in both treatments were compared using the unpaired *t*-test (PROC TTEST SAS Institute, Inc. 2001).

Results

Identification and Behavior of Ants Associated with *D. citri* in *M. paniculata*

Two ant species were identified during this survey, the bigheaded ant, *Pheidole megacephala* Fabricius, and the rover ant, *Brachymyrmex obscurior* Forel. *Pheidole megacephala* was the most common species during both surveys. Both species were active day and night. During the first survey the highest number of ants (n = 5) was observed at 7 PM; this number was significantly different from the evaluation at 9 AM (n = 1.4) (F = 2.03; df = 11,168; P = 0.0288). No significant differences were observed (F = 1.21; df = 11,168; P = 0.2843) during the second survey. The mean number of ants per flush varied from 3.33/flush (9 PM and 3 AM) to 0.93/flush at 1 PM (Fig. 1). Forty two percent of the 180 observed ants were seen feeding on the sugary excretion of the *D. citri* nymphs

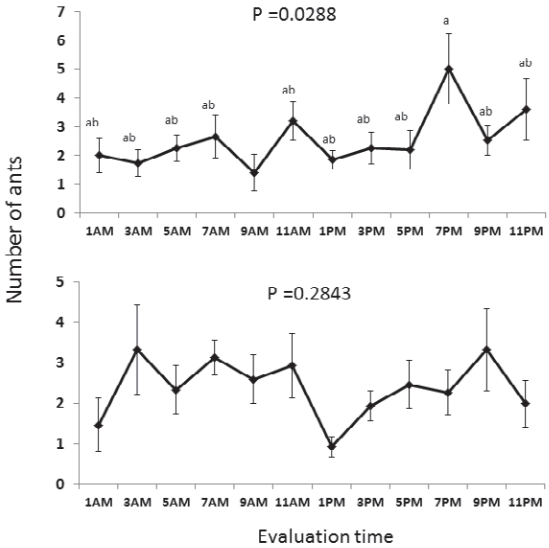

Fig. 1. Number of *Pheidole megacephala* (99% of the observations) and *Brachymyrmex obscurior* (1% of the observations) found in *Murraya paniculata* flushes infested with *Diaphorina citri* during a 24-h period on 11 Oct 2011 (A) and 11 Nov 2011 (B). Different letters represent a significant difference (Tukey's Test, P < 0.05). Error bars represent the standard error of the mean.

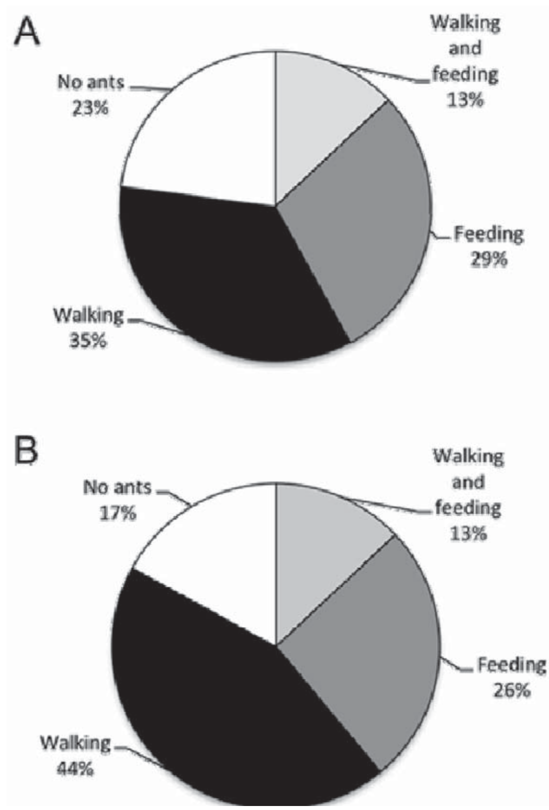

Fig. 2. Behavior of *Pheidole megacephala* (99% of the observations) and *Brachymyrmex obscurior* (1% of the observations) registered in *Diaphorina citri* infested flushes (A) 11 Oct 2011 and (B) 11 Nov 2011.

during the first evaluation. Similarly, 39% were observed feeding during the second evaluation. Ants were also observed walking (35% in the first evaluation and 44% in the second evaluation). No interactions of ants with other arthropods or aggressive behaviors against *D. citri* nymphs or adults were recorded during the surveys (Fig. 2). No statistical correlation was found between the numbers of ants and the numbers of *D. citri* nymphs in the first (b = -0.01, t (178) = -0.653, P = 0.5177) and second (b = 0.02, t (178) = 0.97, P = 0.3876) surveys.

