Effect of Soil Treatments for Cottony Cushion Scale (Hemiptera: Monophlebidae) Control on Nylanderia fulva (Hymenoptera: Formidicae) Survival and Trailing Activity

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Source: Florida Entomologist, 102(1) : 202-206

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.102.0132

Effect of soil treatments for cottony cushion scale (Hemiptera: Monophlebidae) control on *Nylanderia fulva* (Hymenoptera: Formidicae) survival and trailing activity

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Abstract

Nylanderia fulva (Mayr) (Hymenoptera: Formicidae), or tawny crazy ant, is an invasive ant from South America that is spreading in the southern US. Extremely large populations of this ant can inundate urban and natural landscapes, and efficient control methods are lacking. This study was conducted to determine if activity of *N. fulva* would decline after controlling the hemipteran honeydew-producers they were tending. Potted plants infested with cottony cushion scale (*Icerya purchasi* Maskell) (Hemiptera: Monophlebidae) were treated with 2 imidacloprid insecticide formulations, and changes in *N. fulva* survival and foraging behavior were monitored. There were fewer ant trails, lower trailing intensity, less foraging, and less nesting in potted plants treated with either product than in the control pots. The use of low application rates of systemic insecticide to reduce honeydew-producing hemipterans, such as cottony cushion scale, could be an important component of the integrated pest management of *N. fulva*.

Key Words: tawny crazy ant; *Paratrechina pubens*; *Nylanderia pubens*; Caribbean crazy ant; Rasberry [sic] crazy ant; invasive ants, integrated pest management

Resumen

Nylanderia fulva (Mayr) (Hymenoptera: Formicidae), o hormiga loca leonado, es una hormiga invasora de América del Sur que se está disperando en el sur de los EE. UU. Las poblaciones extremadamente grandes de esta hormiga pueden inundar las zonas urbanas y naturales, y faltan métodos eficientes para controlarlas. Se realizó este estudio para determinar si la actividad de *N. fulva* disminuiría después de controlar a los productores hemípteros de mielcilla que las hormigas atienden. Se trataron plantas en maceta infestadas con la cochinilla acanalada (*Icerya purchasi* Maskell) (Hemiptera: Monophlebidae) con 2 formulaciones de insecticidas de imidacloprid y se monitorearon los cambios sobre la sobrevivencia de *N. fulva* y su comportamiento de forrajeo. Hubo menos rastros de hormigas, menor intensidad de arrastre, menos forrajeo y menos nidos en plantas en macetas tratadas con cualquiera de los productos que en las macetas de control. El uso de tasas bajas de aplicación de insecticida sistémico para reducir los hemipteros que producen la mielcilla, como la cochinilla acanalada, podría ser un componente importante del manejo integrado de plagas de *N. fulva*.

Palabras Clave: hormiga loca leonado; *Paratrechina pubens*; *Nylanderia pubens*; hormiga loca del Caribe; hormiga loca de mora; hormigas invasoras; manejo integrado de plagas

An invasive ant from South America, *Nylanderia fulva* (Mayr) (Hymenoptera: Formicidae), or tawny crazy ant, can infest urban and natural landscapes with extremely large populations, and its distribution continues to expand in the southern US (MacGown 2016). Established colonies of *N. fulva* in loose litter or mulched areas may harbor thousands of ants (S. Sharma, personal observation), and are difficult to manage solely by insecticides. Indeed, insecticide-treated surfaces often become covered with ant cadavers, allowing other workers to escape insecticide exposure (Oi et al. 2016; Calibeo et al. 2017). The identification of novel methods and strategies that effectively suppress *N. fulva* is essential to limiting the spread of this invasive pest.

