

EFFICACY OF SELECTED INSECTICIDES USING ARBOREAL BIOASSAYS AND  
RUBIDIUM MARKING OF WHITE-FOOTED ANTS, *Technomyrmex albipes*  
(HYMENOPTERA: FORMICIDAE)

By

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by

J.R. Warner

I testify that through Thee the sovereignty of God and His dominion, and the majesty of God and His grandeur, were revealed, and the Day-Stars of ancient splendor have shed their radiance in the heaven of Thine irrevocable decree, and the Beauty of the Unseen hath shone forth above the horizon of creation.

— Bahá'u'lláh

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Abstract of Dissertation Presented to the Graduate School  
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Chair: Rudolf H. Scheffrahn

Major Department: Entomology and Nematology

Experimental and commercial baits and surface treatments were compared in three field experiments (2002 to 2004) for control efficacy against white-footed ants (WFA, *Technomyrmex albipes*) nesting in and confined to coconut palms (*Cocos nucifera*).

Results were obtained from counts of ants foraging on the wicks of feeding vials from the 2002 experiment. At 23 days after exposure (DAE) to the test products, only the mean numbers of ants on wicks for 600 ppm bifenthrin (BIF) surface treatment and NecDew (ND, University of Florida proprietary liquid ant bait matrix) with 100 ppm thiamethoxam (TX) were significantly lower than a 40% aqueous sucrose (SS) control. At 50 DAE, only counts of ants on wicks for TXND were significantly less than SS. Other products tested included 600 ppm fipronil (FIP) and 50 ppm imidacloprid (IM) liquid ant bait.

During the 2003 experiment, overall trends showed that NecDew with either 100 ppm IM or 100 ppm TX had lower numbers of ants on wicks than the other products tested, including a commercial 10,000 ppm boric acid bait (BA), and surface treatments BIF and FIP, both at 600 ppm. This experiment also showed that WFA foragers preferred SS over BA.

The trend for the 2004 experiment for the numbers of ants on wicks and amount of baits consumed showed no significant differences between 100 ppm TX experimental bait and 50 ppm IM commercial bait, both of which had significantly fewer ants on wicks and were consumed less than the 25% aqueous sucrose solution control. Trailing frequency counts for WFA had high correlation with bait consumption ( $R = 0.931$ ) and numbers of ants on wicks to bait consumption ( $R = 0.850$ ), and bait consumption to trailing frequency ( $R = 0.8330$ ).

Rubidium chloride (RbCl) dissolved in 40% aqueous sucrose solution was tested as a Rb marker for WFA foragers. The RbCl was nontoxic to WFA foragers at 15,000 ppm, was detectible in single ants and was detectible at 16 DAE. Using RbCl to determine foraging distances, WFA foragers were detected foraging ~20 m from the RbCl source. Rubidium was determined as a valid marker for WFA.

## CHAPTER 1 INTRODUCTION

It seems fitting to begin this dissertation with a quote from one of the greatest myrmecologists, William Morton Wheeler (1910): “Ants are to be found everywhere, from the arctic regions to the tropics, from timberline on the loftiest mountains to the shifting sands of the dunes and seashores, and from the dampest forests to the driest deserts.”

Although as individual organisms, ants are small, their collective weight in tropical rainforests and grasslands is estimated to be 10 to 15% of the entire animal biomass (Agosti et al. 2000). With their highly complex systems of chemical communication and social life cycles, ants represent the culmination of insect evolution (Hölldobler and Wilson 1990). About 9,000 described species of ants (Agosti et al. 2000) have successfully adapted throughout nearly all habitats on earth. Most of these species go unnoticed by humans, but there are always a few ants that will, at some time or another, be considered unwanted intruders. These invaders of human structures include a small black ant, with white feet (tarsi).

The white-footed ant (WFA) was described in 1861 by Frederick Smith (1861) from specimens collected by Alfred R. Wallace in Tondano, Sulawesi, Indonesia. Smith named this ant *Formica (Tapinoma) albipes*. It is not known why the subgenus name *Tapinoma* was included by Smith. Smith’s work at the British Museum included descriptions of hundreds of new ant species, but Creighton (1950) notes that not more than one third of these species could actually be recognized by his descriptions.

Much of the “reprehensible work of Smith” was corrected by Gustav Mayr, Julius Roger, C. Emery, W. Nylander and later, W.M. Wheeler and F. Santschi (Creighton 1950). In 1872, the genus *Technomyrmex* was erected by Mayr. Emery (1888) raised *Tapinoma* to full generic status in the subfamily Dolichoderinae, thereby assigning this ant its current name, *Technomyrmex albipes*. Warner (2003) gives further background on the WFA, including its common names, biology and life cycle, and pest status.

*Technomyrmex albipes* is widespread from South Africa to eastern Australia, from Japan to New Zealand and throughout the Pacific (Wilson and Taylor 1967) including Hawaii. In the continental United States and vicinity, the WFA has spread further north from where it was first collected in Homestead, Florida, in 1986 (Deyrup 1991), advancing to northern Florida, with isolated records from Columbia, SC, New Orleans, LA, Savannah, GA, Paradise Island, Bahamas, and Grand Cayman, Cayman Islands (Warner, unpubl. reports). Further information on the distribution of *T. albipes* is given by Warner (2003).

Although the WFA is still considered by both the public and pest control industry as a formidable pest due to the frequent difficulty in managing WFA populations, new pest control products, such as Termidor (9.1% fipronil, BASF Corp., Durham, NC), are being widely used by pest control operators who report fewer complaints from customers (Warner 2005, unpubl. observation). Part of my study documents the results of novel field bioassays comparing the efficacy of fipronil and other products, some of which are commonly used for WFA control.

To investigate control of WFA in structures, it was deemed necessary to first obtain a better understanding of the food preferences of these ants and then determine if any of

these foods could be incorporated into a toxic bait. Sucrose, fructose, glucose, and maltose in aqueous solutions were offered at selected concentrations in binary choice tests to WFA trailing on exterior building walls at University of Florida's, Ft. Lauderdale Research and Education Center (FLREC), in Davie, Florida (26.08010° N, 80.23897° W). Commercial ant baits and four NecDew™ (University of Florida) formulae, a proprietary sweet bait, all without toxicants, were also tested against the sugar solutions. White-footed ant foragers preferred NecDew<sub>4</sub> to sucrose solutions, and sucrose solutions (25% ≤ 40%) were preferred over other sugar solutions tested. In tests with solutions containing the toxicant disodium octaborate tetrahydrate (DOT), NecDew<sub>4</sub> with 1% DOT was preferred over Uncle Albert's Super Smart Ant Bait (A Safe Pest Eliminators, Inc., Miami, FL) a commercial bait with 1% DOT. Additionally, no repellency was observed in 25% sucrose solutions containing up to 7% DOT (Warner and Scheffrahn, 2004).

The preference tests led to a series of laboratory experiments which showed that liquid sweet baits were most effective for WFA control (Warner and Scheffrahn, 2005). These tests compared experimental and commercial baits, gels, surface treatments, one insecticidal dust, and an ultrasonic pest repeller for control efficacy against containerized WFA subcolonies. NecDew<sub>4</sub> with 10 ppm thiamethoxam (Syngenta Crop Protection, Greensboro, NC), reached 62% mortality at 8 days after exposure (DAE), and 100% mortality at ~35 DAE, and would therefore likely achieve an acceptable level of control in the field. Other baits yielding high mortality (64-79% at ~30 DAE) were imidacloprid (Bayer Environmental Sciences, Montvale, NJ) (w/v) in 25% sucrose solution, NecDew™ with 10,000 ppm DOT, (Tim-bor®, U.S. Borax, Los Angeles, CA), 10 ppm

thiamethoxam in 25% (w/v) sucrose solution, and Terro Ant Killer II® (54,000 ppm sodium borate decahydrate (borax), Senoret Chemical Co. Minneapolis, MN).

Results using the other baits were unsatisfactory (11 to 62% mortality at ~30 DAE), including:

- Drax Liquidator® (10,000 ppm orthoboric acid, Waterbury Companies, Waterbury, CT), a commercial ready-to-use liquid bait;
- Imidacloprid ant bait instant granules (50 ppm imidacloprid) in deionized water (3:1, water: granules);
- Pre-Empt® (50 ppm imidacloprid);
- PT381B Advance Liquid Ant Bait (54,000 ppm borax), Whitmire Micro-Gen Research Laboratories, Inc., St. Louis, MO).

Results from surface treatments were unsatisfactory, including:

- Conserve® SC (800 ppm spinosad, Dow AgroSciences, Indianapolis, IN);
- Demand® CS (600 ppm lambda cyhalothrin, Syngenta Crop Protection);
- Indoxacarb (500 ppm, DuPont, Wilmington, DE);
- Talstar® Lawn and Tree Flowable (600 ppm bifenthrin, FMC Corporation, Philadelphia, PA);
- Termidor® SC (600 ppm and 1,200 ppm fipronil, Aventis Environmental Science, Montvale, NJ).

Results from gels and other products were unsatisfactory, including:

- Combat® Quick Kill, (100 ppm fipronil, Combat Insect Control Systems, Oakland, CA), over-the-counter ant bait stations;
- DeltaDust® (an insecticidal dust of 500 ppm deltamethrin, Aventis Environmental Science);
- Indoxacarb (500 ppm, DuPont, Wilmington, DE) as a suspension in honey water (1:1);
- Maxforce® Ant Bait Gel (10 ppm fipronil, Maxforce Insect Control Systems, Oakland, CA);
- Noviflumuron (5,000 ppm, Dow AgroSciences, Indianapolis, IN), suspended in a loose bait gel using 5,000 ppm Phytigel® (Sigma, St. Louis, MO) in 25% (w/v) sucrose-water;
- Noviflumuron (5,000 ppm, Dow AgroSciences), used as a suspension bait in honey-water (1:1);
- Ultrasonic Pest Repellers (Lentek International, Inc., Orlando, FL).

The surface insecticides tested under laboratory conditions had unsatisfactory control results. However, in view of their popularity with pest control operators, it was decided to test them in field trials, along with selected liquid baits. Since the WFA is considered a residential pest, a field trial simulating a residential scenario was considered desirable. During the summer of 2000, a field trial was attempted in Boca Raton, FL, (Palm Beach County) using 12 houses infested with WFA. Significant differences in structures, landscaping, and climatic factors made it difficult or impossible to adequately monitor treatment effects on WFA populations therefore, the results of this test were not conclusive. For valid field trial results, the test sites should be in close proximity to each other. Thus one could monitor population effects under the same climatic conditions, save time, and avoid changing weather conditions when driving from house to house. Hourly weather changes affect ant trailing and hence population monitoring efforts. It is also possible that homeowners might interfere with treatments.

Monitoring stations applied to the exterior sides of residential structures were a special concern because each location had its own microclimate (e.g., some are shaded at a certain time of day, while others are exposed to direct sunlight), and caused far too much variability for an effective test. To resolve these issues, an experimental plot was created and a bioassay was developed using coconut palms (*Cocos nucifera* L.) located on the FLREC campus.

The design we developed (hereafter referred to as an “arboreal bioassay”) aimed to simulate a residential habitat. A sticky barrier applied on the lower trunks of coconut palms prevented the escape of WFA populations nesting in the tree crowns and also prevented other ant species from entering the trees. The details of this novel bioassay are

described in Chapter 2 (Materials and Methods). Monitoring stations containing vials filled with sweet liquids were placed on the trunks between the sticky barriers and tree crowns.

Data collected during a series of field experiments performed between 2002 and 2005 included counting the numbers of ants feeding on the wicks of vials containing sweet liquids, measuring the weight losses of these vials, and counting numbers of ants crossing a point in 60 seconds (trailing frequency) as they trailed from their nests to the feeding sites. The null hypothesis was that there was no significant difference between insecticidal and noninsecticidal (control) treatments.

To increase our understanding of WFA preferences and the effects of various toxicants, we attempted to better understand WFA trailing behavior. A practical method of marking the ants was needed to study WFA foraging. After literature review, rubidium (Rb), as rubidium chloride (RbCl) was selected as an elemental marker for field experiments. The Broward County Environmental Protection Department (EPD), Environmental Monitoring Division (EMD) analyzed ant tissues for Rb content using inductively coupled plasma (ICP) optical emission spectroscopy. The null hypothesis was that no significant difference existed in the amount of Rb contained in ants that fed on RbCl-containing liquid versus those that did not.

## CHAPTER 2 ARBOREAL BIOASSAY 2002

### **Introduction**

In January 2002, laboratory tests were performed at the University of Florida's Ft. Lauderdale Research and Education Center (FLREC), Broward County, FL, to examine the efficacy of selected insecticides against WFA. The efficacy of thiamethoxam dissolved in NecDew, a University of Florida (UF) bait matrix, was greater than most of the other products tested (Warner 2003, Warner and Scheffrahn 2005). Ants feeding on thiamethoxam (10 ppm) in NecDew suffered 98% mortality at 29 days after exposure (DAE) (Warner and Scheffrahn 2005). In the present test, thiamethoxam (100 ppm) in NecDew (TXND) was compared with a commercial imidacloprid (IM) bait in a field trial against WFA. In addition, two compounds used in commercial surface treatments, bifenthrin and fipronil, frequently used to control WFA, were also evaluated.

### **Materials and Methods**

Several test plots at FLREC (Davie, Florida, 26.085° N., 80.238° W), were chosen for WFA control studies. These areas had established plantings of coconut palms. For about 2 years, unsuccessful attempts were made to establish WFA populations in a grove of coconut palms on the FLREC campus until naturally infested palms were discovered.

Isolated populations of WFA in palms simulated a landscape/household scenario in which WFA could forage to experimentally supplied food sources but also had natural food sources and a natural nesting and foraging environment. A critical aspect of these experiments was to isolate WFA populations in the palms and exclude other ant species

present in the grove, especially *Solenopsis invicta* Buren (red imported fire ant (RIFA)), and also others including *Paratrechina longicornis* (Latreille) (crazy ant), *Camponotus floridanus* (Buckley), and *C. tortuganus* (Emery) (Florida carpenter ants), *C. planatus* Roger (compact carpenter ant), *Tapinoma melanocephalum* (Fabr.) (ghost ant), *Dorymyrmex* spp. (pyramid ants), and *Brachymyrmex* spp. (rover ants). This was accomplished by applying a 10.2 cm wide sticky barrier of Tree Tanglefoot (The Tanglefoot Company, Grand Rapids, Michigan) around each trunk. It was necessary to continually monitor this barrier and remove wind-blown debris (especially leaves) that would otherwise act as bridges allowing ants to escape from the palm.

It was also important to monitor the rapidly growing palm fronds that (if left untrimmed) would eventually contact neighboring palms and permit ants to quickly escape to other palms. Since many of the palms were high (up to 20 m), on two occasions we rented a boom lift to be able to trim fronds. Smaller palms were continually pruned using a standard pole pruner and a tree saw. The grass around the palms was maintained at a low height and periodically the palms were fertilized with 10–4–10 or similar fertilizer at ~2.3 kg/palm. The test areas were not irrigated. An electric fence (Parmak Energizer Model DF-SP-LI, Parker McCrory MFG. Co., Kansas City, MO) was installed to keep animals (such as raccoons) from entering the research plots.

Five treatments assigned randomly with 4 replicates each, were applied to the palms on 16 September 2002. Some of the products were purchased over-the-counter, others were supplied by chemical manufacturers, and one other (NecDew Formula 4) was developed at the University of Florida as a liquid bait for pest ants. Liquid baits included 10 ppm thiamethoxam SC (Syngenta Crop Protection, Greensboro, NC) dissolved in

NecDew (TXND) (on 2 October 2002, the thiamethoxam concentration was increased to 100 ppm), and 50 ppm imidacloprid (IM)(Pre-Empt) sweet liquid bait (Bayer Environmental Sciences, Montvale, NJ). Surface treatments included 600 ppm fipronil (FIP) (Termidor® SC, Aventis Environmental Science, Montvale, NJ), and 600 ppm bifenthrin (BIF) (Talstar®, FMC Corporation, Philadelphia, PA). Control treatment consisted of 40% (w/v) aqueous sucrose solution (SS40).

Foraging ant populations were monitored in 13.5 x 13.5 x 9 cm “Sandwich Plus” plastic boxes (Home Products International, Chicago, IL), fitted with 5 holes that were strapped to the palm trunks with cable ties (Thomas and Betts Corp., Memphis, TN) just above the Tanglefoot barrier (Figs. 1 and 2). Glass shell vials (6 mL capacity) with Titeseal® plastic caps (Fisher Scientific, Hampton, NH) were modified for use as bait containers by drilling 6 mm diam holes in the caps, inserting cotton dental wicks (38.1 x 9.53 mm, no. 2 medium cotton roll, Crosstex International, Hauppauge, NY), to minimize bait desiccation and entrapment by ants, and then adding 4.5 mL bait solution. Two bundles of three vials each held together with rubber bands were placed on shelves in the stations that were strapped to the palms. When the vials were filled with 4.5 mL of either sugar water or one of the sweet toxic baits being tested, ants would quickly arrive to feed on the wicks. Ant counts were taken every 3 to 4 days at dawn after placement of freshly filled vials into the stations. Digital photos were taken of each station in which the ants on the wicks of the 6 vials could clearly be observed. A flashlight was often used to illuminate the wick areas and condensation was removed with a paper towel. Digital photos were displayed on a computer screen and the numbers of ants on the wicks were counted using a hand counter. The plastic stations were replaced as needed. Ant

precunts using only sucrose solution in the vials were taken from the palms for 18 days before treatment applications to determine a baseline count for each palm. Although it was impossible to determine the actual population of each palm, counts of foragers on the wicks of the vials would provide relative assessments of populations from palm to palm.