Ant Exclusion Experiments in *M. paniculata* and *C. latifolia*

During the time of this experiment, *Pheidole megacephala* was the only ant species found in *M. paniculata*, but *P. megacephala*, *B. patagonicus* and *Solenopsis invicta* were observed in *C. latifolia*. The number of *P. megacephala* found in the unprotected flushes in *M. paniculata* fluctuated between 0.15/flush (6 Jan) and 0.5/flush (2 Jan and 8 Jan). In *C. latifolia* the number of ants, pooled over all species, varied between 1.44 (31 Mar) and 6.61 (25 Mar) (Fig. 3). During the first 3 evaluations, the most abundant species was *P. megacephala* but in the rest of the evaluations it was displaced by *B. patagonicus* (Fig. 4). In both hosts the flushes smeared with Tanglefoot® remained free of ants during the experiment. No other arthropods were seen during the evaluations, except on 6 Jan when a *Zelus longipes* female was observed feeding on the honeydew of a *D. citri* nymph in a Tanglefoot® - treated flush in *M. paniculata*; in *C. latifolia* a *Z. longipes* fifth instar nymph was seen in an unprotected flush on 2 Apr. In untreated flushes, the number of *D. citri* nymphs was not significantly higher than in the flushes treated with Tanglefoot® in *M. paniculata* (t = -0.95; df = 34; P = 0.35) or *C. latifolia* (t = 2.07; df = 34; P = 0.0513). However, in both host plants the percentage of nymphs parasitized by *T. radiata* was significantly higher in the flushes where the ants were physically excluded by the use of the Tanglefoot® sticky barrier. In *M. paniculata* treated flushes 20.36% of the nymphs were parasitized compared to 0.39% parasitism in the untreated control flushes (t = -3.35; df = 34; P = 0.002). Fifty-eight percent of the psyllid nymphs were parasitized in the *C. latifolia* Tanglefoot® treated flushes compared with 8.57% parasitism in the control (t = -0.47; df = 34; P = 0.0003).

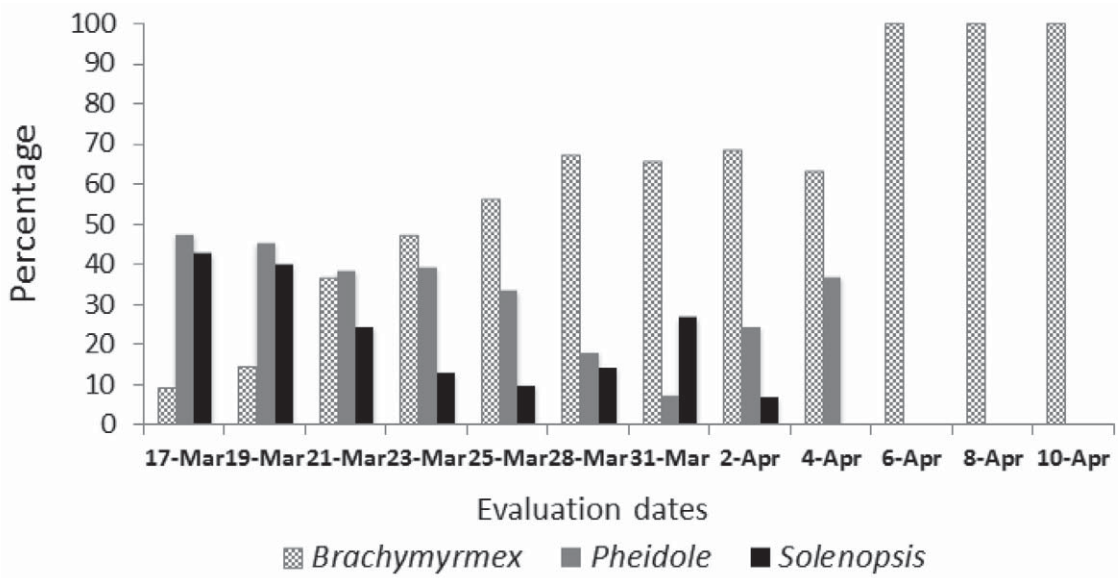

Fig. 3. Number of ants found in unprotected flushes in *Murraya paniculata* (*Pheidole megacephala* 100 % of the observations) (A) and *Citrus latifolia* (*Brachymyrmex patagonicus* 55%, *P. megacephala* 29% and *Solenopsis invicta* 15% of the observations) (B). Error bars represent the standard error of the mean.

Effect of Ant Control on *D. citri* Parasitism

The ant species *P. megacephala* and *B. patagonicus* were observed tending *D. citri* nymphs. No differences in ant densities were recorded until the last evaluation date when the number of ants was significantly lower in the trees treated with the granular bait (1.22 ants per flush) compared with the untreated trees (4.02 ants per flush) (t = 2.3; df = 16; P = 0.036) (Fig. 5). In the treated trees, *P. megacephala* was the dominant species during the evaluations of 5 May, 4 Jun, 8 Jun and 12 Jun 2012; in the control trees this species was also the most common except during the evaluations of 31 May and 6 Jun 2012 in which *B. patagonicus* was the most abundant species (Fig. 6). The number of *D. citri* nymphs was not statistically higher in the control trees compared with the trees treated with Extinguish Plus® (t = 1.40; df = 16; P = 0.1792). The percentage of parasitism by *T. radiata* was statistically higher (19.56%) in the trees treated with the granular bait in comparison with the control trees which had in average 3.62% parasitism (t = -3.26; df = 16; P = 0.004).