The cottony cushion scale, *Icerya purchasi* Maskell (Hemiptera: Monophlebidae), is a large, polyphagous insect that primarily infests citrus (*Citrus* spp.) (Rutaceae) and pittosporum (*Pittosporum tobira* [Thunb]) (Pittosporaceae) in Florida (Caltagirone & Doutt 1989; Thórarinsson 1990). *Nylanderia fulva* was observed foraging on the honeydew produced by *I. purchasi* on pittosporum hedges and bushes in Gainesville, Florida, USA, which had not been previously documented (Sharma et al. 2013). By consuming the nutrient-rich honeydew (Way 1963; Buckley 1987; Larsen et al. 1992; Delabie 2001), the ants presumably minimized the buildup of sooty mold on the host plants, and kept the hemipteran's environment cleaner (Haines & Haines 1978; Fokkema et al. 1983). As with other ant-hemipteran interactions, *N. fulva* could potentially protect *I. purchasi* from its natural enemies, and allow the scale populations to increase (Nixon 1951; El-Ziady & Kennedy 1956).

The effects of excluding ants from the hemipterans they tend for honeydew has been previously studied with several other ant and hemipteran species (Kenne et al. 2003; Altfeld & Stiling 2006; Piñol et al. 2009; Vanek & Potter 2010), but not with *N. fulva* or any of the

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hemipterans that its workers tend. For example, when invasive Argentine ant, *Linepithema humile* Mayr (Hymenoptera: Formicidae), foragers were excluded from the terrapin scale, *Mesolecanium nigrofasciatum* (Pergande) (Hemiptera: Coccidae), the ants relocated their nests from the base of the host red maple trees due to the lack of access to their immediate carbohydrate resource. As a result, the number of Argentine ant nests was reduced at the base of these trees (Brightwell & Silverman 2009). In addition, limiting honeydew or nectar resources may increase the attractiveness of nonrepellent, liquid carbohydrate-based baits to the ants (Silverman & Brightwell 2008). The objective of this study was to determine if *N. fulva* activity would decrease in plants treated for cottony cushion scale infestation.

Materials and Methods

LABORATORY TEST

A laboratory test was conducted to evaluate the effect of 2 imidacloprid formulations used for hemipteran control on *N. fulva*. The test used a completely randomized design with 4 replications. Each replicate consisted of 3 plastic trays (27.2 × 19.6 × 9.4 cm) with sides coated with Fluon® and filled to a depth of 2.5 cm with untreated soil (50% autoclaved sand and 50% potting mix; Fafard®, Agawam, Massachusetts, USA). Two 0.47 L pots filled with the same soil were placed at the center of each tray. The soil in 1 pot was treated with either CoreTect® or Merit® 2F (20% and 21.4% imidacloprid, respectively) (Bayer Environmental Science, Research Triangle Park, North Carolina, USA), whereas the other pot remained untreated. One CoreTect® tablet was placed 5.1 cm below the soil surface to follow the label rate of 1 tablet per 3.75 L or smaller pot. Merit® 2F was applied as a soil drench with a pipette at the rate of 146.2 µL per m^2. After treatment, all pots, including controls, received 25 mL of water.

A *N. fulva* colony fragment consisting of 500 workers and 2 queens, originating from source colonies collected in Gainesville, Alachua County, Florida, USA, was placed on the soil in each tray. The colony was provided water and a 10% sucrose solution through cotton-plugged 20 mL vials, and frozen house crickets were provided as a protein source. Ant mortality was assessed after 2 wk. The soil from each pot and tray was spread separately in larger tubs (45.5 × 34.3 × 7.6 cm) and allowed to dry. Because ants would move from dry soil to moist areas, artificial nests were established to entice living ants out of the soil, which facilitated the determination of ant survivorship. Placed in the middle of each tub, the artificial nest consisted of an inverted black plastic container (6 × 17 × 12 cm; Sterling King Products, Lyons, Illinois, USA), with holes on each side that covered 2 nest tubes. A nest tube was a 20 mL glass test tube, half filled with water and plugged with cotton and dental plaster (Castone®, Dentsply International, York, Pennsylvania, USA) (Banks et al. 1981).

The number of live *N. fulva* in the nest tubes were counted after 48 h and the percentage of surviving ants relative to the initial number of ants was calculated. Analysis was conducted on percent survival after square-root transformation. Differences in *N. fulva* survival among the 2 imidacloprid treatments and untreated control were determined by a 1-way analysis of variance (R Core Team 2012).