Within each station containing a toxic bait, the three vials on the left side contained the toxic bait while the three vials on the right side were always SS40, thereby giving the ants a choice of feed. Counts were taken of the numbers of ants feeding on the vials to determine any feeding preferences. To uniformly provide 2 bundles of 3 vials in all stations, the control stations contained SS40 on both the left and right sides.

Surface treatments were applied to a 12.7 cm band painted with brown exterior latex house paint (BEHR Process Corporation, Santa Ana, CA). Actual amounts of surface sprays applied were measured gravimetrically. Surface treatments were made using a mist sprayer bottle containing the products at label or manufacturer requested rates, and applied to run-off. Plastic sheeting with masking tape temporarily placed above and below the treatment bands previous to the chemical applications, insured that product was applied only to the designated painted area (Fig. 3).

All bait tubes were replaced twice per week and additional baby food (Turkey and Turkey Broth, BeechNut Nutrition Corp., Canajoharie, NY) (~1 g in a glass vial placed inside the monitoring stations) was provided as a protein source for the ants on all palms twice weekly. The surface treatments were applied only once. Liquid baits were applied (Fig. 4) as described above, until 7 May 2003. Counts were taken twice weekly until 7 May 2003.

To normalize the data and stabilize the variance, the numbers of ants foraging on wicks were square root-transformed and analyzed using general linear models. Means were separated by the Waller-Duncan K-ratio *t*-Test (SAS Institute 1998). A *t*-test was used to detect significant differences in left-right feeding preferences.

Daily weather data came from a Florida Automated Weather Network (FAWN, University of Florida, IFAS) station on the FLREC campus, located ~200 m east of the west coconut grove.



Figure 1. 2002 experimental set-up for surface treatments of coconut palm. A) Sticky band. B) Five-inch wide painted treatment band for surface product, and population monitoring station containing 2 bundles of 3 vials.

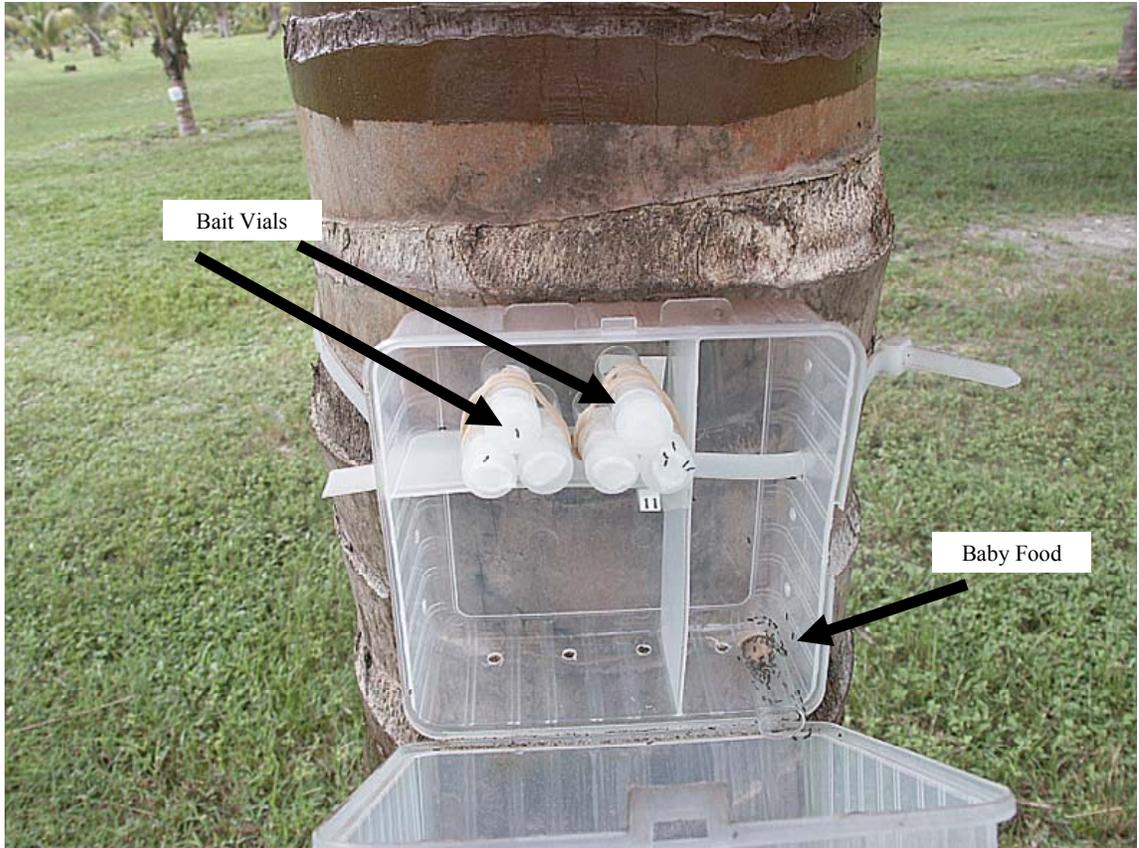


Figure 2. Population monitoring station opened to show 2 bundles of 3 vials each. The WFA are seen foraging on vial wicks and by vial containing supplemental baby food. Three vials on the left are treatment and 3 vials on the right are sugar water (40% w/v aqueous).



Figure 3. The author applies surface product to the painted band. Note that the monitoring station is covered with plastic to protect it from being sprayed.



Figure 4. Inserting bundles of vials into population-monitoring station.

### Results

The mean numbers of ants foraging on wicks for each treatment (total of six vials) for 10 days pretreatment; and 14, 23, 50, and 170 days after exposure (DAE) were selected to represent the exposure time course (Table 1). There were no significant differences for ants on wicks during the pre-treatment period for palms assigned to their respective treatments. At 14 DAE, there were no significant differences among SS40 ( $52.00 \pm 32.43$ ), FIP ( $42.25 \pm 37.48$ ), BIF ( $25.13 \pm 11.61$ ) and IM ( $20.88 \pm 21.20$ ); but TXND ( $5.38 \pm 7.89$ ) had significantly fewer ants on wicks than did SS40 or FIP. At 16 DAE, the concentration of thiamethoxam in TXND was increased from 10 ppm to 100 ppm. At 23 DAE, SS40 ( $25.88 \pm 12.59$ ) and FIP ( $17.50 \pm 10.20$ ) had significantly more

ants on wicks than did IM ( $6.75 \pm 6.41$ ), BIF ( $3.75 \pm 6.18$ ); or TXND ( $2.38 \pm 5.53$ ); but TXND, BIF, and IM were not significantly different from each other. At 50 DAE, there were more ants on wicks of SS40 ( $13.50 \pm 5.66$ ), FIP ( $12.00 \pm 14.95$ ), and BIF ( $4.63 \pm 2.50$ ) that were not significantly different from each other, but were significantly different from IM ( $0.50 \pm 1.41$ ) and TXND ( $0.00 \pm 0.00$ ); and BIF and IM were not significantly different from each other. At 170 DAE, there were significantly more ants on wicks for FIP ( $31.13 \pm 26.20$ ), BIF ( $25.75 \pm 9.77$ ), and SS40 ( $25.75 \pm 16.64$ ) than for IM ( $2.75 \pm 3.81$ ) and TXND ( $0.00 \pm 0.00$ ), which were not significantly different from each other. There were no significant left-right preferences for any treatment during the days examined in Table 1.

Figure 5 shows a smoothed line graph of the numbers of ants on wicks for over 200 DAE for the treatments, including linear regressions. Although TXND clearly shows lower numbers of ants throughout the experiment, there were usually no significant differences between TXND and IM. At 3 DAE, BIF had significantly fewer ants on wicks than the other treatments, with the exception of FIP, but the numbers of ants increase quickly in the following days. TXND reduced ant numbers more than the other treatments, reaching its lowest level at about 50 DAE, and the numbers remained low throughout the experimental period. The numbers of ants for all the other treatments remained higher, with IM approaching TXND after 200 DAE, while after 100 DAE BIF, FIP, and SS40 increased in a similar manner.

### **Discussion**

The arboreal bioassay developed in my study is a novel method for performing tests to control populations of arboreal ants. The bioassay allows tests to be performed in the ants' natural environment while simulating a structural setting.

A residential setting may not be ideal for conducting controlled field experiments because it is subject to several sources of bias. A research design for testing an insecticide ideally requires replications of a treated plot in which each plot is tested under the same conditions. In a residential setting, each house (i.e. test plot) is different, and likely has a unique landscape ecology. Additional difficulties include potential interference from residents, varying weather, and travel time from site to site. The possible entrance or exit of target pests from adjacent properties is of special concern.

The arboreal assay design used in this experiment, had a naturally acquired population of the target species isolated from other ant species, nesting under similar conditions, and exposed to virtually identical climatic factors. The design was meant to simulate a residential scenario, without the biasing variables mentioned above. The WFA nesting in the palms could forage on honeydew-producing insects and nectars, and travel up and down the trunk, much in the same way as they would forage in a suburban landscape. Exterior house paint was used for the bands for surface treatments to simulate the exterior of a structure; and ants foraging inside the monitoring stations simulated foraging inside a structure.

Researchers have previously performed experiments involving ant control in trees and vineyards, but their purpose has usually been to exclude ants. Often these tests were performed to prevent ants from tending mealybugs, scales, aphids, and other insects that were the primary targets for control.

Stevens et al. (2002) listed three approaches to controlling ant populations in citrus groves: ground sprays, trunk barriers and baiting. Most ant control studies used one of these three approaches, or broadcast applications of various products. Young citrus trees

have been killed by the red imported fire ant (RIFA) *Solenopsis invicta* Buren, which girdles the tree trunks. Banks et al. (1991) tested an insect growth regulator, fenoxycarb (broadcast via tractor) and found that significantly more trees were killed in untreated plots vs. those treated with the insect growth regulator.

Reese and Reese (1981) tested tree wraps to protect young citrus trees from insect damage, including damage caused by ants. A tree wrap having a central chamber lined with polystyrene containing packets of insecticide was placed around young citrus tree trunks. Diazinon packets placed within tree wraps were found to be very effective in controlling ants within the wraps for 11 months and nests at the base of these trees. The authors did not state what species of ants were found.

A study done by Moreno et al. 1987, tested chlorpyrifos and diazinon barriers to Argentine ants (*Linepithema humile* (Mayr)) foraging on citrus trees. Argentine ants were excluded from citrus trees for 8 months by placing granular diazinon around the trees. Applications of limonene to pruned trees and diazinon to unpruned trees were not effective in preventing ants from entering trees. The authors state that any breaks in the continuity of the diazinon around the trees permitted ants to climb the trunks.

Phillips and Sherk (1991) tested chlorpyrifos and diazinon to exclude Argentine ants from entering vineyards to tend honeydew-producers. Diazinon AG500 (3%), and chlorpyrifos 4E (3% and 6%) sprays were applied around vines. Chlorpyrifos 6% solution was significantly more effective than diazinon and a water control at excluding ants from vine canopies over an 8 month period, but was not significantly different than chlorpyrifos 3%.

Tests to control the populations of the Argentine ant in citrus groves and *Formica perpilosa* Wheeler in vineyards were performed in California by Klotz et al. 2003. There was a significant reduction in ants in vineyards in the chlorpyrifos treated vs. untreated areas, and found that chlorpyrifos provided a significant reduction in ants for nine weeks. In citrus, there were significantly more ants in the control plots than on the thiamethoxam or fipronil treated plots. Insecticidal sprays to the base of vineyards were effective against foraging ants, but did not eliminate the colony. For Argentine ant control, the authors suggest the use of a liquid bait, because 99% of the food brought back to nests was in liquid form (Markin 1970).

Tollerup et al. (2004) found that chlorpyrifos together with skirt-pruning was of limited value because the Argentine ant queen(s) and the vast majority of workers were not affected, and that using sticky bands around trunks was too labor intensive to be of practical value. They found that the foraging activity of field ants (*Formica*, spp.) was significantly reduced in plots treated with Maxforce (1.0% hydramethylnon) and an anchovy bait containing 0.05% imidacloprid at 10 DAE, but the activity returned to the pre-treatment level by 18 DAE. Maxforce significantly reduced foraging activity below that of the control at 93 and 122 DAE, while at 122 DAE, both anchovy baits reduced the foraging activity similarly, however only the lower rate of imidacloprid (0.005%) reduced the foraging activity significantly lower than the control.

Abamectin (0.011% granular bait) was used by Johnson (2004) to control ants in almond orchards. Applied at 1.12 Kg/Ha the bait significantly reduced the populations of the pavement ant *Tetramorium caespitum* L., and the southern fire ant, *Solenopsis xyloni* Buren, for 163 days compared to untreated control plots. The author does not state if ant

population monitoring vials were placed on the ground or in the trees. No barriers were utilized to prevent ants from entering trees.

James et al. 1995 tested methods of excluding *Iridomyrmex* spp. ants from entering trees by skirt-pruning, sticky bands, insecticidal baits, and chemical treatments. Polybutene-based sticky bands applied directly to tree trunks or over a protective base excluded ants for 3 to 4 months. AntCaps™ (Cape Agricultural Products, South Africa) kept trees mostly ant-free for up to 7 months, while a slow-release chlorpyrifos-impregnated band that was stapled to tree trunks, was effective after 27 months. Various emulsifiable concentrate surfaces applied to tree trunks were only effective at preventing ant access for a few weeks. The authors also tested baits, which they considered the “most attractive option” for controlling ants in citrus. A Japanese bait (name not provided) gave 3 to 6 months of ant control.

Shorey et al. 1992, 1996 and 1993, tested various ant-repellent semiochemicals to exclude Argentine ants from citrus and *Formica aerata* Francoeur from plum trees. Farnesol, a sesquiterpene compound, at 0.8 to 2 g/tree, which, when mixed with Stickem Special (Seabright, Emeryville, CA), gave effective disruption for 14 weeks, while farnesol mixed with Tree Tanglefoot was only effective for 7 weeks (Shorey et al. 1992). In another test, farnesol provided the best control (>60 days) when mixed with Stickem applied to cotton twine (40g each) and wrapped around tree trunks. Several tests were performed on plum trees and on wooden stakes to determine which chemical was most active in disrupting ant foraging. Cotton twine permeated with Stickem and farnesol was the most effective, providing 53 days of ant exclusion when wrapped around the trunk of trees (Shorey et al. 1993).

A common observation is even though few or no ants are visible foraging on palm trunks or leaves, a residual population with brood is often found when leaf petioles are pulled back. The food brought back to the nest by foragers is probably the only food for the colony and is therefore critical to colony survival. Controlling the residual population that remains in the nest after foragers are killed by insecticidal treatments therefore becomes key to the control of the colony. We hypothesize that by eliminating the population of foragers using toxic baits, the adults (intercastes and workers) remaining in the nest tending the brood are required to forage, and that by offering a supply of fresh bait, these foragers would be eliminated leading to further reduction or total elimination of the colony.

For a WFA bait to be effective, a toxicant must be mixed into a bait matrix that is palatable to the target species and preferred over other available foods. Since trophallaxis has not been observed in WFA, the speed of kill is probably not important, as long as foragers have enough time to recruit more foragers to the bait before dying, an action requiring only a few minutes (Warner, unpubl. observ.). In species that engage in trophallaxis, a slower speed of kill is preferable permitting time for the toxicant to circulate throughout the colony.

Warner and Scheffrahn (2004) showed that thiamethoxam at 10 ppm in NecDew<sub>4</sub> was an effective bait for the control of WFA, and therefore it was used in this test. Sixteen days after testing began the concentration of thiamethoxam in the NecDew was increased from 10 ppm to 100 ppm, because it was felt that a concentration of 100 ppm would be necessary for effectiveness in the field where there were fewer controlled variables such as temperature, rainfall, and alternative food sources. The results of the

test showed that TXND was again a good combination of a toxicant with a bait matrix and although the numbers of ants on wicks of TXND and IM in most cases were not significantly different from each other.

Counts were initially low on wicks at 3 DAE for BIF ( $0.13 \pm 0.35$ ), which was not significantly different than FIP ( $6.38 \pm 11.25$ ), which in turn was not significantly different than IM ( $13.75 \pm 11.88$ ). Nearly all of the data for the two surface treatments (FIP and BIF) after 3 DAE were not significantly different than SS40, with the one exception at 23 DAE when BIF was  $3.75 \pm 6.18$ , which was not significantly different than the two liquid baits.

Figures 5 and 6 show much daily variability in the counts of ants foraging on wicks for all treatments, including the SS40 control. This variability is probably due to climatic conditions. Data collection was mostly done just after dawn when the ants were very active, but on occasions when the palm trunk was wet from rain or condensation, there was reduced or no foraging. The regression line for the control (Fig. 5) shows a slight decrease over time, FIP and BIF show a slight increase, while the two liquid baits, IM and TXND have a greater negative slope indicating more effective foraging population reduction. There was a drop in ant counts for all treatments and the controls beginning at about 21 DAE (7 Oct. 2002), this decrease was probably due to other factors. Daily maximum and minimum temperatures and rainfall data are plotted with data for ants foraging on wicks in Fig. 6. The minimum daily temperatures occurred at close to dawn, when data were collected. Although there is an overall decline in minimum temperatures after 21 DAE at the commencement of winter, there is no immediate correlation with the

decrease in ant counts. There did not seem to be a correlation between numbers of foraging ants and daily precipitation.

The decline in numbers was likely due to lower temperatures of the winter. This decline continued until the end of January 2003, followed by an increase in the control, FIP and BIF. Although numbers also increased for TXND and IM by ~170 DAE they dropped close to zero where they remained, while the other treatments increased to pre-treatment levels. Because ant activity is reduced in winter, it is probably best to initiate long-term experiments such as this one in south Florida in the late winter or early spring.