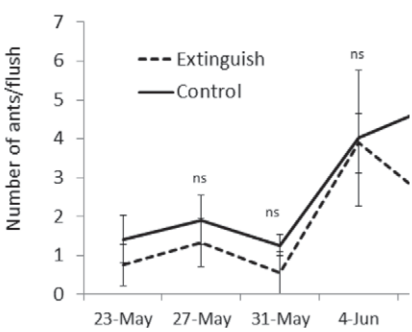

Fig. 4. Proportion of ants species found in unprotected flushes in *Citrus latifolia*.

Discussion

In this study we found 4 species of ants tending *D. citri*; *P. megacephala* and *B. obscurior* in *M. paniculata,* and *P. megacephala*, *B. patagonicus* and *S. invicta* in *C. latifolia*. *Pheidole megacephala* and *B. obscurior* have been previously reported tending *D. citri* (Michaud 2004; Peña et al. 2008). Here we report for the first time *B. patagonicus* tending *D. citri*. All of the ant species are invasive species in the USA. *Pheidole megacephala* is a native of Africa and the other 3 species are natives of South America. *Pheidole megacephala* is an omnivorous opportunistic species with a broad range of food preferences such as tending sap-sucking Hemiptera and eating their sugary excretions (Deyrup et al. 2000; MacGown et al. 2007). This behavior was confirmed by our observations in both hosts. The species involved in this system have been found tending other hemipterans, i.e., *B. osbcurior* tending *Dalbulus quinquenotatus* Delong & Nault (Cicadellidae) (Larsen et