FIELD TEST

Twenty-four pittosporum plants (< 1 m tall) in 11.34 L pots were kept in a greenhouse together with pittosporum naturally infested with *I. purchasi* until all the plants were equally infested (approximately 1 mo). The plants were then transferred to a location in Gainesville, Florida, that was infested with *N. fulva*. In Aug 2012, pots were placed on the soil surface 2 m apart between a hedge and a road. *Nylanderia fulva* naturally moved into the potted plants. One wk after the pots were placed on site, the mean number of *I. purchasi* from 3 randomly selected branches per plant was determined to characterize the scale infestation. *Nylanderia fulva* activity was indicated by the following: (a) the mean number of *N. fulva* on 2 sausage slices (22 mm diam × 33 mm wide) placed at the base of the main stem, or trunk, of each plant for approximately 30 min; (b) trailing intensity reflected by the mean number of trailing ants crossing a single spot in 20 s; and (c) the mean number of ant trails per main plant stem (Sharma et al. 2013). The initial counts for each of the variables above were not significantly different ($P > 0.05$) among the plants.

The treatments were assigned to pots in a completely randomized design, with 8 replications per treatment. The scale and ant-infested pittosporum pots were randomly assigned 1 of the 2 imidacloprid treatments used in the laboratory test, or designated as a control. For the CoreTect® treatment, we inserted 3 tablets, spaced equally apart, about 5 cm deep into each pot (about 15 cm from the base of the plant), and watered (28.8 mL per pot). Merit® 2F was applied as a soil drench following the label rate of 6 mL of Merit® 2F in 28.8 mL water per 30.5 cm of shrub height. The untreated control pots received 28.8 mL of water. All pots were drip-irrigated daily for 10 min.

Icerya purchasi and *N. fulva* survival and activity in the infested plants were monitored weekly for 2 mo when ambient temperatures were > 21 °C and favorable for ant activity. Because plants were small (≤ 1 m tall), destructive sampling was not conducted; instead, 3 branches were randomly flagged on each plant before the study began and the mean number of *I. purchasi* per 3 branch sample was determined weekly.

Two mo after treatment, the plants were removed from their pots, placed in fluon-coated tubs (45.5 × 34.3 × 7.6 cm), the roots were removed, and the soil was air-dried in the laboratory. The ants were supplied with water, a 10% sucrose solution, and frozen house crickets. All live ants that moved from the soil into the nest tubes were counted after 48 h, and the mean number of *N. fulva* per pot was determined for each treatment.

Repeated measures analysis of variance was conducted to assess differences in the numbers of *I. purchasi* on the pittosporum, and the number of *N. fulva* counted, on the sausage slices, on trailing intensity (20 s counts), and on the number of trails among treatments for the pre-treatment and weekly post-treatment data. If significant, weekly means were separated with the Tukey HSD test (R Core Team 2012). Pearson's correlation was used to assess the association between the number of *I. purchasi* and *N. fulva* trailing intensity for each treatment.

Results

LABORATORY TEST

Nylanderia fulva survival when exposed to soil treated with either imidacloprid formulation did not differ significantly from the untreated control. Mean percent survivorship ranged from 71 to 86% of the 500 ants initially exposed to the treatments (Table 1). Because a majority of *N. fulva* survived both treatments, these treatments were used in the field test to examine *N. fulva* activity relative to the treatment of *I. purchasi*.

Table 1. Mean (± SE) numbers of live *Nylanderia fulva* workers and percent survival of workers given access to treated and untreated soil in the laboratory after 2 wk of exposure (n = 4 pots per treatment).

Treatment	No. ants[a]	% Survival[b]
Merit 2F®	390.0 ± 21.6	78 ± 4
CoreTect®	357.6 ± 22.9	71 ± 5
Control	432.5 ± 15.0	87 ± 3

[a]Means were not significantly different by analysis of variance ($F = 16.54$; df = 2, 6; $P > 0.05$).
[b]Percent survival of initial count of 500 live workers per replicate.