The results for this experiment are shown most clearly in Figure 7, which shows smoothed lines of the least squared mean numbers of ants on wicks. This mathematical transformation serves to greatly reduce the daily variations caused by weather conditions and permits an overall view of the experimental trends, with SS40 producing the highest numbers until the spring of 2003. The trend for FIP was only slightly below that of SS40, indicating very little reduction in populations, followed by BIF with somewhat greater population reduction. Overall, the two liquid baits reduced foraging populations more than the surface treatments did, with TXND achieving the highest population reduction, most likely due to the preferred NecDew bait matrix (Warner and Scheffrahn 2004). The surface vs. bait treatment lines clearly diverge (Fig. 7) after ~120 DAE, with the surface treatment data nearly paralleling that of SS40 going into the spring population increase, while the liquid bait treatments continue to decline. Based on further experiments (discussed below) we found the WFA populations in these palms eventually recovered to a number that could be used for further experiments. Therefore, it seems apparent that sufficient reproductive ants survived to assure colony survival.

The data suggest surface treatments left a residual populations that was able to return to previous, or even greater, numbers at  $\sim >130$  days, while the bait treatments suppressed the populations during the period that they were provided. The surface treatments were applied only once, but the baits were applied twice weekly on a continual basis during the entire experiment. Several applications of surface treatments might have a cumulative effect, and therefore is more economical than long-term bait applications, which are more time and labor intensive. Testing this hypothesis would require applying the surface treatments on a regular basis, perhaps quarterly, and testing their performance against liquid baits.

Table 1. September 2002 to May 2003, mean number ( $\pm$ SD)<sup>1</sup> of *Technomyrmex albipes* adult ants foraging on wicks (total of 6 vials per station) 10 days before and after 14, 23, 50, and 170 days exposure to 5 treatments in a field test.

Treatment	DAE <sup>3</sup>				
	10 Pretreat <sup>2</sup>	14	23	50	170
BIF	48.25 $\pm$ 16.26 a	25.13 $\pm$ 11.61 ab	3.75 $\pm$ 6.18 c	4.63 $\pm$ 2.50 ab	25.75 $\pm$ 9.77 a
FIP	44.00 $\pm$ 8.08 a	42.25 $\pm$ 37.48 a	17.50 $\pm$ 10.20 ab	12.00 $\pm$ 14.95 a	31.13 $\pm$ 26.20 a
IM	52.50 $\pm$ 30.01 a	20.88 $\pm$ 21.20 ab	6.75 $\pm$ 6.41 bc	0.50 $\pm$ 1.41 bc	2.75 $\pm$ 3.81 b
SS40	65.25 $\pm$ 25.71 a	52.00 $\pm$ 32.43 a	25.88 $\pm$ 12.59 a	13.50 $\pm$ 5.66 a	25.75 $\pm$ 16.64 a
TXND	34.25 $\pm$ 22.95 a	5.38 $\pm$ 7.89 b	2.38 $\pm$ 5.53 c	0.00 $\pm$ 0.00 c	0.00 $\pm$ 0.00 b
Treatment Effects Statistics					
F	1.14	2.72	6.78	6.45	11.63
df	4,20	4,20	4,20	4,20	4,20
P	0.3770	0.0694	0.0025	0.0032	0.0002

<sup>1</sup>Means of 4 replicates. Means within a column followed by the same letter are not significantly different (Waller-Duncan K-ratio *t*-Test) at P = 0.05. BIF = bifenthrin (600 ppm), FIP = fipronil (600 ppm), IM = imidacloprid (50 ppm) sweet liquid ant bait, SS40 = 40% (w/v) aqueous sucrose solution, TXND = thiamethoxam (10 ppm initially; increased to 100 ppm on Day 16) in NecDew<sub>4</sub>.

<sup>2</sup>Days before exposure.

<sup>3</sup>DAE = days after exposure

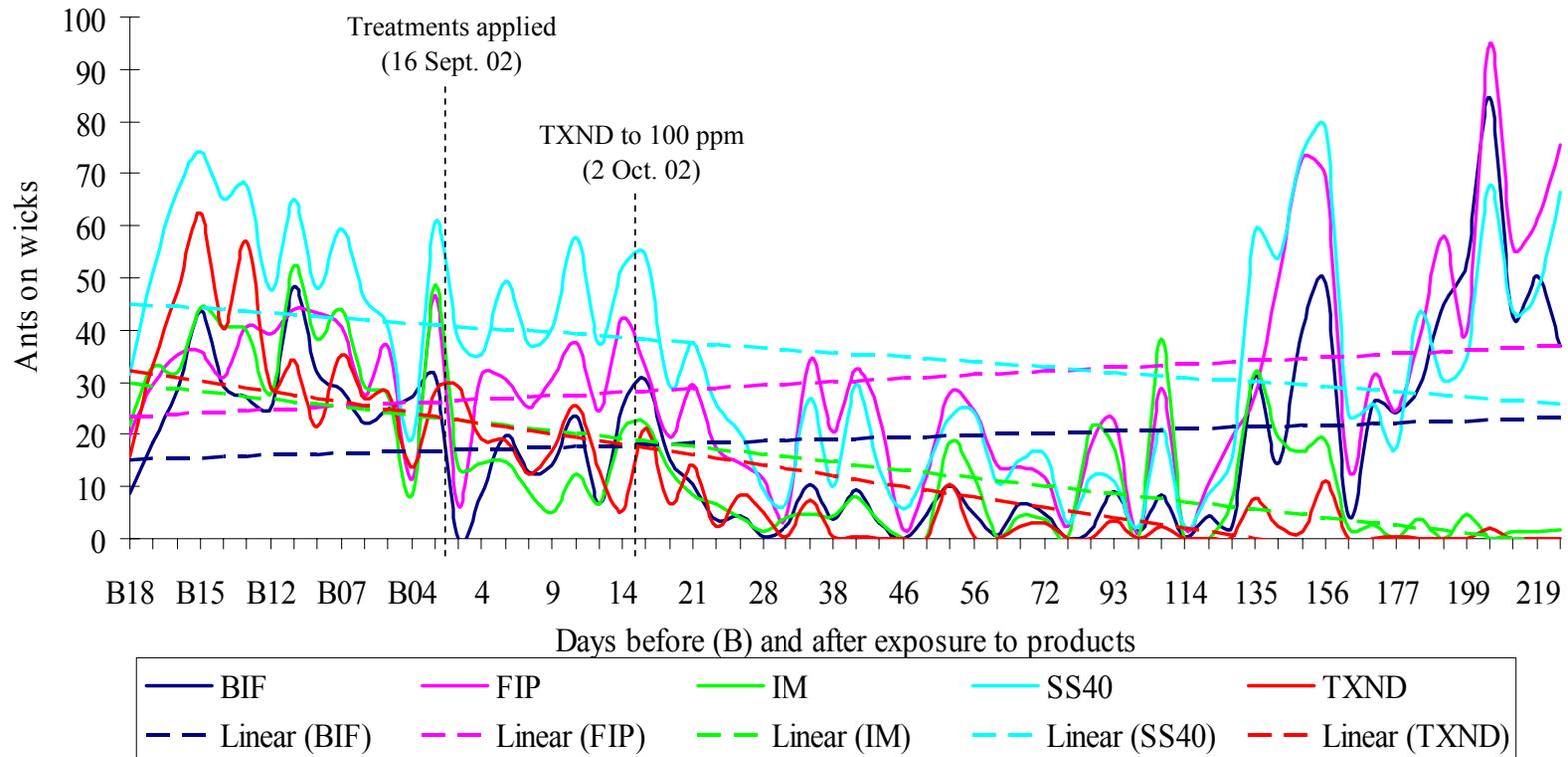


Figure 5. Mean number of ants on wicks before and after treatment with sprays or baits. BIF = bifenthrin (600 ppm), FIP = fipronil (600 ppm), IM = imidacloprid (50 ppm) sweet liquid ant bait, SS40 = 40% (w/v) aqueous sucrose solution (control), TXND = thiamethoxam (10 ppm initially; increased to 100 ppm on Day 16) in NecDew<sub>4</sub>. Liquid baits were applied weekly until 7 May 03.

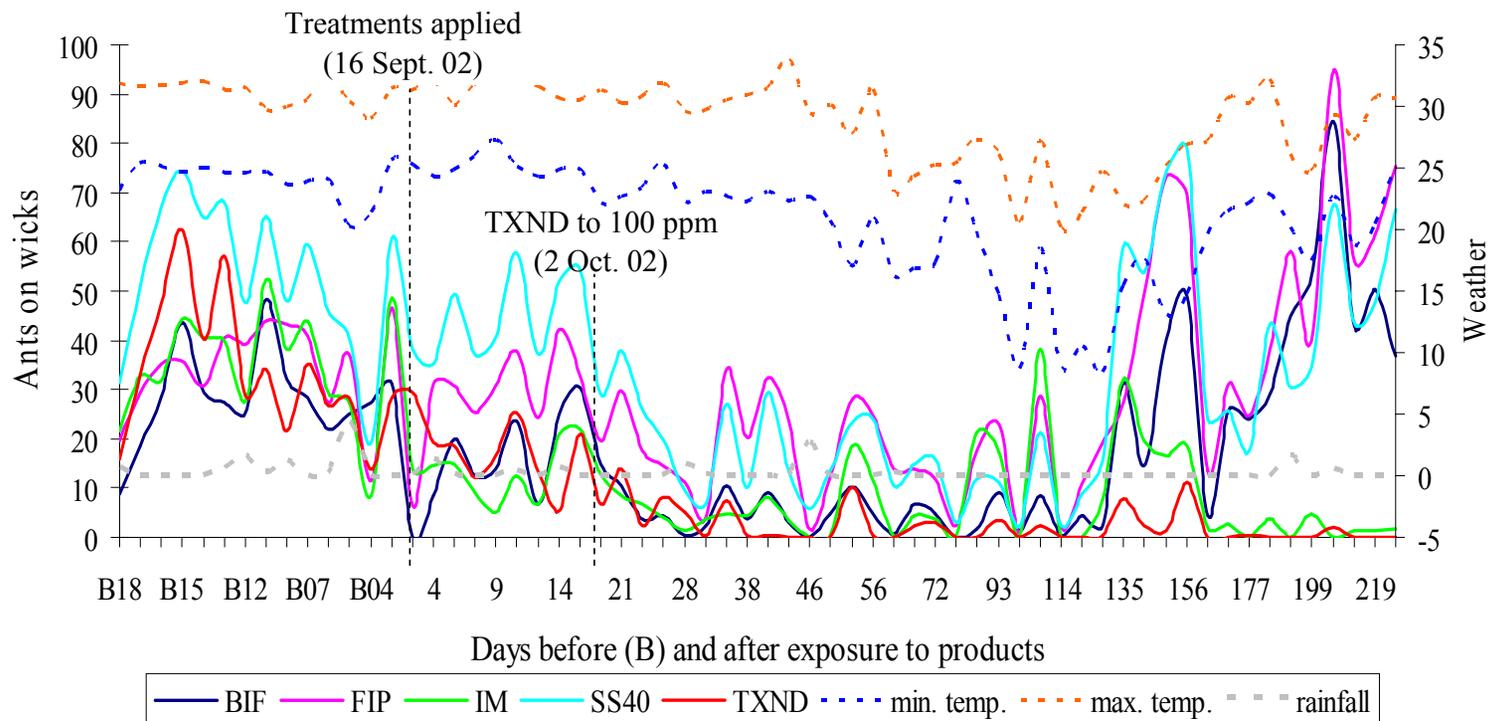


Figure 6. Smoothed line of mean number of ants on wicks of vials during pre-treatment (B) and Test Period with weather data: temperature (min., max., °C) and rainfall (cm). BIF = bifenthrin (600 ppm), FIP = fipronil (600 ppm), IM = imidacloprid (50 ppm) sweet liquid ant bait, SS40 = 40% (w/v) aqueous sucrose solution (control), TXND = thiamethoxam (10 ppm initially; increased to 100 ppm on Day 16) in NecDew<sub>4</sub>. Liquid baits were applied weekly until 7 May 03.

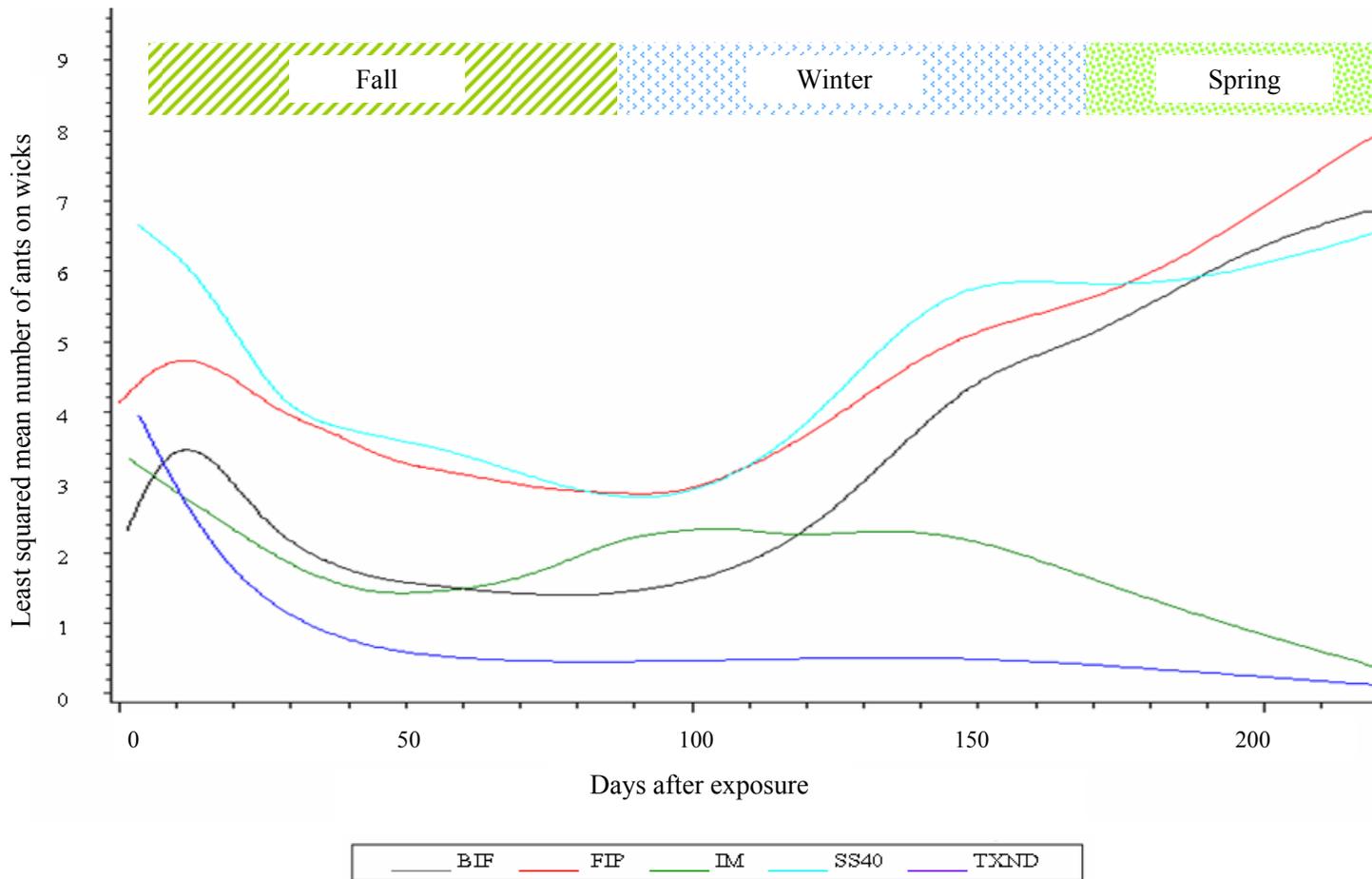


Figure 7. Least squared mean number of ants on wicks of vials during test period. DAE = days after exposure, BIF = bifenthrin (600 ppm), FIP = fipronil (600 ppm), IM = imidacloprid (50 ppm) sweet liquid ant bait, SS40 = 40% (w/v) aqueous sucrose solution (control), TXND = thiamethoxam (10 ppm initially; increased to 100 ppm on Day 16) in NecDew4. Liquid baits were applied weekly until 7 May 03

## CHAPTER 3 ARBOREAL BIOASSAY 2003

### **Introduction**

A field test done between September 2002 and May 2003 (previous chapter), indicated the need for further testing with bifenthrin, fipronil, imidacloprid, and thiamethoxam. At 50 DAE, only the liquid baits caused a decline in the numbers of ants foraging on the wicks of vials significantly greater than that caused by the surface treatments. In the test reported here, six substances were compared for their effects on WFA foragers. These were imidacloprid dissolved in NecDew, thiamethoxam dissolved in NecDew, bifenthrin and fipronil surface treatments and control.

### **Materials and Methods**

Populations of WFA inhabiting 24 coconut palms located in several groves at the FLREC, Broward County, FL were isolated from other ant species and restricted to the palms by sticky bands of Tree Tanglefoot<sup>®</sup> Pest Barrier placed around the trunks (Fig. 8). Individual fronds were pruned to prevent fronds of one palm from touching those of nearby palms or the ground which would permit population mixing. A solar-charged electric fence was placed around the grove to prevent raccoons from removing foods provided for the ants. The ant populations had been provided 40% (w/v) aqueous sucrose solution (SS40) administered in plastic tubes of varying types and baby food (Turkey & Turkey Broth, BeechNut Nutrition Corp., Canajoharie, NY) for protein, before testing. The grass around the palms was periodically mowed and the palms were fertilized with 10-4-10 or similar fertilizer at ~2.3 kg/palm. The test area was not irrigated.