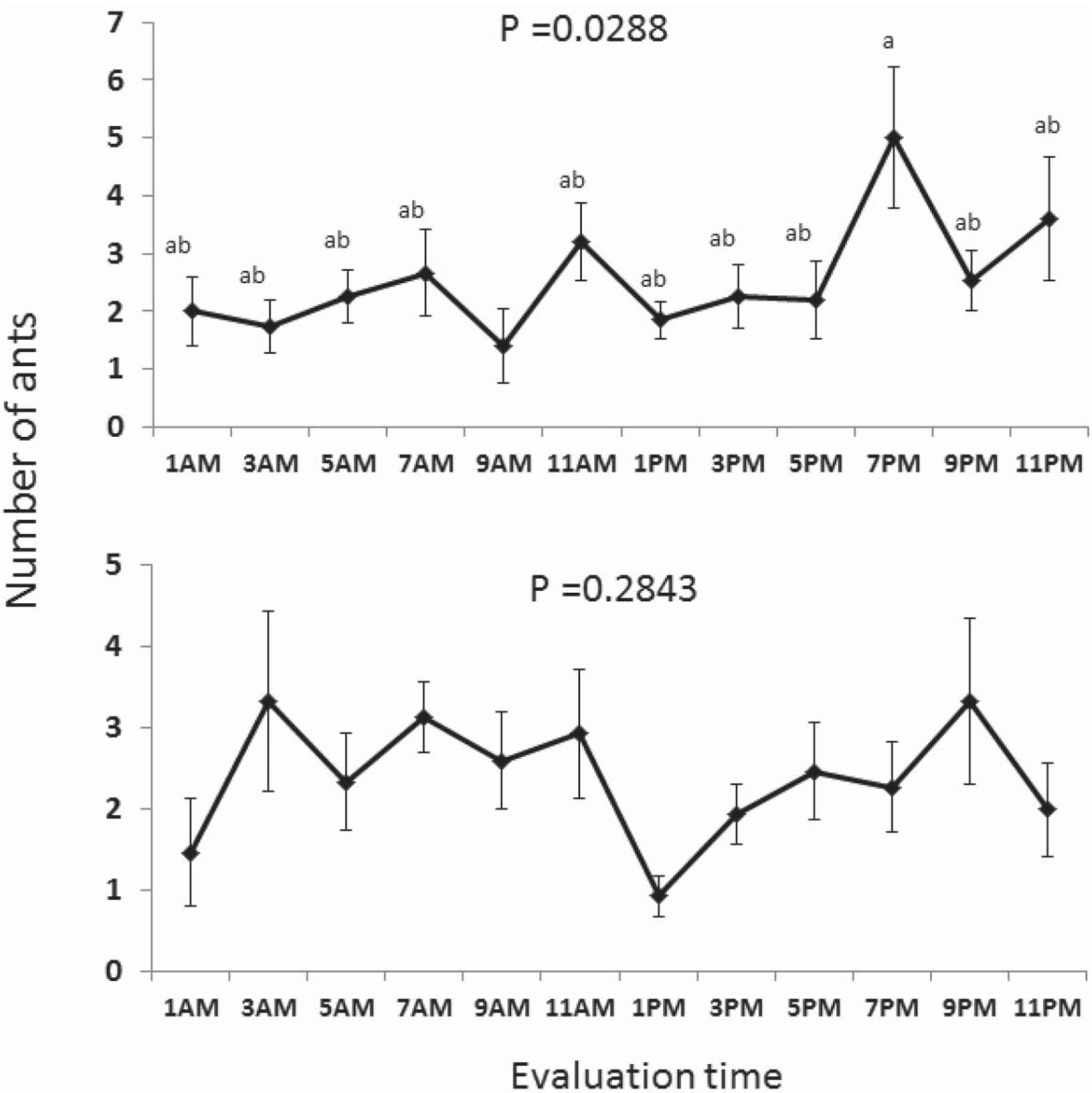

Fig. 5. Number of ants found in flushes of *Citrus latifolia* trees with and without the use of granular insecticidal ant bait . Error bars represent the standard error of the mean. Different letters represent significant differences, ns means no significant differences (independent *t*-test, $P < 0.05$).

al. 1991), *B. patagonicus* tending *Aphis gossypii* Glover (Aphididae) (Barnum 2008), *P. megacephala* tending *Coccus viridis* (Coccidae) (Reimer et al. 1993), and *S. invicta* tending *Phenacoccus solenopsis* Tinsley (Pseudococcidae) (Zhou et al. 2012).

We observed that *P. megacephala* and *B. obscurior* can forage in the infested flushes during night and day. This report is in agreement with the observations made by Cogni & Freitas (2002), Bestelmeyer (2008) and Dejean et al. (2007).

The ant exclusion experiments in both host plants showed that the ant presence in the flushes infested by *D. citri* had a negative effect on the performance of the parasitoid *T. radiata*. Although we did not observe (visually) a direct interaction between ants and the parasitoid, the difference in percentage of parasitism was significant. On the other hand we did not see any aggressive behavior of ants against nymphs of *D. citri*, and the number of *D. citri* nymphs in both treatments did not show a statistical difference, therefore, we assume that in this experiment the ants did not eat the tended psyllids. According to Way (1963), ants tending Hemiptera may feed on their protected trophobionts if sources of proteins

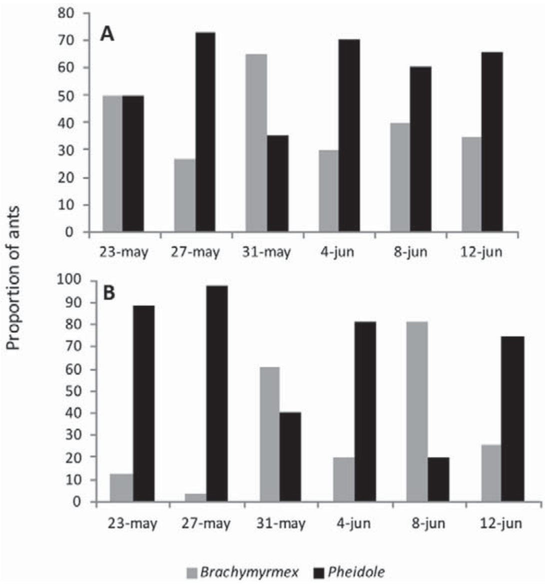

Fig. 6. Proportion of ants species found in flushes of *Citrus latifolia* trees with (A) and without (B) the use of granular ant bait.

and lipids are scarce. Qureshi & Stansly (2009) also found a higher percentage of parasitism by *T. radiata* in flushes protected with a sticky barrier in comparison with other exclusion treatments in *Citrus sinensis* L., but they did not associate ants with this phenomenon.

In the last experiment, we proved that the use of granular bait can help to reduce ant populations and consequently increase the percentage of parasitism. In this trial we could not totally prevent the access of ants to *D. citri* nymphs, as we did in the previous experiment using Tanglefoot®, however, we could decrease ant populations during the most susceptible stage for parasitism of *D. citri* (4th to 5th instar), and apparently this was enough to increase the activity of *T. radiata*. The use of different kinds of chemical treatments has been tested with success in the control of ants interfering with parasitoids of the grape mealybug (*Pseudococcus maritimus* (Ehrhorn)) in California and South Africa (Addison 2002; Klotz et al. 2003). We encourage similar studies in Florida citrus to determine if ant control in this agroecosystem will be a practical IPM practice against *D. citri*.

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