FIELD TEST

Numbers of *I. purchasi* Scales on Branches

The numbers of *I. purchasi* were significantly higher on the control, compared to both imidacloprid treatments on all sampling dates ($F = 8.9$; df = 2, 21; $P < 0.001$). However, statistical differences were not observed for the numbers of *I. purchasi* between the 2 insecticide treatments. The numbers of scales on treated plants began to decrease 1 wk post-treatment, and it was almost zero at 7 wk post-treatment. Scale density decreased by 67% to an average of 20 scales per plant in Sep and Oct in the control pots (Fig. 1), possibly due to declining temperatures. In Sep and Oct, the mean average daily temperatures were 24 and 20 °C, respectively, compared to 26 °C in Aug (Florida Automated Weather Network).

Activity of *N. fulva* on Potted Plants

The numbers of *N. fulva* workers foraging on the sausage slices placed at the base of the main stem of each potted plant were significantly lower in the 2 imidacloprid treatments than the control over all sampling dates ($F = 11.1$; df = 2, 21; $P < 0.0005$). The mean numbers of ants in the pots treated with either CoreTect® or Merit® 2F did not differ significantly (Fig. 2). The trailing intensities were significantly lower (2 ants per 20 s) in the treated plants than in the control plants (15 ants per 20 s) ($F = 20.6$; df = 2, 21; $P < 0.0001$). However, the trailing intensities did not differ significantly between the imidacloprid treatments (Fig. 3). Similarly, significantly fewer ant trails occurred on the main stems of treated plants than on the stems of control plants ($F = 26.0$; df = 2, 21; $P < 0.0001$). The average number of trails between the 2 insecticide treatments did not differ significantly (Fig. 4). The mean number

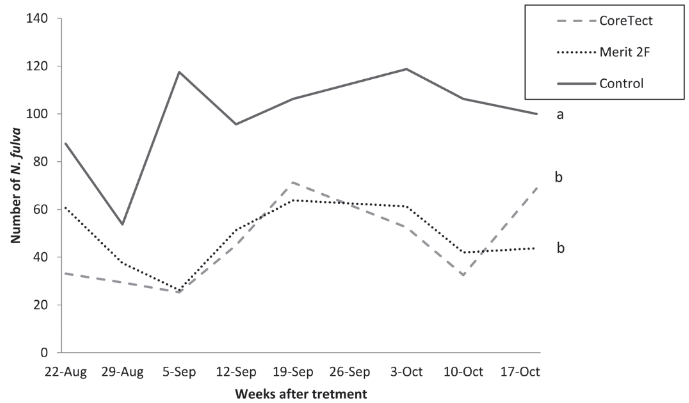

Fig. 2. Mean (n = 8) numbers of *Nylanderia fulva* counted from sausage samples placed near the base of the trunk of pittosporum plants. Different letters indicate significantly different ($P < 0.05$) means by repeated measures analysis of variance and Tukey HSD test (R Core Team 2012).

of *I. purchasi* and the *N. fulva* trailing intensity counts were positively correlated in the pots treated with CoreTect® ($r = 0.88$) and Merit® 2F ($r = 0.88$), and to a lesser extent, the control ($r = 0.60$) (Fig. 5).

Nesting by *N. fulva* in Potted Plants

After 2 mo in the *N. fulva* infested area, all pots were examined for the presence of *N. fulva* nests. All untreated control pots either had nests or ants present in them. Half (n = 4) of the untreated pots had tunnels and brood present, and contained an average of 545 ± 222 (± SE) *N. fulva* workers per pot. The remaining control pots contained 250 ± 86 adults but lacked brood. Three (19%) of the treated pots (2 with Merit® 2F, and 1 with CoreTect®) contained a small number of worker ants averaging 100 ± 50 workers per pot. The rest of the treated pots did not harbor any *N. fulva*.