Plastic boxes having seven holes to allow access by ants and containing six vials each filled with SS40 were strapped to the palms (Fig. 2). The vials were 6 mL glass shell vials with Titeseal® plastic caps (Fisher Scientific) having a 7 mm hole through which a cotton dental wick (38.1 x 9.53 mm, no. 2 medium cotton roll, Crosstex International, Hauppauge, NY) was inserted. Vials were filled with ~4.5 mL SS40; a bundle of three vials was held together with a rubber band. Two vial bundles were placed into each plastic box. Vials were replaced with fresh SS40 twice per week and ~10 mL baby food supplement once a week.

Pictures were taken of the ants feeding on the vial wicks with a digital camera (Fig. 9). Photographs of each box were taken at dawn and the number of ants were counted from the image displayed on a computer monitor. An active trail(s) was identified on each palm. After dawn, ants would be counted for 30 seconds as they crossed a mark on the trunk while foraging from their nesting sites in the palm crown to and from the food source provided in the monitoring station.

Six randomly assigned treatments of 4 replicates each, were applied to the ants on 25 June 2003. Liquid baits included 100 ppm thiamethoxam SC (Syngenta Crop Protection, Greensboro, NC) dissolved in NecDew<sub>4</sub> (TXND), 100 ppm imidacloprid (Bayer Environmental Sciences, Montvale, NJ) dissolved in NecDew<sub>4</sub> (IMND), and 10,000 ppm orthoboric acid (BA) (Drax Liquidator®, Waterbury Companies, Inc., Waterbury, CT). Surface treatments included 600 ppm fipronil (FIP) (Termidor® SC, BASF, Durham, NC), and 600 ppm bifenthrin (BIF) (Talstar®, FMC Corporation, Philadelphia, PA). The control treatment consisted of 40% aqueous sucrose solution (SS40).

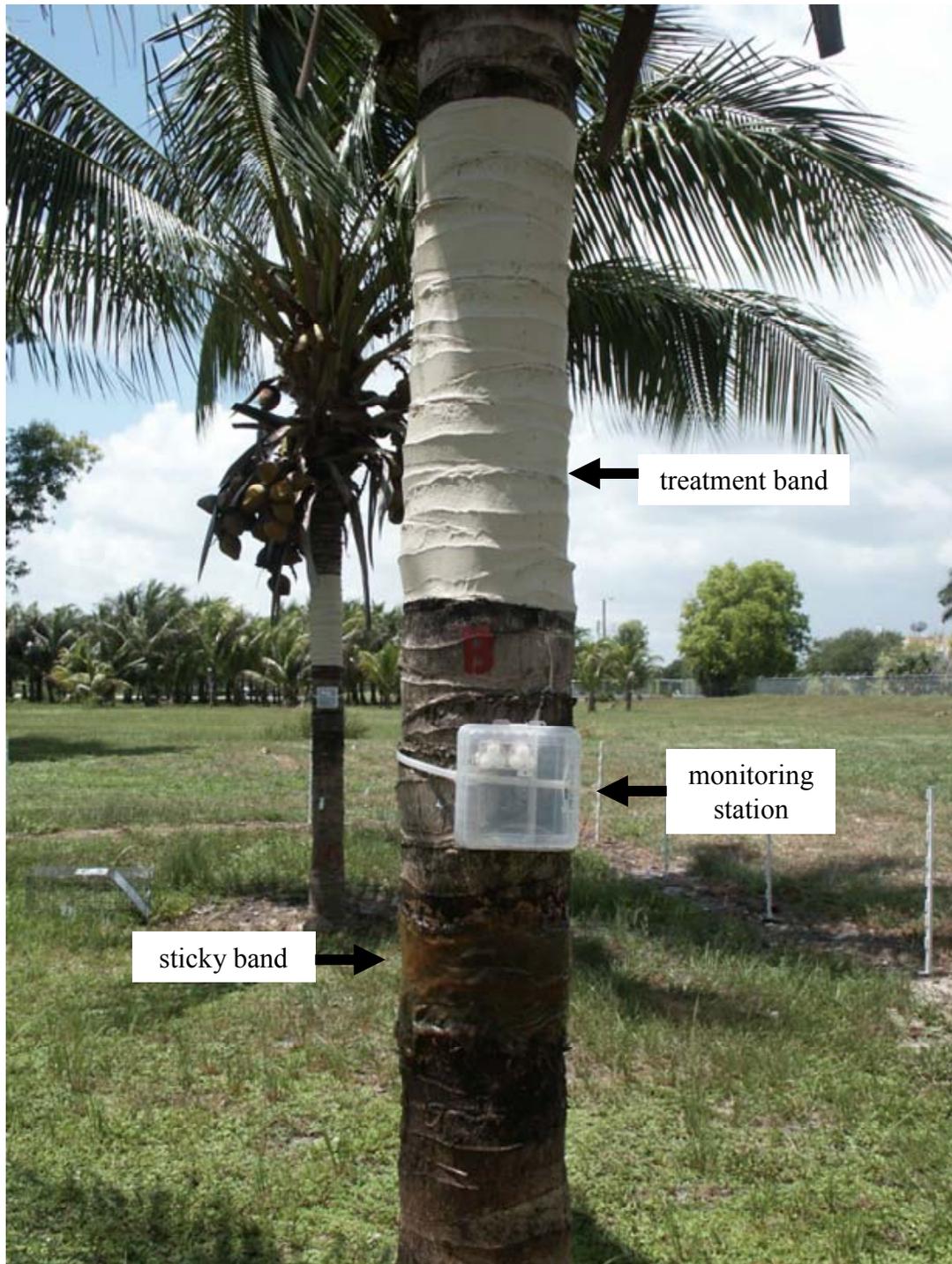


Figure 8. 2003 experimental design for surface treatments of coconut palm. Painted treatment band (61 cm wide) for surface product, population monitoring station containing 2 bundles of 3 vials each, and sticky band to isolate ant population.

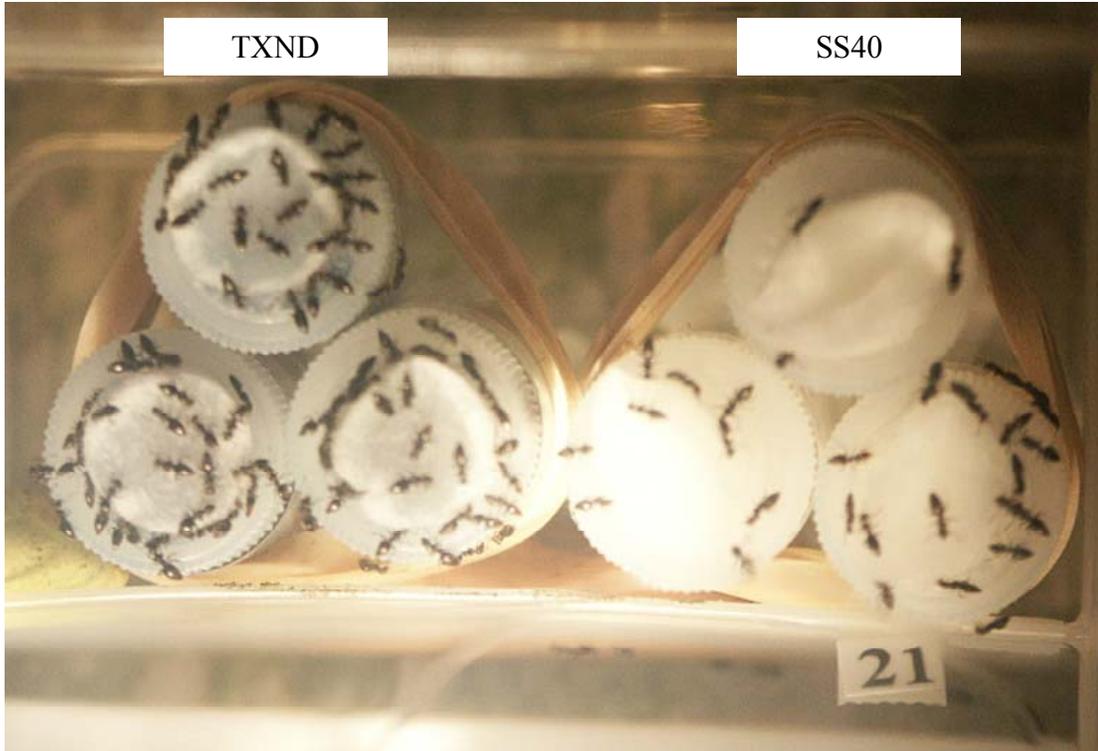


Figure 9. Ants on vials containing TXND (left bundle) and 40% sugar water (SS40) (right bundle).

The liquid baits, including the control, were applied using the vials described above. For each box containing a toxic bait, the three vials held on the left side of the box contained the toxic bait while the three vials on the right side of the box were always SS40, thereby permitting the ants to feed from the preferred material. Counts taken of the numbers of ants feeding on the vials would therefore demonstrate feeding preferences, if any. The control boxes contained SS40 on both the left and right sides.

In a previous experiment (Chapter 2), surface treatments were applied to a 12.7 cm band that was painted with brown latex exterior house paint. In this experiment, as requested by one of the chemical suppliers, the band width was increased to 61 cm, and painted beige with latex exterior paint (BEHR Process Corporation, Santa Ana, CA) making it easier to observe WFA trails (Fig. 8). Painted bands were only applied to trees receiving surface treatments. Surface treatments were applied to runoff with a sprayer

bottle containing the residual spray products at label or manufacturer requested rates (Table 2). Plastic sheeting and masking tape placed above and below the treatment bands previous to the chemical application insured that product was applied only to the designated pre-painted area (Fig. 10)

All baits were replaced twice per week and additional baby food (Turkey and Turkey Broth, BeechNut Nutrition Corp., Canajoharie, NY) was provided to the ants on all palms twice per week. The surface treatments were first applied as described above on 25 June 2003, but on 6 August 2003 (i.e. 42 DAE), palms selected for the surface treatments were sprayed from a boom lift, from the top to the bottom of the crown, including ~10-20 cm from the stem along the petioles, with the respective product, (Figs. 11 and 12). Data were collected two times per week until 9 April 2004.

Numbers of ants foraging on wicks, including left-right vial-wick counts, were square root-transformed and analyzed using ANOVA general linear models. Means were separated using the Waller-Duncan K-ratio *t*-Test (SAS Institute 1998).

## Results

The mean total numbers of ants foraging on wicks (summing left and right vial bundles) for each treatment for the pre-treatment period (pooled data), and 8, 13, 21, 43 and 49 DAE were selected to be representative of the exposure time course and are given in Table 3. The numbers of ants on the wick bundles on the left and right sides were not significantly different during the pretreatment period.

At 8 DAE, there were no statistical differences between any of the treatments in the numbers of ants foraging on wicks. At 13 DAE, FIP ( $23.25 \pm 22.69$ ) had significantly more ants than did BA ( $4.88 \pm 5.69$ ), IMND ( $0.63 \pm 0.74$ ), or TXND ( $0.00 \pm 0.00$ ), but was not statistically different than SS40 ( $15.38 \pm 6.48$ ) or BIF ( $16.76 \pm 13.63$ ).

Table 2. Surface treatments applied to painted bands<sup>1</sup> on palms

Treatment	Palm no.	Trunk circum. cm	Band			
			treated cm <sup>2</sup>	net mix <sup>3</sup> applied g	g mix <sup>3</sup> per cm <sup>2</sup>	g a.i. per cm <sup>2</sup>
BIF	8	70	4261	64.3	0.0151	9.05.E-04
BIF	30	71	4338	62.2	0.0143	8.60.E-04
BIF	39	70	4300	57.8	0.0134	8.07.E-04
BIF	40	68	4145	39.0	0.0094	5.65.E-04
mean			4261	55.8	0.0131	7.84.E-04
FIP	3	69	4183	54.0	0.0129	7.74.E-04
FIP	12	69	4183	42.9	0.0103	6.15.E-04
FIP	34	74	4493	43.0	0.0096	5.74.E-04
FIP	38	74	4493	53.0	0.0118	7.08.E-04
mean			4338	48.2	0.0111	6.68.E-04

<sup>1</sup>61 cm wide<sup>2</sup>band width<sup>3</sup>mix = 0.06% A.I.



Figure 10. Application of the surface products to the 61-cm painted treatment band.



Figure 11. Surface treatment applied from the top of the crown to the monitoring stations (covered by plastic) using a hand mister while standing on a boom lift.



Figure 12. Surface treatments applied to the coconuts and crown using a hand mister while standing on a boom lift.

Treatments BA, BIF and SS40 were not statistically different from each other, and TXND, IMND and BA were not statistically different from each other. At 21 DAE, only IMND ( $0.63 \pm 0.74$ ) was statistically different than SS40 ( $22.75 \pm 13.13$ ), but was not statistically different than any of the other treatments.

At 43 DAE, one day after the crowns were sprayed, numbers of ants in all treatments were statistically different than SS40 ( $22.25 \pm 11.42$ ), while numbers of ants in BA ( $4.13 \pm 5.72$ ) and FIP ( $1.50 \pm 3.21$ ) were not statistically different from each other, and numbers of ants in FIP, BIF ( $0.13 \pm 0.35$ ), IMND ( $0.00 \pm 0.00$ ), and TXND ( $0.00 \pm 0.00$ ), were not statistically different from each other. By 49 DAE, numbers of ants in BA ( $25.88 \pm 14.51$ ) were statistically different than both IMND ( $0.50 \pm 0.76$ ) and TXND ( $1.88 \pm 2.23$ ), which were not statistically different from each other, and there were no statistical differences among the remaining treatments.

Figure 13 shows the mean number of ants crossing lines in 30 seconds on palms treated with BIF and FIP. These two surface treatments show similar trailing results until the time the crowns were treated at 42 DAE, after which there is some divergence, with BIF showing somewhat lower counts. The regression slope for FIP is slightly negative for the entirety of the experiment, while BIF is more negative.

Figure 14 shows a line graph of ants on wicks for ~90 DAE, including rainfall and minimum-maximum temperature data. Figure 15 graphs the least squared mean number of ants on wicks.

Significant feeding preferences, that is, toxic baits vs. control (SS40) (Figs. 16 to 19), or surface treatments left (SS40) vs. right (SS40) (Figs. 20 and 21), can be observed in selected DAE. The SS40 treatment had no significant left-right feeding preferences

(i.e. directional bias), during the selected DAE (Fig. 19). In BA treatments, there were significant preferences for SS40 over BA at 8, 13, 21, and 43 DAE (Fig. 16). The IMND bait had no significant left-right feeding preferences (Fig. 17). The TXND bait had a significant preference for TXND over SS40 at 8 DAE (Fig. 18). For the surface treatments, having SS40 on both left and right sides, the only significant preferences were observed for FIP at 13 DAE for the right side (Fig. 21).

### **Discussion**

In a previous field experiment (Chapter 2), the surface treatments were applied to bands that were 12.7 cm, but in this study, at the request of chemical manufacturers that supplied some of the tested products, surface treatments were applied to bands that were 61 cm wide (2 feet) because the results from the previous test (Chapter 2) did not show these treatments to be very effective. Since the ants were nesting in the crowns, usually under older leaf petioles, foragers would cross the bands twice as they went to the food sources provided in the monitoring stations and then returning to the crown. Initially it was thought that if the product was efficacious, the foragers would receive a lethal dose during this transit, but by 40 DAE with little effect being observed it was thought that an additional application of the surface products would be necessary, therefore the crowns were treated. There was a reduction in the numbers of ants on the BIF and FIP palms after the crown treatments, but foraging activity again increased shortly thereafter.

Minutes after the initial application of treatments to the 61 cm band, ants were observed foraging over the treated areas, indicating the products were not repellent (Fig. 22). Active trails were observed 2 and 30 DAE on most of the palms treated with the surface treatments (Fig. 23).

Table 3. 25 June to 30 September 2003, mean number ( $\pm$ SD)<sup>1</sup> of *Technomyrmex albipes* foragers feeding on wicks, pretreatment<sup>2</sup>, and after 8,13,21,43, and 49 days exposure to 6 treatments in a field test.

Treatment <sup>4</sup>	DAE <sup>3</sup>					
	Pretreatment	8	13	21	43	49
BA	22.21 $\pm$ 15.58 a	21.00 $\pm$ 12.73 a	9.75 $\pm$ 9.81 bc	29.75 $\pm$ 29.39 ab	8.25 $\pm$ 6.70 b	51.75 $\pm$ 29.26 a
BIF	37.17 $\pm$ 19.39 a	34.00 $\pm$ 36.40 a	33.50 $\pm$ 28.91 ab	39.25 $\pm$ 37.85 ab	0.25 $\pm$ 0.50 c	45.75 $\pm$ 69.40 ab
FIP	32.67 $\pm$ 22.34 a	29.75 $\pm$ 37.35 a	46.50 $\pm$ 48.59 a	50.75 $\pm$ 61.21 ab	3.00 $\pm$ 6.00 bc	39.75 $\pm$ 39.21 ab
IMND	22.29 $\pm$ 11.66 a	5.25 $\pm$ 5.74 a	1.25 $\pm$ 1.50 c	1.25 $\pm$ 0.50 b	0.00 $\pm$ 0.00 c	1.00 $\pm$ 1.41 b
SS40	26.33 $\pm$ 16.23 a	30.25 $\pm$ 12.97 a	30.75 $\pm$ 13.89 ab	45.50 $\pm$ 26.86 a	44.50 $\pm$ 19.19 a	33.50 $\pm$ 26.45 ab
TXND	31.08 $\pm$ 12.07 a	12.75 $\pm$ 13.25 a	0.00 $\pm$ 0.00 c	5.25 $\pm$ 8.62 ab	0.00 $\pm$ 0.00 c	3.75 $\pm$ 4.50 b
Treatment Effects Statistics						
F	0.57	1.24	6.76	2.49	23.30	2.63
df	5,24	5,24	5,24	5,24	5,24	5,24
P	0.7255	0.3323	0.0010	0.0702	<0.0001	0.0592

<sup>1</sup>Means of 4 replicates. Means within a column followed by the same letter are not significantly different (Waller-Duncan K-ratio *t*-Test) at P = 0.05.

<sup>2</sup>Pooled data.

<sup>3</sup>Days after exposure.

<sup>4</sup>BA = orthoboric acid (10,000 ppm), BIF = bifenthrin (600 ppm), FIP = fipronil (600 ppm), IMND = imidacloprid (100 ppm) in NecDew<sub>4b</sub>, SS40 = 40% (w/v) aqueous sucrose solution, TXND = thiamethoxam (100 ppm) in NecDew<sub>4b</sub>.

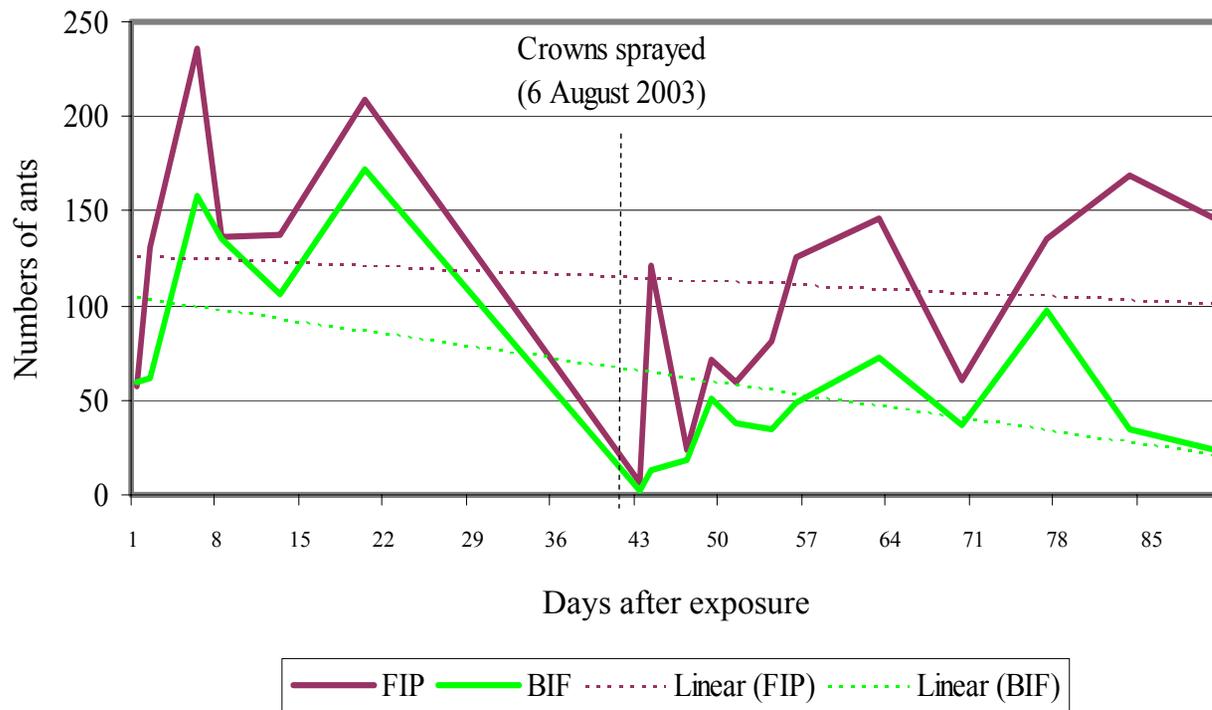


Figure 13. Mean number of *Technomyrmex albipes* adult ants trailing frequency for two surface treatments applied to a 61-cm band on palm trees on 25 June 2003, with additional application to crowns at 42 DAE (6 August 2003). DAE= days after exposure, FIP = fipronil (600 ppm), BIF = bifenthrin (600 ppm).

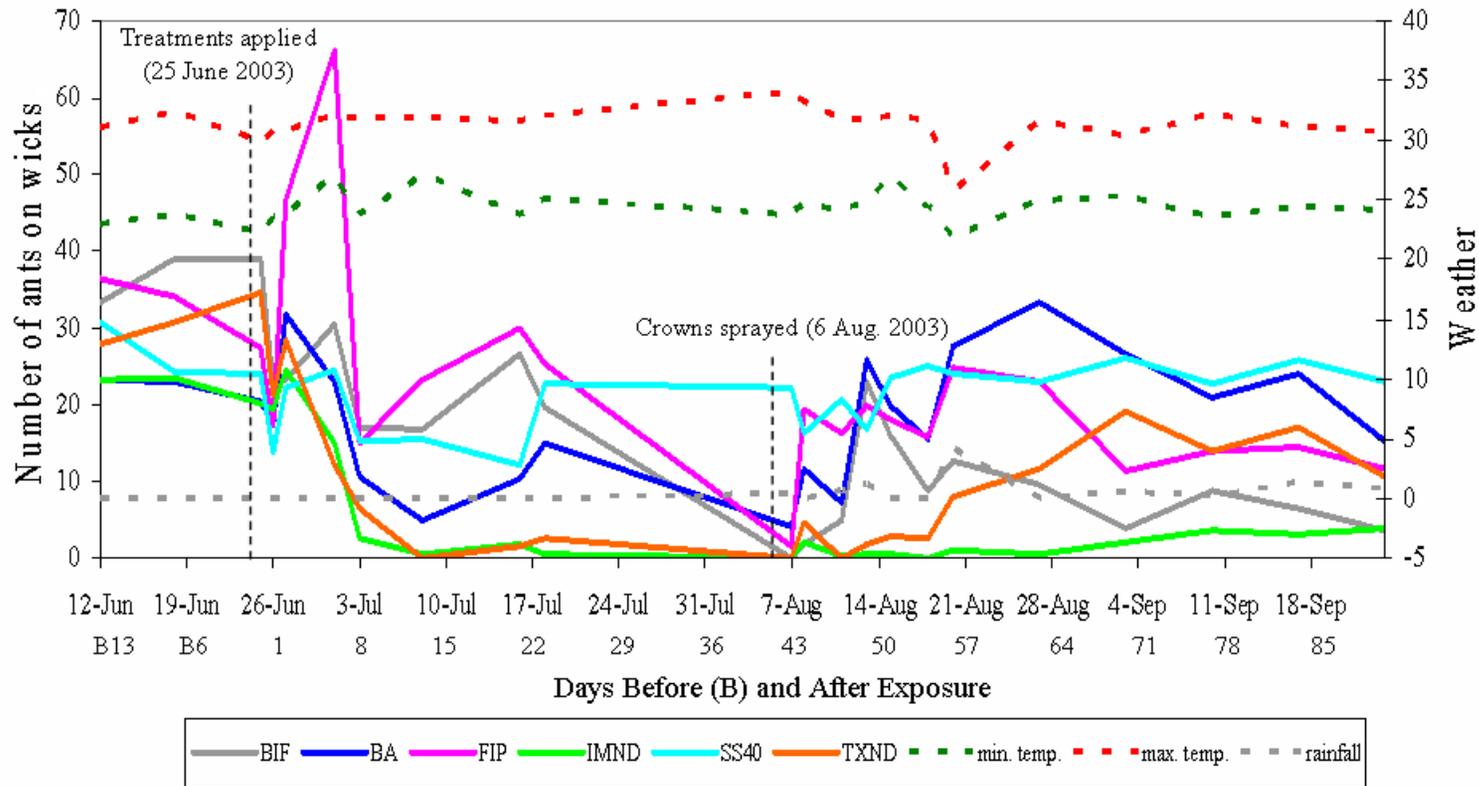


Figure 14. Mean number of ants on wicks of vials during pre-treatment (B) and Test Period, with weather data. Temperature (min., max., °C) and rainfall (cm). BA = orthoboric acid (10,000 ppm), BIF = bifenthrin (600 ppm), FIP = fipronil (600 ppm), IMND = imidacloprid (100 ppm) in NecDew<sub>4b</sub>, SS40 = 40% (w/v) aqueous sucrose solution, TXND = thiamethoxam (100 ppm) in NecDew<sub>4b</sub>.

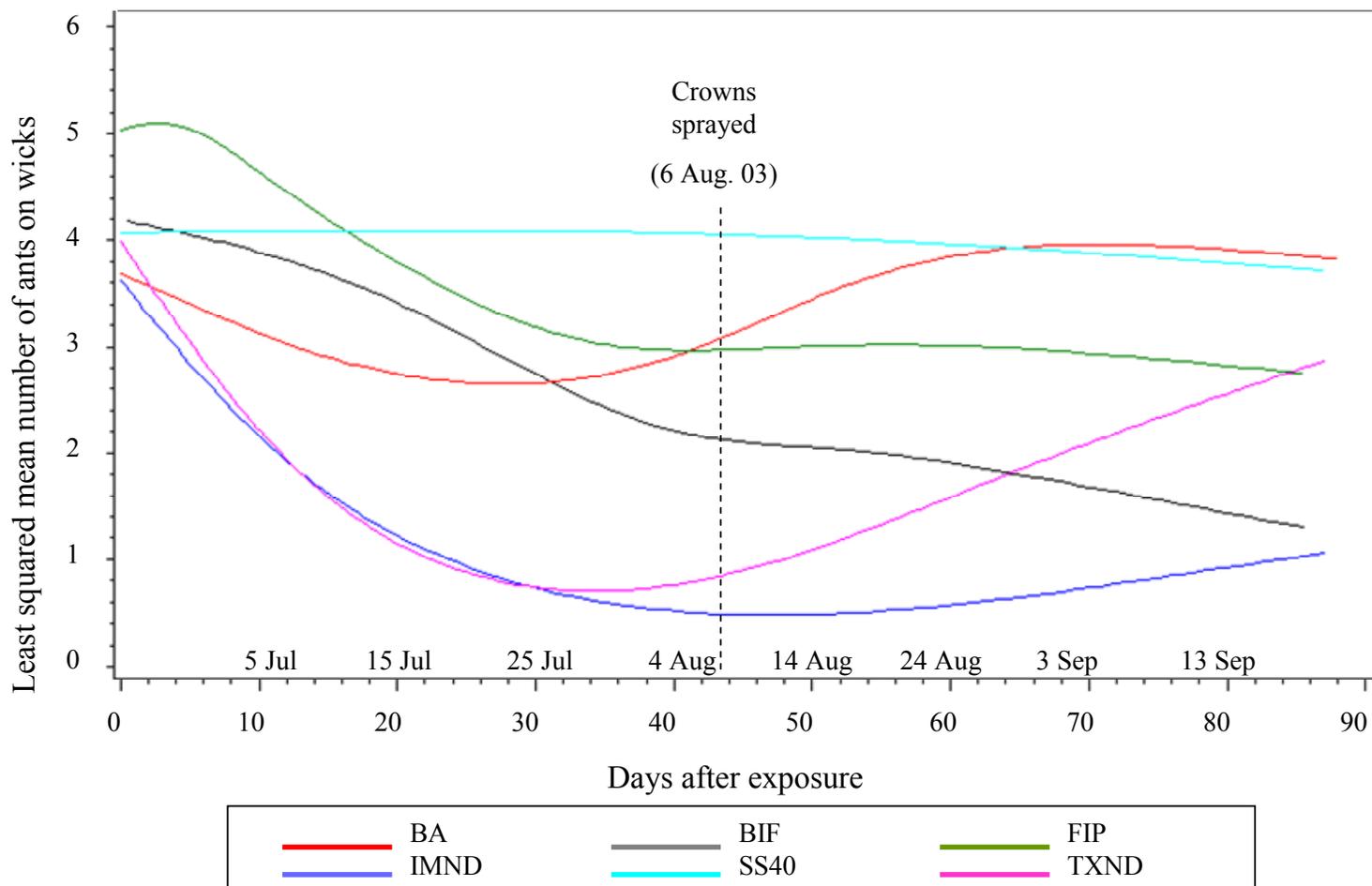


Figure 15. Smoothed line of least squared mean number of ants on wicks of vials during Test Period. DAE = days after exposure, BA = orthoboric acid (10,000 ppm), BIF = bifenthrin (600 ppm), FIP = fipronil (600 ppm), IMND = imidacloprid (100 ppm) in NecDew<sub>4b</sub>, SS40 = 40% (w/v) aqueous sucrose solution, TXND = thiamethoxam (100 ppm) in NecDew<sub>4b</sub>.

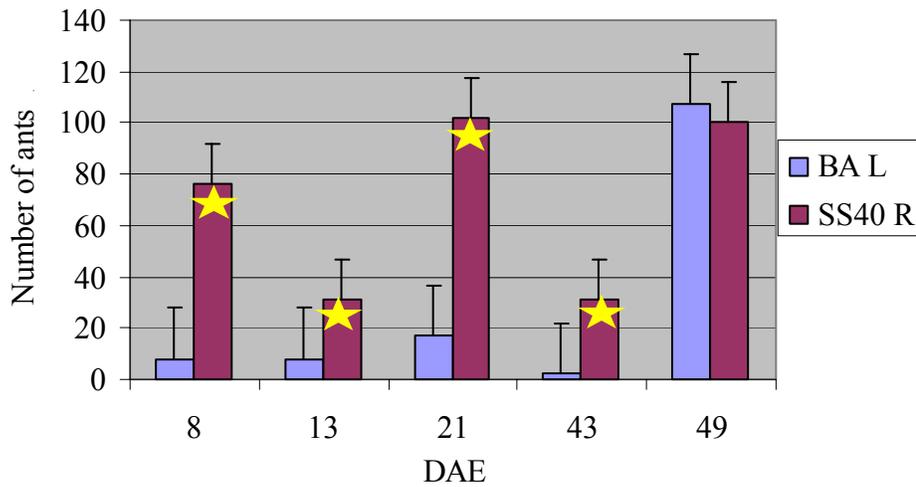


Figure 16. Treatment BA. Number of *Technomyrmex albipes* foragers feeding on wicks of vials at 8, 13, 21, 43, and 49 DAE showing left (BA) and right (SS40) preferences at  $\alpha = 0.05$ . Stars indicate significant preference. SS40 = 40% (w/v) aqueous sucrose solution. BA = orthoboric acid (10,000 ppm) sweet liquid ant bait. L = 3 vials on left side of box; R = 3 vials on right side of box. DAE = Days after exposure to test products.

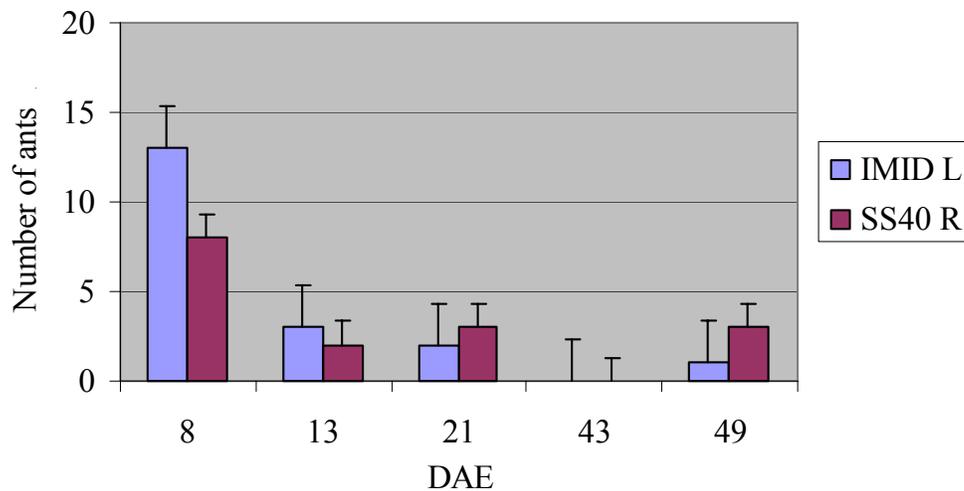


Figure 17. Treatment IMND. Number of *Technomyrmex albipes* foragers feeding on wicks of vials at 8, 13, 21, 43, and 49 DAE showing left (IMND) and right (SS40) preferences at  $\alpha = 0.05$ . SS40 = 40% (w/v) aqueous sucrose solution. IMND = imidacloprid (100 ppm) in NecDew<sub>4b</sub>. L = 3 vials on left side of box; R = 3 vials on right side of box. DAE = Days after exposure to test products.

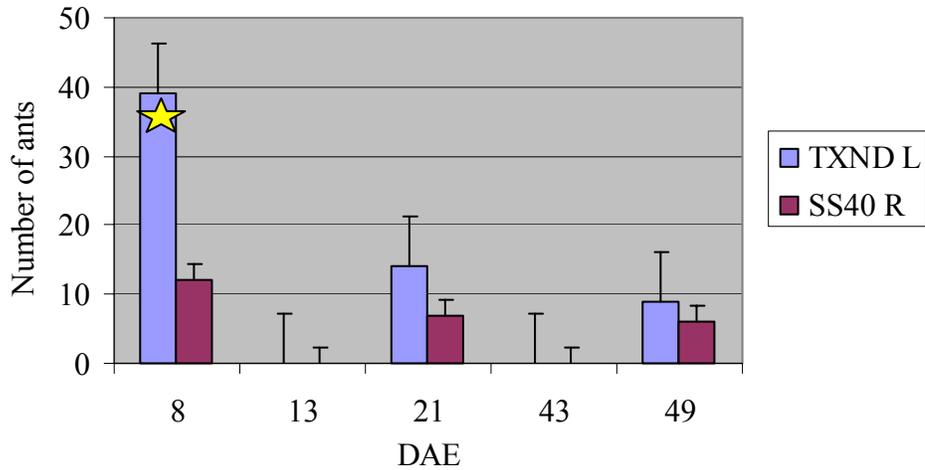


Figure 18. Treatment TXND. Number of *Technomyrmex albipes* foragers feeding on wicks of vials at 8, 13, 21, 43, and 49 DAE showing left (TXND) and right (SS40) preferences at  $\alpha = 0.05$ . Star indicates significant preference. SS40 = 40% (w/v) aqueous sucrose solution. TXND = thiamethoxam (100 ppm) in NecDew<sub>4b</sub>. L = 3 vials on left side of box; R = 3 vials on right side of box. DAE = Days after exposure to test products.