Discussion

In the laboratory test, ant survivorship averaged 72% and 78% in the imidacloprid treatments. Some of the observed mortality could be due to initial contact with treated soil. However, the low

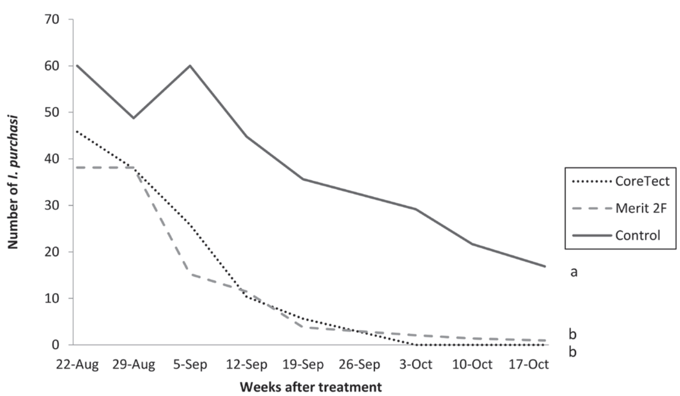

Fig. 1. Mean (n = 8) numbers of *Icerya purchasi* per pittosporum plant counted on 3 branches of each plant. Different letters indicate significantly different ($P < 0.05$) means by repeated measures analysis of variance and Tukey HSD test (R Development Core Team 2012).

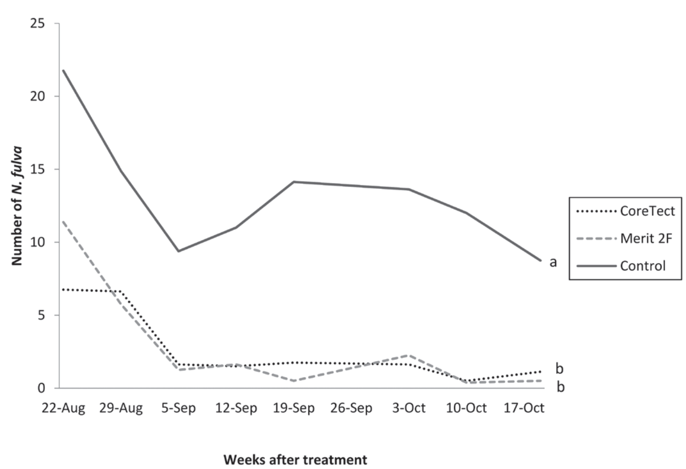

Fig. 3. Mean (n = 8) numbers of *Nylanderia fulva* crossing a specific point on the main stems of pittosporum plants in 20 s (trailing intensity). Different letters indicate significantly different ($P < 0.05$) means by repeated measures analysis of variance and Tukey HSD test (R Core Team 2012).

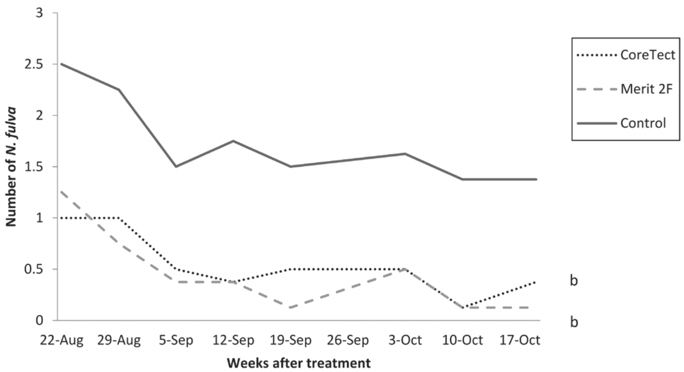

Fig. 4. Mean numbers of *Nylanderia fulva* trails on the trunk of pittosporum plant. Different letters indicate significantly different ($P < 0.05$) means by repeated measures analysis of variance and Tukey HSD test (R Core Team 2012).