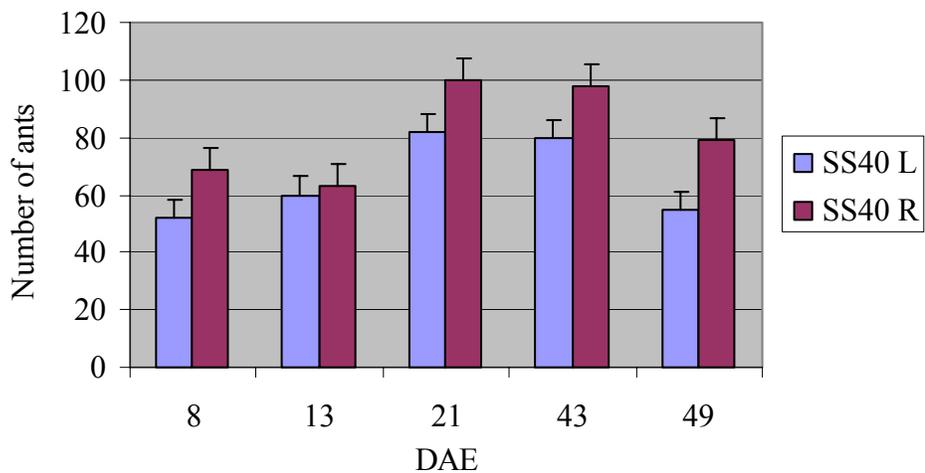


Figure 19. Treatment SS40 (control). Number of *Technomyrmex albipes* foragers feeding on wicks of vials at 8, 13, 21, 43, and 49 DAE showing left (SS40) and right (SS40) preferences at  $\alpha = 0.05$ . SS40 = 40% (w/v) aqueous sucrose solution. L = 3 vials on left side of box; R = 3 vials on right side of box. DAE = Days after exposure to test products.

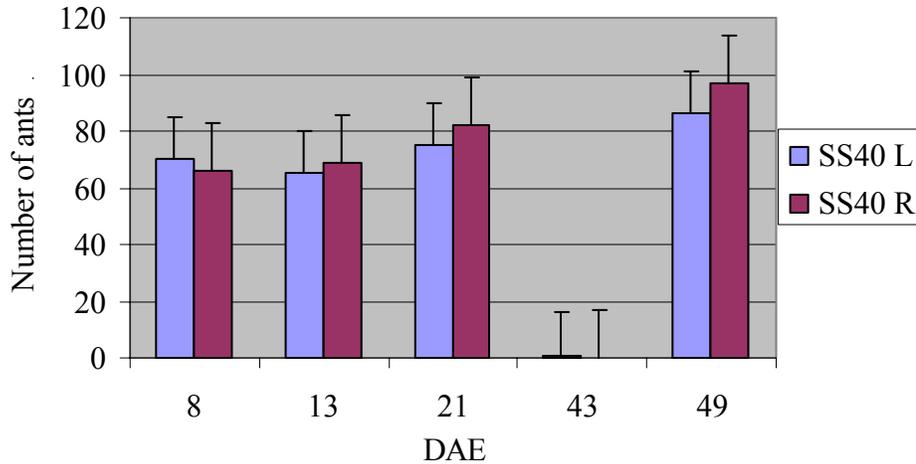


Figure 20. Treatment BIF (surface treatment). Number of *Technomyrmex albipes* foragers feeding on wicks of vials at 8, 13, 21, 43, and 49 DAE showing left (SS40) and right (SS40) preferences at  $\alpha = 0.05$ . SS40 = 40% (w/v) aqueous sucrose solution. BIF = bifenthrin (600 ppm). L = 3 vials on left side of box; R = 3 vials on right side of box. DAE = Days after exposure to test products.

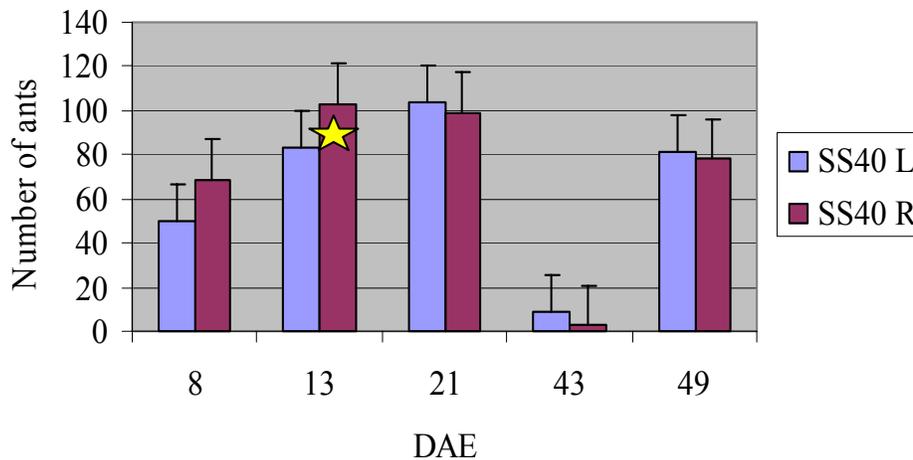


Figure 21. Treatment FIP (surface treatment). Number of *Technomyrmex albipes* foragers feeding on wicks of vials at 8, 13, 21, 43, and 49 DAE showing left (SS40) and right (SS40) preferences at  $\alpha = 0.05$ . Star indicates significant preference. SS40 = 40% (w/v) aqueous sucrose solution. FIP = bifenthrin (600 ppm). L = 3 vials on left side of box; R = 3 vials on right side of box. DAE = Days after exposure to test products.

In the 2003 bioassay, Fig. 15, with data transformed to show the least squared mean number of ants on wicks, permits a clearer view of the effects of the treatments over time by reducing the amount of daily variation shown in Fig. 14. The SS40 control is seen in Fig. 15 as nearly a horizontal line throughout the duration of the experiment, with the

highest point at the beginning of August when yearly temperatures were highest, and declined as winter approached. At about 30 DAE there was a clear separation in the treatments, with the two liquid baits, TXND and IMND well below all the others, including the third liquid bait, BA, which, at about this point increased to SS40 levels.



Figure 22. Ants continue to cross BIF treated surface minutes after mist was dry.

The crowns were sprayed at 42 DAE because ants were observed foraging over the trees with surface treatments, and a more aggressive treatment was considered necessary. Figure 15 shows a slight decline in ant numbers on the palms treated with BIF after the crown application, while those treated with FIP show little change. It was concluded that the effects of the liquid baits were superior to those of the surface sprays.

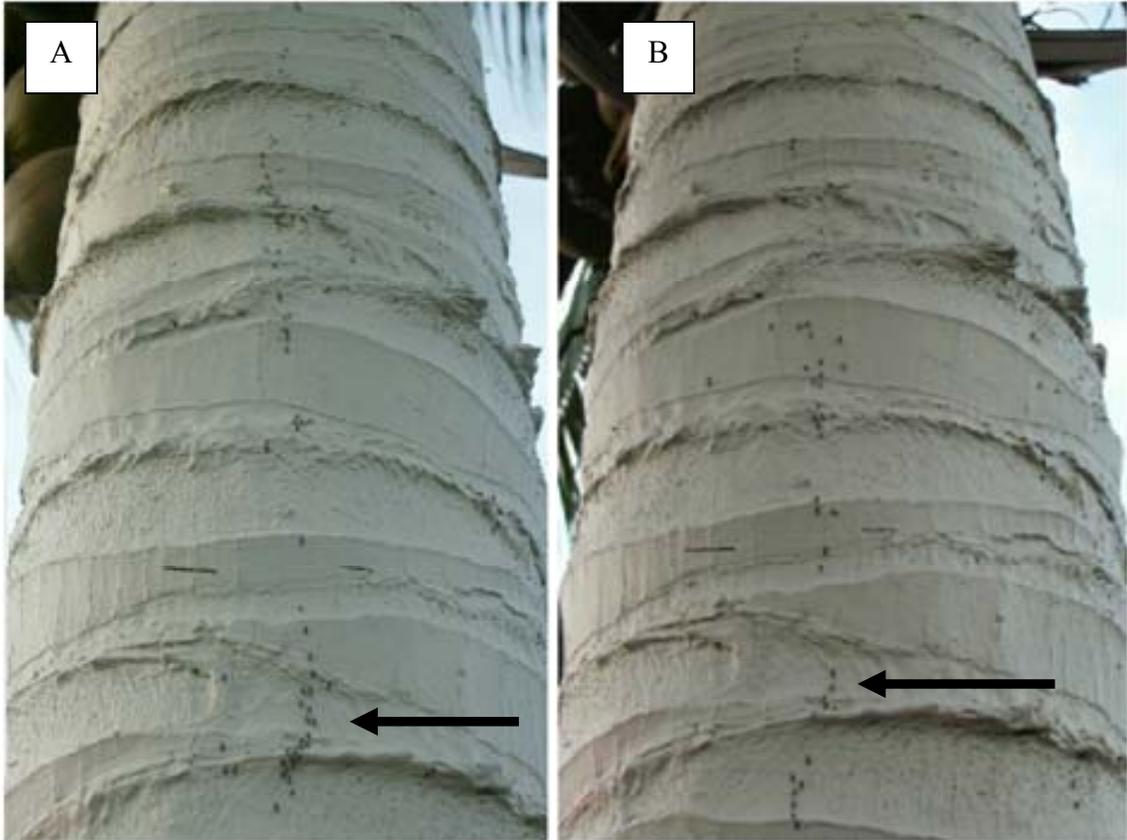


Figure 23. Ants trailing after application of surface treatment (FIP). A) 2 days after application. B) 30 days after application. Arrows point to ant trails.

## CHAPTER 4 ARBOREAL BIOASSAY 2004

### **Introduction**

In the test reported here, 50 ppm thiamethoxam (TX) in an experimental proprietary sweet bait matrix was compared with 50 ppm imidacloprid (IM) (Pre-Empt) commercial sweet liquid bait in a field trial against WFA. This test differs from those reported in chapters 2 and 3 in that it does not include surface treatments, and, in addition to counts of ants feeding on wicks and trailing frequency, includes one additional mechanism of data collection: consumption of baits by weight.

### **Materials and Methods**

This arboreal bioassay was similar to the one described in the previous chapter. Differences include the kind of plastic monitoring boxes used, the additional step of weighing the baits, including establishing evaporation controls, as part of data collection, and the products that were tested. Plastic boxes (11 x 6.5 x 7.5 cm, Organized Living, Boca Raton, FL) having seven 3 mm holes to allow access by ants and containing 6 vials filled with 25% aqueous sucrose (w/v) (SS) were strapped to the palms (Figs. 24 to 26). Vials were 6 mL glass shell vials with Titeseal® plastic caps (Fisher Scientific). Each cap was fitted with a 7 mm hole through which a cotton dental wick (38.1 x 9.53 mm, no. 2 medium cotton roll, Crosstex International, Hauppauge, NY) was inserted. Vials were filled with ~4.5 mL SS; three vials were held together with a rubber band. Two vial bundles were placed into each plastic box. Vials were replaced with fresh SS and ~10 mL baby food was supplied twice per week. Pretreatments consisting of SS and baby

food only, which were provided to WFA colonies from 12 to 19 July 2004. To correct for evaporative loss of liquids provided for ant consumption, evaporation controls (5 boxes) were placed on a wooden pole and isolated with Tree Tanglefoot (Fig. 24).

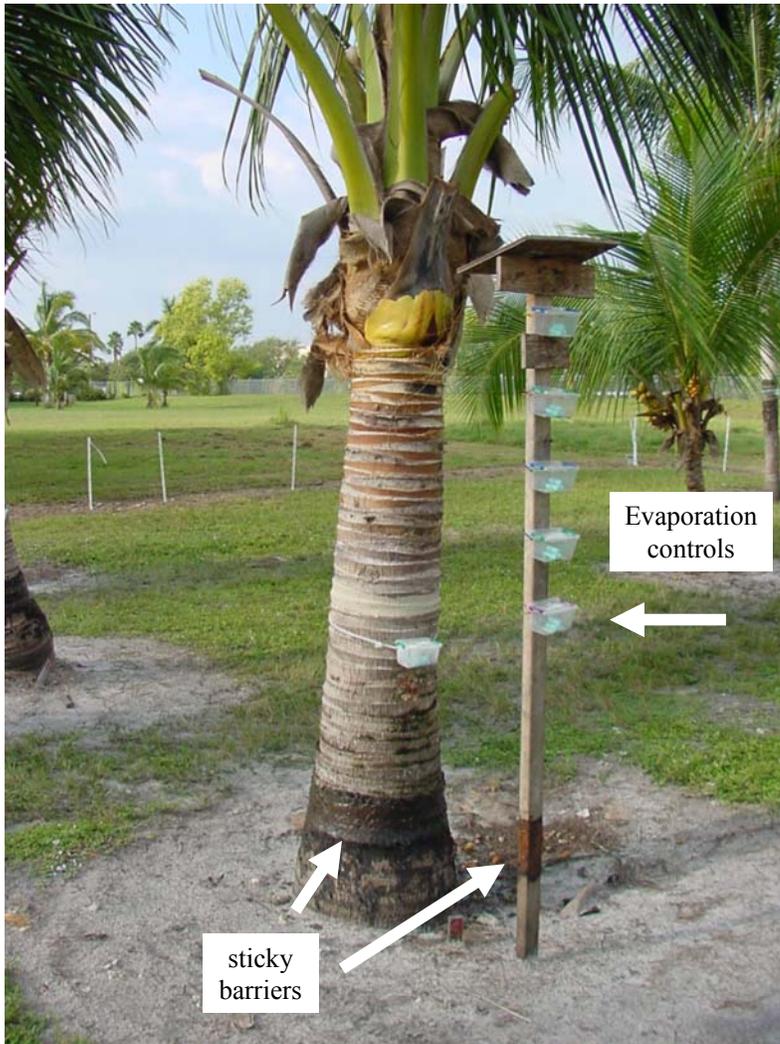


Figure 24. Coconut palm with sticky barrier and evaporation controls.

The number of ants feeding on the wicks was recorded with digital photographs (Fig. 26). Photographs of each box were taken at dawn; later ants on wicks were counted while viewed on a computer monitor. The number of WFA crossing a line in a 60-second span was recorded (trailing frequency). An active trail(s) was selected on each palm, and a mark was placed on the palm trunk where ants would be counted after dawn

as they trailed from their nesting sites in the palm crown to and from the food source provided (Fig. 25).



Figure 25. Monitoring station for *Technomyrmex albipes* foragers. Plastic box is strapped to a coconut palm having 2 bundles of 3 vials containing treatment solutions. White-footed ants are seen on the vial wicks and trailing above the box.

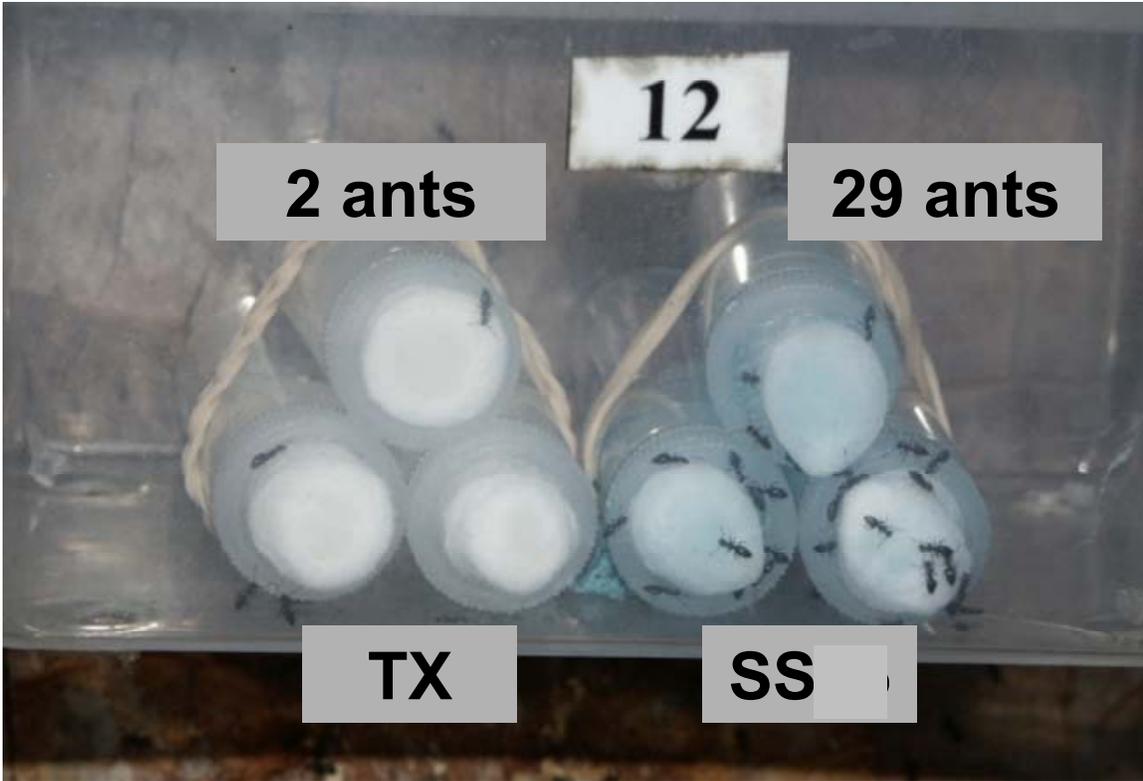


Figure 26. Bait is delivered to *Technomyrmex albipes* foragers using a plastic box containing 3 vials of toxic bait (TX) (left) and 3 vials of SS (right). TX = thiamethoxam (100 ppm) sweet liquid ant bait, SS = 25% (w/v) aqueous sucrose solution.

On 20 July 2004, 5 replications each of TX, IM, and SS were applied to 15 randomly selected palms. In each box, the vial bundle on the left side was filled with either TX, IM, or SS, while the vials in the bundle on the right side in all boxes was filled with SS (Fig. 26), allowing the ants to feed by preference on the bait in either bundle. Since WFA prefer to feed on sweet liquids (Warner and Scheffrahn 2004), they would forage among the vials and feed either on the toxic baits on the left side or on SS on the right side. Our hypothesis is that feeding on the toxic baits would kill enough foragers to cause a significant reduction in the overall WFA population compared to foragers feeding only on SS.

The baby food supplement was provided twice per week as a smear under each monitoring station. Baits were weighed with a TR-403 electronic balance ( $d = 0.001\text{g}$ , Denver Instrument Co., Denver, CO) and replaced twice per week. Vial weights, number of ants feeding on wicks, and trailing frequency were recorded every 2 or 3 days. The mean weight loss from the baits due to evaporation was determined from the change in weight of the vials in the 5 evaporation control boxes and subtracted from the corresponding treatment vials to determine the net amount of liquids consumed by WFA. At 32 DAE, toxic baits were removed and replaced with SS and the populations were monitored until May 2005. Numbers of ants observed feeding on the wicks of vials and trailing frequency were recorded during the 32 day test period and when toxic baits were replaced with SS.

Numbers of ants foraging on wicks, including left vs. right vial bundle preferences, and trailing frequency were square root-transformed and analyzed using ANOVA general linear models. Means were separated by the Waller-Duncan K-ratio  $t$ -Test (SAS Institute 1998). Analysis of residuals for the consumption data, corrected for evaporative losses, were analyzed by ANOVA general linear model and means separated by the Waller-Duncan K-ratio  $t$ -Test.

## Results

### Ants on Wicks

The mean total numbers of ants foraging on wicks (summing left and right vial bundles) for each treatment for the pre-treatment period (pooled data) and 6, 11, 21, 32, and 68 DAE were selected to be representative of the exposure time course and are given in Table 4. There were no significant differences in the numbers of ants on wicks during the pre-treatment period for palms assigned to their respective treatments. At 6 DAE SS

had the highest number of ants ( $83.6 \pm 41.0$ ), but this mean was not significantly different from that of TX ( $54.0 \pm 48.6$ ), however the IM mean total ( $24.6 \pm 19.3$ ) was significantly less than for SS. On Day 11, the three treatments were each significantly different from each other, with the SS having the highest mean number of ants ( $75.6 \pm 30.3$ ), followed by TX ( $41.6 \pm 16.6$ ), and then IM ( $14.0 \pm 13.1$ ). On Day 21, the number of ants on SS ( $113.4 \pm 58.6$ ) was significantly higher than both TX ( $19.6 \pm 31.4$ ) and IM ( $10.4 \pm 8.6$ ), with the latter two not significantly different from each other. The results on Day 32 were similar to that of Day 21; the number of ants on SS ( $105.4 \pm 57.0$ ) was significantly higher than on either TX ( $15.2 \pm 13.3$ ) or IM ( $7.4 \pm 12.0$ ). At Day 68, 36 days into the recovery period, IM ( $7.6 \pm 10.74$ ) had significantly fewer ants on wicks than both SS ( $38.2 \pm 16.89$ ) and TX ( $41.4 \pm 37.07$ ), which were not significantly different from each other.

Figure 27 shows the overall period of the test, including the pre-treatment period and part of the recovery period, including the differences in feeding choice between the left (treatment) and right (SS) side counts in each box. The two toxic baits, TX and IM, followed a similar course. The separation in the TX, right side (SS) and the TX, left side (toxic bait), and the IM, right side (SS) and IM, left side (toxic bait) show that ants were observed more on the SS vial wicks, especially between ~23 July to 1 August, at which time there tends to be a convergence in the lines, showing fewer left-right preferences, but also showing that the numbers of ants are near zero on the TX and IM wicks, while the SS wick counts remained high, with daily variations.

Figure 28 shows the least squared mean number of ants foraging on wicks during the test period and ~70 days of the recovery period. This figure does not graph left-right preferences, only the over-all treatment effects. TX had an initial increase followed by a

Table 4. July to August, 2004, mean number ( $\pm$ SD)<sup>1</sup> of *Technomyrmex albipes* foragers feeding on wicks, pretreatment<sup>2</sup>, and after 5, 6, 11, 21, and 32 days exposure to three treatments in a field test.

Treatment <sup>4</sup>	DAE <sup>3</sup>					
	Pretreatment	6	11	21	32	68 (recovery period)
IM	73.40 $\pm$ 44.84 a	24.6 $\pm$ 19.3 b	14.0 $\pm$ 13.1 c	10.4 $\pm$ 8.6 b	7.4 $\pm$ 12.0 b	7.6 $\pm$ 10.74 b
SS	70.20 $\pm$ 31.48 a	83.6 $\pm$ 41.0 a	75.6 $\pm$ 30.3 a	113.4 $\pm$ 58.6 a	105.4 $\pm$ 57.0 a	38.2 $\pm$ 16.89 a
TX	110.40 $\pm$ 63.80 a	54.0 $\pm$ 48.6 ab	41.6 $\pm$ 16.6 b	19.6 $\pm$ 31.4 b	15.2 $\pm$ 13.3 b	41.4 $\pm$ 37.07 a
Treatment Effects Statistics						
F	0.84	2.96	10.46	10.84	12.46	2.55
df	2,15	2,15	2,15	2,15	2,15	2,15
P	0.4546	0.0903	0.0023	0.0020	0.0012	0.1190

<sup>1</sup>Means of 5 replicates. Means within a column followed by the same letter are not significantly different (Waller-Duncan K-ratio *t*-Test) at P = 0.05.

<sup>2</sup>Pooled data, 15 replicates.

<sup>3</sup>Days after exposure.

<sup>4</sup>TX = thiamethoxam (100 ppm) sweet liquid ant bait, IM = imidacloprid (50 ppm) sweet liquid ant bait, SS = 25% (w/v) aqueous sucrose solution. IM and TX substituted with SS at 32 DAE to initiate recovery period.

rapid decrease in ants foraging on wicks, with the lowest level at about 20 DAE, followed by an increase that continued into the recovery period, reaching pre-exposure level at nearly 60 DAE. The IM treatment on the other hand, had a steady decline in numbers of ants on wicks, with the lowest number (about 20% lower than the lowest reached by TX), at about 30 DAE. This level for IM remained low during the recovery period and at 90 DAE did not recover to pre-exposure foraging populations.

Significant feeding preferences between toxic baits and SS, can be observed in selected DAE values (Figs. 29 to 31). The SS treatment had no significant left-right feeding preferences during the selected DAE (Fig. 29). In TX treatments, there were significant preferences for SS over TX at 5, 6, 11, and 32 DAE (Fig. 30).

### **Bait Consumption**

Bait consumption was related to the numbers of ants foraging on wicks (Figs. 32 to 34). In SS controls, there were no significant feeding preferences (Fig. 32). In TX treatments, SS was significantly preferred (Fig. 33). In the IM treatment, there were no significant feeding preferences at 10, 14, and 17 DAE, but IM was significantly preferred over SS at 28 DAE, and SS was significantly preferred over IM at 32 DAE (Fig. 34). There were no significant differences in bait consumption during the pre-treatment period (pooled data) for palms assigned to their respective treatments. Considering the total net daily consumption of liquids for 6 vials by treatment at 10, 14, 17, 32, and 67 DAE (Table 5), SS was always preferred during the test period, while the preference between IM and TX was statistically the same, with one exception, only at 14 DAE, when IM ( $9.19 \pm 1.91$  g) was consumed significantly more than TX ( $5.80 \pm 3.34$  g). At 67 DAE, during the recovery period when all vials contained only SS, there were no significant differences in consumption.

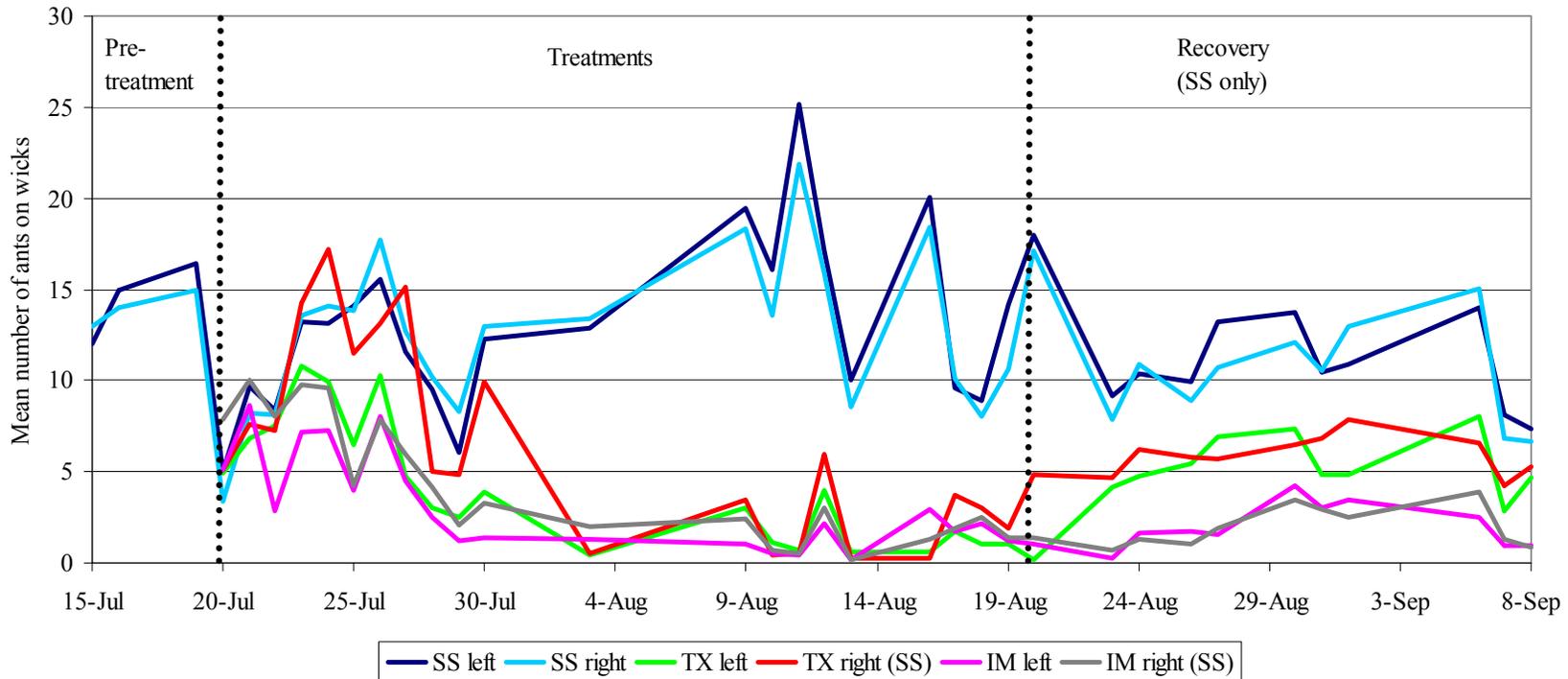


Figure 27. Mean number of *Technomyrmex albipes* foragers feeding on wicks of vials, pretreatment (12 to 19 July 2004), test (19 July to 20 August 2004) and recovery period beginning 20 Aug. 2004). Pretreatment period used SS only. Test period had a treatment (TX, IM or SS) on the left side of each box and SS vials on the right side. The recovery period had SS on both sides, but chart color lines remain the same as during the test period to distinguish treated population effects. TX = thiamethoxam (100 ppm) sweet liquid ant bait, IM = imidacloprid (50 ppm) sweet liquid ant bait, SS = 25% (w/v) aqueous sucrose solution.

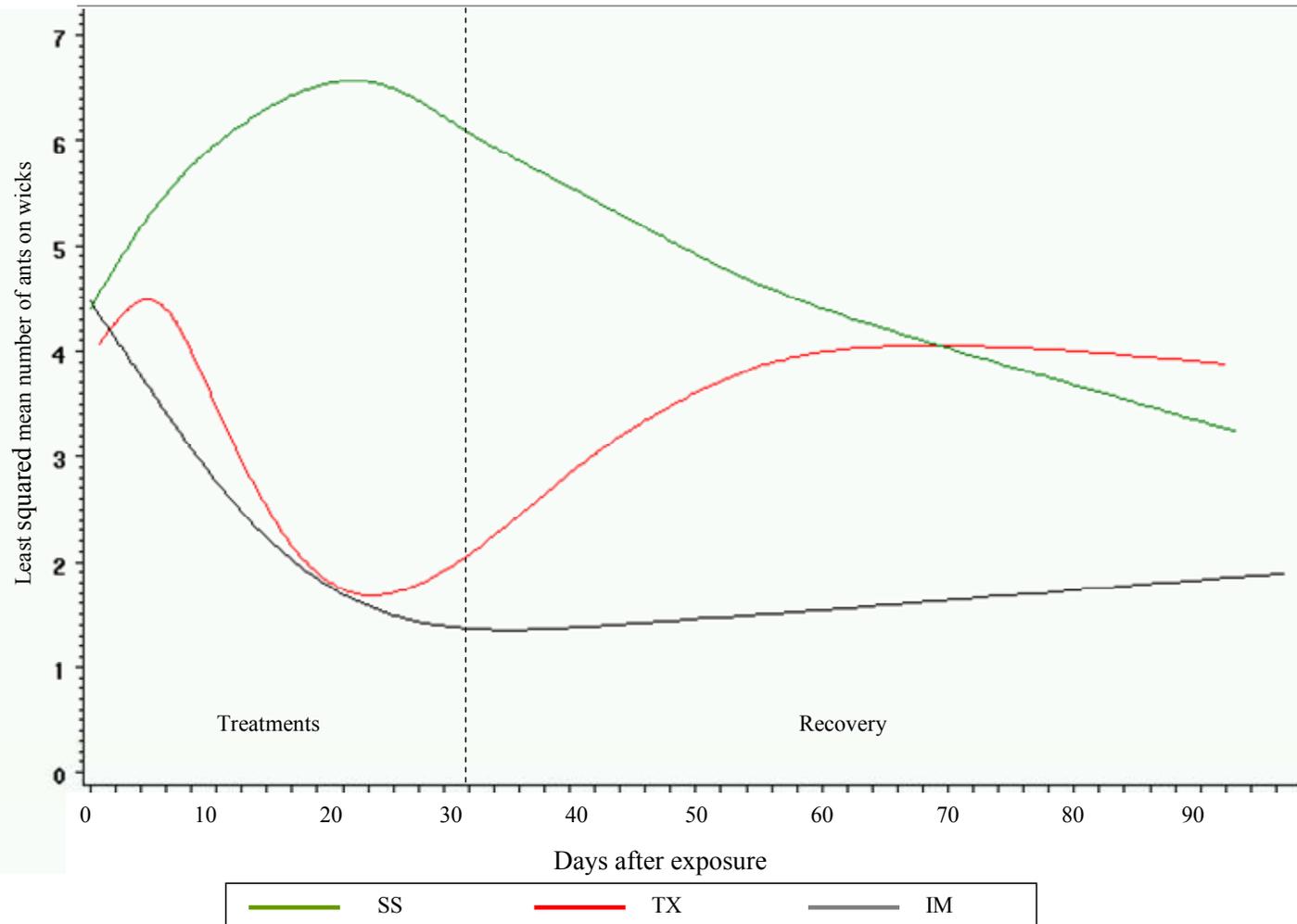


Figure 28. Smoothed line of ants foraging on wicks during test and recovery period. TX = thiamethoxam (100 ppm) sweet liquid ant bait, IM = imidacloprid (50 ppm) sweet liquid ant bait, SS = 25% (w/v) aqueous sucrose solution

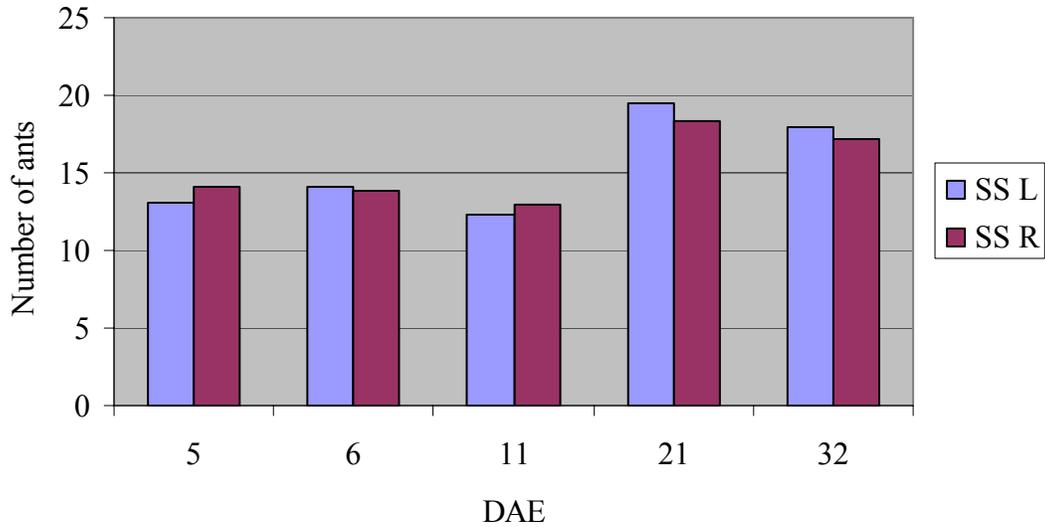


Figure 29. Treatment SS. Numbers of *Technomyrmex albipes* foragers feeding on wicks of vials at 5, 6, 11, 21, and 32 DAE showing left (SS) or right (SS) preferences. There were no significant feeding preferences. SS = 25% (w/v) aqueous sucrose solution. L = 3 vials on left side of box; R = 3 vials on right side of box. DAE = Days after exposure to test products.