concentrations of imidacloprid present in CoreTect® and Merit® 2F formulations (20% and 21.6%, respectively) may have allowed some survivorship of *N. fulva* because substantial mortality of *N. fulva* was observed in a preliminary test where potted soil was treated with higher concentrations of imidacloprid (Merit® 75 WP; S. Sharma, unpublished data). Costa and Rust (1999) noted that even though Argentine ants showed avoidance toward the insecticide-treated soil in pots, ants would initially excavate the treated soil, thereby contacting the insecticide, before displaying avoidance. We also observed avoidance behavior by *N. fulva* of the imidacloprid-treated soil inside the pots. Although *N. fulva* nested within the potting soil of the untreated pots, they nested beneath the pots treated with imidacloprid. This suggested that the treated soil deterred *N. fulva* from nesting in the potted soil, even though imidacloprid is considered a non-repellent insecticide for ants (Rust et al. 2004). Oi and Williams (1996) reported red imported fire ants, *Solenopsis invicta* Buren, clinging to the exterior of pots containing pyrethroid-treated soil, apparently due to the repellent effects of the insecticide.

In the field test, it was observed that most of the *N. fulva* on the sausages at the base of the control plants emerged from the potting soil within min of sausage placement. In contrast, *N. fulva* found on sausages at the base of treated plants appeared to originate in the surrounding area beyond the pots. Because these ants were not nesting in the pots, searching and recruitment times to the sausages would take longer, thus resulting in the significantly lower counts for the treated soil. These results are consistent with the laboratory test where *N. fulva* tended to nest under the pots, rather than in the treated soil.

The reduced trailing intensity and the lower number of ant trails on the treated plants may be attributed to the lower abundance of *I. purchasi*, resulting in less availability of honeydew as a food resource. This is supported by the positive correlation between *I. purchasi* counts and the trailing intensity of *N. fulva* (Fig. 5). This also is consistent with the results of Sharma et al. (2013), where ants and hemipteran numbers were positively correlated throughout a sampling season, presumably due to the availability of honeydew. As seen with other ant species, when the number of hemipterans decreased, honeydew became scarce, which may have triggered the ants to search for food elsewhere (Bach 1991; Blüthgen et al. 2000). We also documented fewer *N. fulva* nests in treated pots, which also could be due to the reduced availability of honeydew. Establishment of nests near food sources has been reported for Argentine ants (Holway & Case 2000).

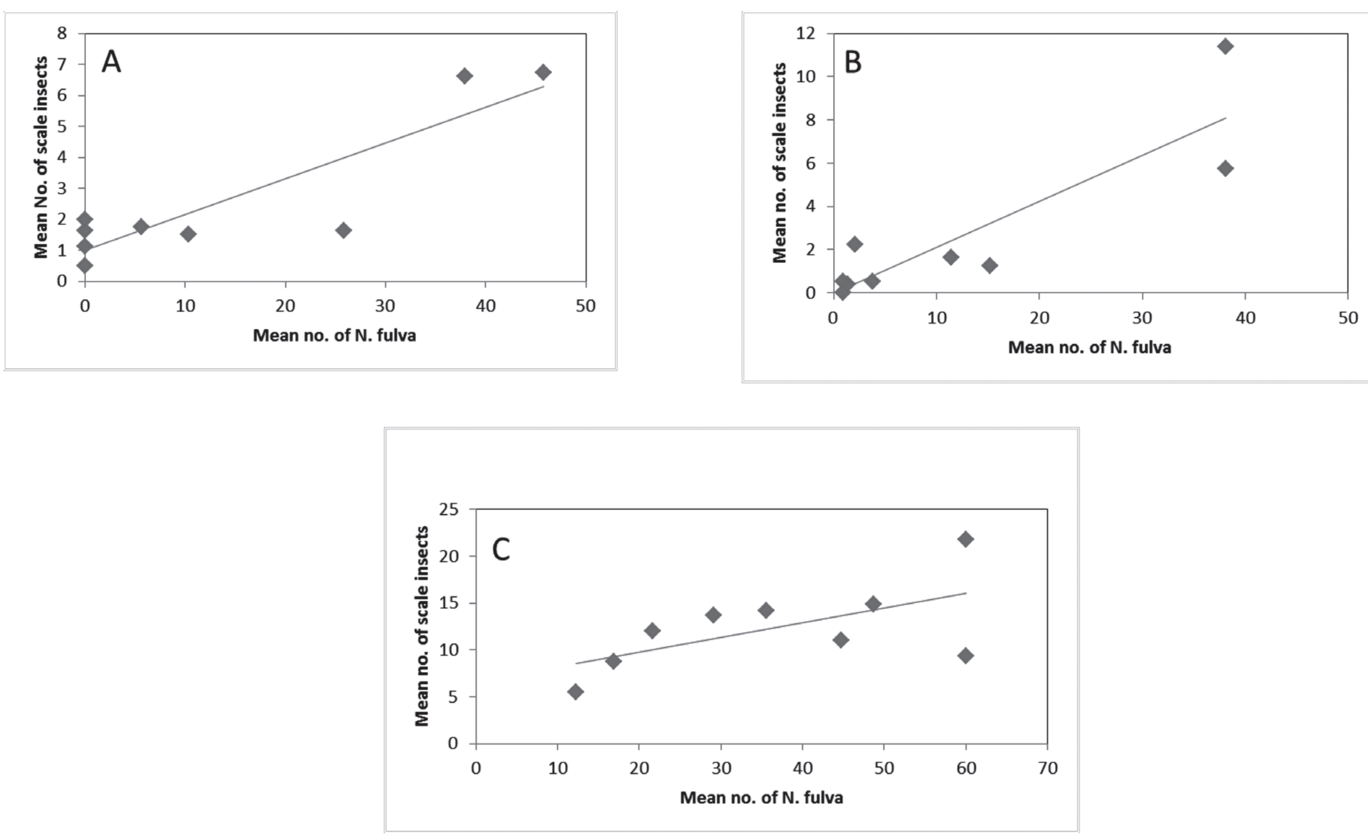

Fig. 5. Correlations between the mean numbers of *I. purchasi* and *N. fulva* in pots treated with (A) CoreTect® ($r = 0.88$; n = 8), (B) Merit® 2F ($r = 0.88$; n = 8), and (C) control (untreated) ($r = 0.60$; n = 8).

Removal of hemipterans to reduce honeydew resources for invasive ant control also is being attempted for yellow crazy ants, *Anoplolepis gracilipes* (Smith) (Green et al. 2011, 2013). In addition, Brightwell et al. (2010) recorded reductions in ground foraging, a decline in nest density, and canopy foraging by Argentine ants when honeydew-producing scale insects were reduced with the use of trunk-injected insecticide. Our study showed that *N. fulva* activity was reduced by managing *I. purchasi* on pittosporum. These results suggested that eliminating honeydew-producing insects in landscapes may contribute to the control of *N. fulva*. However, because soil applications of imidacloprid were used to systemically reduce *I. purchasi*, we cannot conclude that the sole removal of honeydew producing hemipterans can significantly reduce *N. fulva* activity. Indeed, the partial avoidance of imidacloprid-treated soil by *N. fulva* may be an important component of the observed decline in *N. fulva* activity, but it was not a predominant factor because foraging on sausage was not completely excluded in treated pots. *Nylanderia fulva* avoidance of insecticide residues has been documented for several insecticides used to control *N. fulva* (Calibeo et al. 2017). Sustained reductions of overwhelming numbers of invasive ants such as *N. fulva* most likely will require an integrated pest management program consisting of several control tactics (Oi et al. 2016). Eliminating natural food sources, such as honeydew producing hemipterans, either directly with insecticides, or by preventing food access via repellents, could be part of an integrated pest management program for *N. fulva*.

Acknowledgments

We are grateful for the assistance provided by P. Ruppert, S. Rachel, and C. Rachel (formerly with the University of Florida Entomology and Nematology Department). An earlier draft of this manuscript was reviewed by E. Gilman (formerly of the University of Florida Horticultural Sciences Department). Funding was provided by the USDA-NIFA Tropical and Subtropical Agricultural Research Award # 2010-34135-21096. Mention of trade names or commercial products in this article are for the information and convenience of the reader, and does not imply recommendation or endorsement by the University of Florida or the US Department of Agriculture.

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