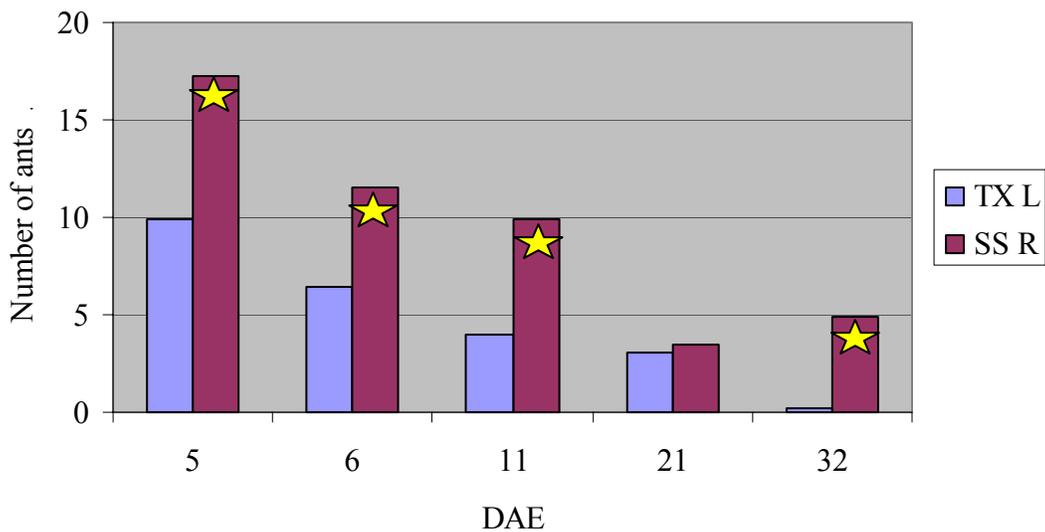


Figure 30. Treatment TX. Number of *Technomyrmex albipes* foragers feeding on wicks of vials at 5, 6, 11, 21, and 32 DAE showing left (TX) or right (SS) preferences. Star indicates significant preference at  $\alpha = 0.05$ . TX = thiamethoxam (100 ppm) sweet liquid ant bait, SS = 25% (w/v) aqueous sucrose solution. L = 3 vials on left side of box; R = 3 vials on right side of box. DAE = Days after exposure to test products.

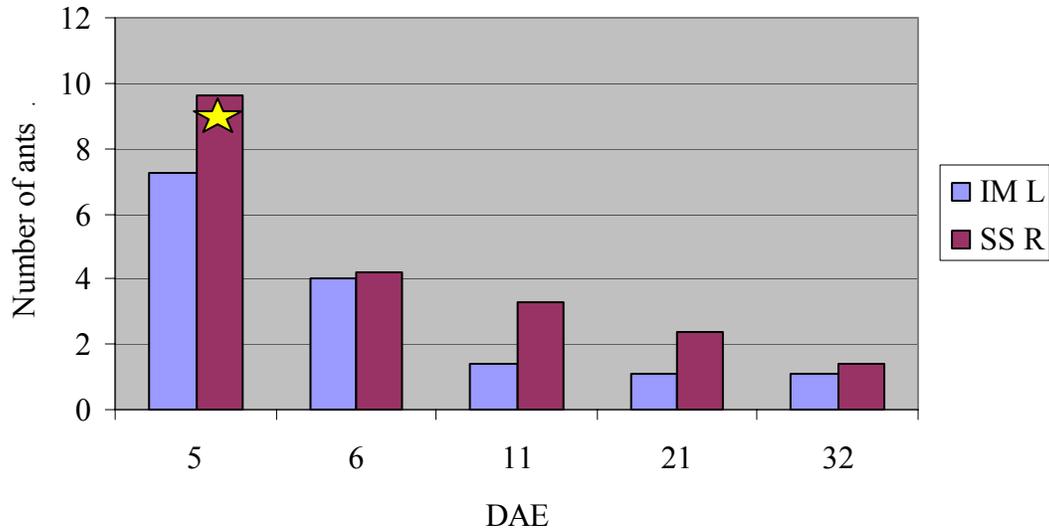


Figure 31. Treatment IM. Number of *Technomyrmex albipes* foragers feeding on wicks of vials at 5, 6, 11, 21, and 32 DAE showing left (IM) or right (SS) preferences. Star indicates significant preference at  $\alpha = 0.05$ . IM = imidacloprid (50 ppm) sweet liquid ant bait, SS = 25% (w/v) aqueous sucrose solution. L = 3 vials on left side of box; R = 3 vials on right side of box. DAE = Days after exposure to test products.

### Trailing Frequency

The mean numbers for the trailing frequency for each treatment for the pre-treatment period (pooled data) and 7, 17, 21, 32, and 68 DAE were selected to be representative of the exposure time course and are given in Table 6. There were no significant differences for trailing frequency during the pre-treatment period for palms assigned to their respective treatments. At 7 DAE, SS had the highest number of ants crossing lines during 60 seconds ( $107.4 \pm 51.2$ , mean  $\pm$  S.D.), and was significantly different from IM ( $40.2 \pm 39.3$ ) which was not significantly different from TX ( $60.8 \pm 38.8$ ). On Days 17, 21, and 32, SS was significantly higher than treatments, IM, and TX, which were not significantly different from each other. At 68 DAE, during the recovery period, TX had the highest number of ants crossing lines ( $75.6 \pm 65.42$ ) which was not significantly different from SS ( $71.8 \pm 34.23$ ), but these were significantly higher than IM ( $8.4 \pm 12.03$ ).

## Correlations of Data

The results for numbers of ants on wicks, bait consumption, and trailing frequency were correlated (Figs. 35 to 37). The correlation (Pearson Correlation Coefficients,  $N = 90$ ) of counts of ants on wicks to bait consumption was  $R = 0.84915$ , of bait consumption to trailing frequency, was  $R = 0.83313$ , and of trailing frequency to counts of ants on wicks, was  $R = 0.93127$ .

## Discussion

Comparing the results of the three arboreal bioassays discussed in this paper (Chapters 2 to 4) has limitations because they were performed during different years and tested different products and application methods. Even so, because the test locations, including climatic trends and palm trees, and ant populations were so similar, including similarities of the products tested, it might be valuable to discuss some comparisons in results and then draw some conclusions.

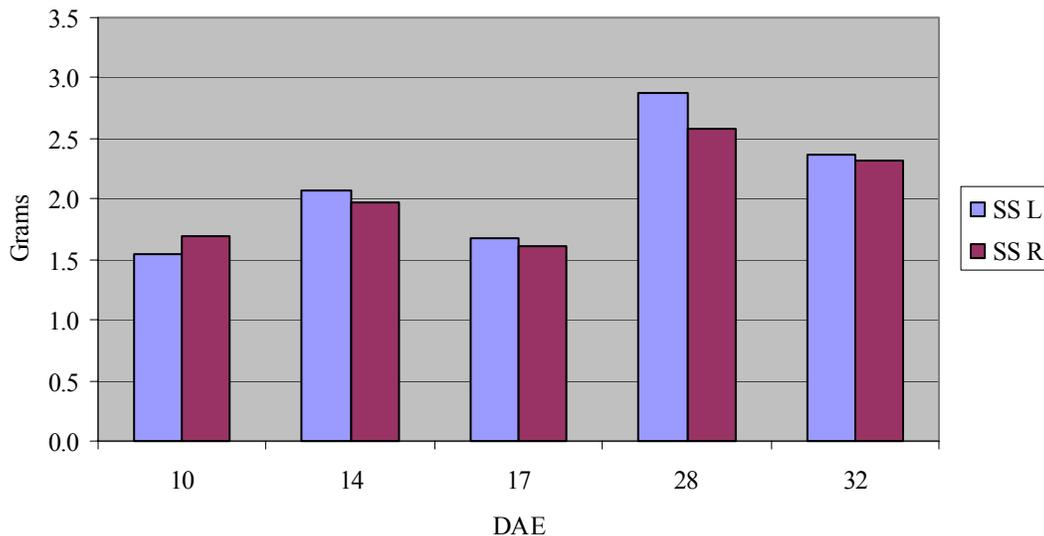


Figure 32. Treatment SS. Net consumption of SS by *Technomyrmex albipes* foragers 10, 14, 17, 28 and 32 DAE, showing bait preferences. There were no significant bait preferences at  $\alpha = 0.05$ . Left and right for each day are SS. SS = 25% (w/v) aqueous sucrose solution. L = 3 vials on left side of box; R = 3 vials on right side of box. DAE = Days after exposure to test products.

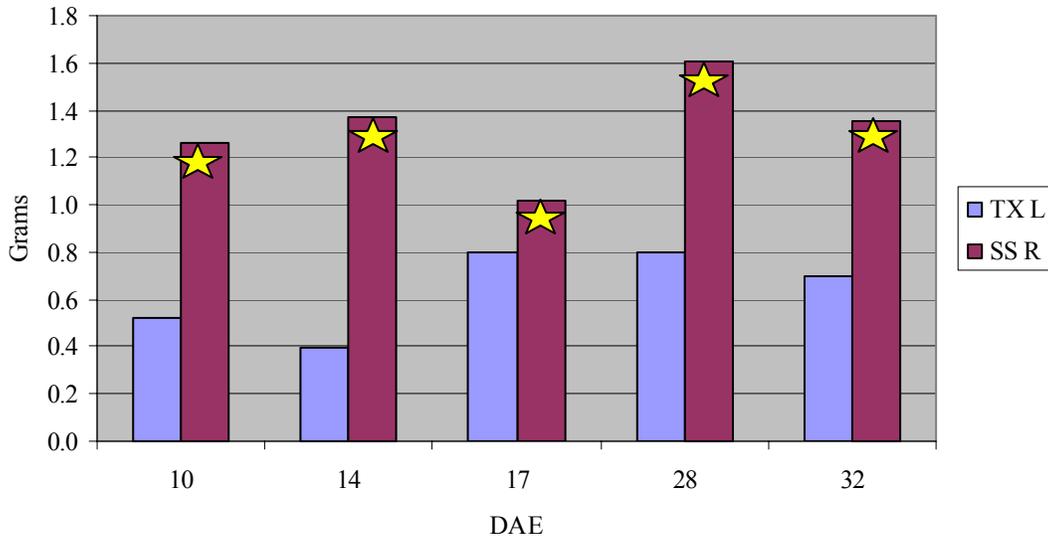


Figure 33. Treatment TX. Net consumption of TX by *Technomyrmex albipes* foragers for 10, 14, 17, 28 and 32 DAE showing bait preferences. Star indicates significant preference at  $\alpha = 0.05$ . TX = thiamethoxam (100 ppm) sweet liquid ant bait, SS = 25% (w/v) aqueous sucrose solution. L = 3 vials on left side of box; R = 3 vials on right side of box. DAE = Days after exposure to test products.

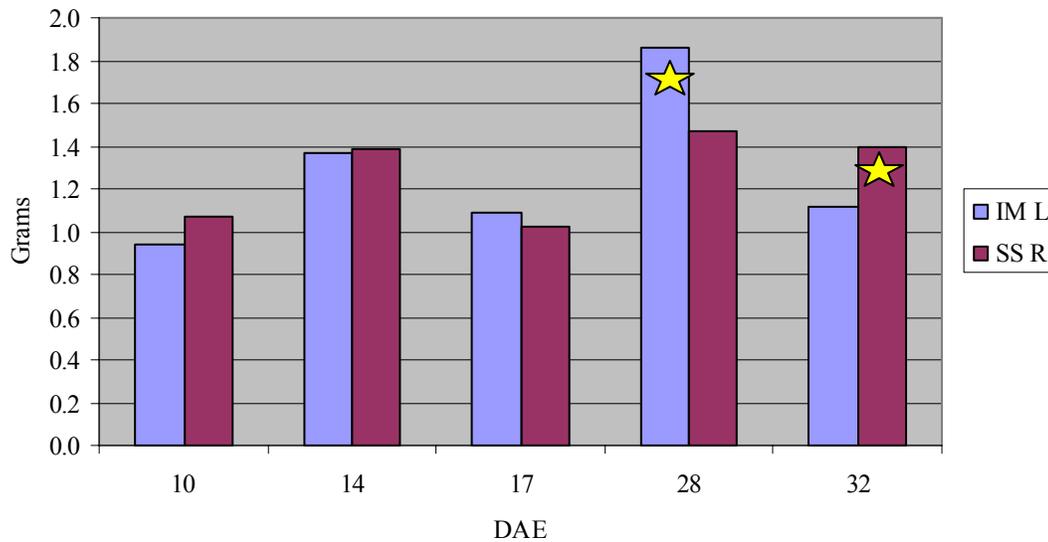


Figure 34. Treatment IM. Net consumption of IM by *Technomyrmex albipes* foragers for 10, 14, 17, 28 and 32 DAE showing bait preferences. Star indicates significant preference at  $\alpha = 0.05$ . IM = imidacloprid (50 ppm) sweet liquid ant bait, SS = 25% (w/v) aqueous sucrose solution. L = 3 vials on left side of box; R = 3 vials on right side of box. DAE = Days after exposure to test products.

Table 5. Mean net daily bait consumption for 6 vials (grams  $\pm$ SD)<sup>1</sup> by *Technomyrmex albipes* foragers of test products, pretreatment<sup>2</sup>, and after 10, 14, 17, 32, and 67 days exposure to three treatments in a field test, July to August, 2004.

Treatment <sup>4</sup>	Pretreatment	DAE <sup>3</sup>				
		10	14	17	32	67 (recovery period)
IM	3.62 $\pm$ 0.94 a	1.85 $\pm$ 0.38 b	1.96 $\pm$ 0.40 b	2.01 $\pm$ 0.32 b	1.80 $\pm$ 0.54 b	2.52 $\pm$ 0.83 a
SS	3.42 $\pm$ 0.83 a	3.19 $\pm$ 1.07 a	3.05 $\pm$ 0.59 a	3.26 $\pm$ 0.83 a	3.60 $\pm$ 1.21 a	2.87 $\pm$ 0.56 a
TX	4.24 $\pm$ 0.98 a	1.72 $\pm$ 0.44 b	1.25 $\pm$ 0.83 c	1.78 $\pm$ 0.19 b	1.52 $\pm$ 0.14 b	2.49 $\pm$ 1.17 a
Treatment Effects Statistics						
F	2.00	7.22	11.10	13.65	12.25	0.275
df	2,15	2,15	2,15	2,15	2,15	2,15
P	0.1630	0.0087	0.0019	0.0008	0.0013	0.764

<sup>1</sup>Means of 5 replicates. Means within a column followed by the same letter are not significantly different (Waller-Duncan K-ratio *t*-Test) at P = 0.05.

<sup>2</sup>Pooled data.

<sup>3</sup>Days after exposure.

<sup>4</sup>TX = thiamethoxam (100 ppm) sweet liquid ant bait, IM = imidacloprid (50 ppm) sweet liquid ant bait, SS = 25% (w/v) aqueous sucrose solution. IM and TX substituted with SS at 32 DAE to initiate recovery period.

Table 6. Mean number ( $\pm$ SD)<sup>1</sup> of *Technomyrmex albipes* adult ants trailing frequency<sup>2</sup>, pretreatment<sup>3</sup>, and after 7, 11, 17, 21 and 32 days exposure to three treatments in a field test, July to August, 2004.

Treatment <sup>5</sup>	DAE <sup>4</sup>					
	Pretreatment	7	17	21	32	68 (recovery period)
IM	83.86 $\pm$ 50.46 a	40.2 $\pm$ 39.3 b	7.8 $\pm$ 7.2 c	8.0 $\pm$ 7.6 b	3.6 $\pm$ 5.0 b	8.4 $\pm$ 12.03 b
SS	99.89 $\pm$ 51.05 a	107.4 $\pm$ 51.2 a	63.4 $\pm$ 23.7 a	114.0 $\pm$ 61.8 a	76.4 $\pm$ 50.5 a	71.8 $\pm$ 34.23 a
TX	100.83 $\pm$ 64.59 a	60.8 $\pm$ 38.8 ab	21.8 $\pm$ 17.7 b	24.2 $\pm$ 32.3 b	18.2 $\pm$ 23.5 b	75.6 $\pm$ 65.42 a
Treatment Effects Statistics						
F	1.02	3.13	15.12	9.95	7.11	6.01
df	2,15	2,15	2,15	2,15	2,15	2,15
P	0.3630	0.0800	0.0005	0.0028	0.0092	0.0156

<sup>1</sup>Means of 5 replicates. Means within a column followed by the same letter are not significantly different (Waller-Duncan K-ratio *t*-Test) at  $\alpha = 0.05$ .

<sup>2</sup>Crossing a line in 60 seconds.

<sup>3</sup>Pooled data.

<sup>4</sup>Days after exposure.

<sup>5</sup>TX = thiamethoxam (100 ppm) sweet liquid ant bait, IM = imidacloprid (50 ppm) sweet liquid ant bait, SS = 25% (w/v) aqueous sucrose solution. IM and TX substituted with SS at 32 DAE to initiate recovery period.

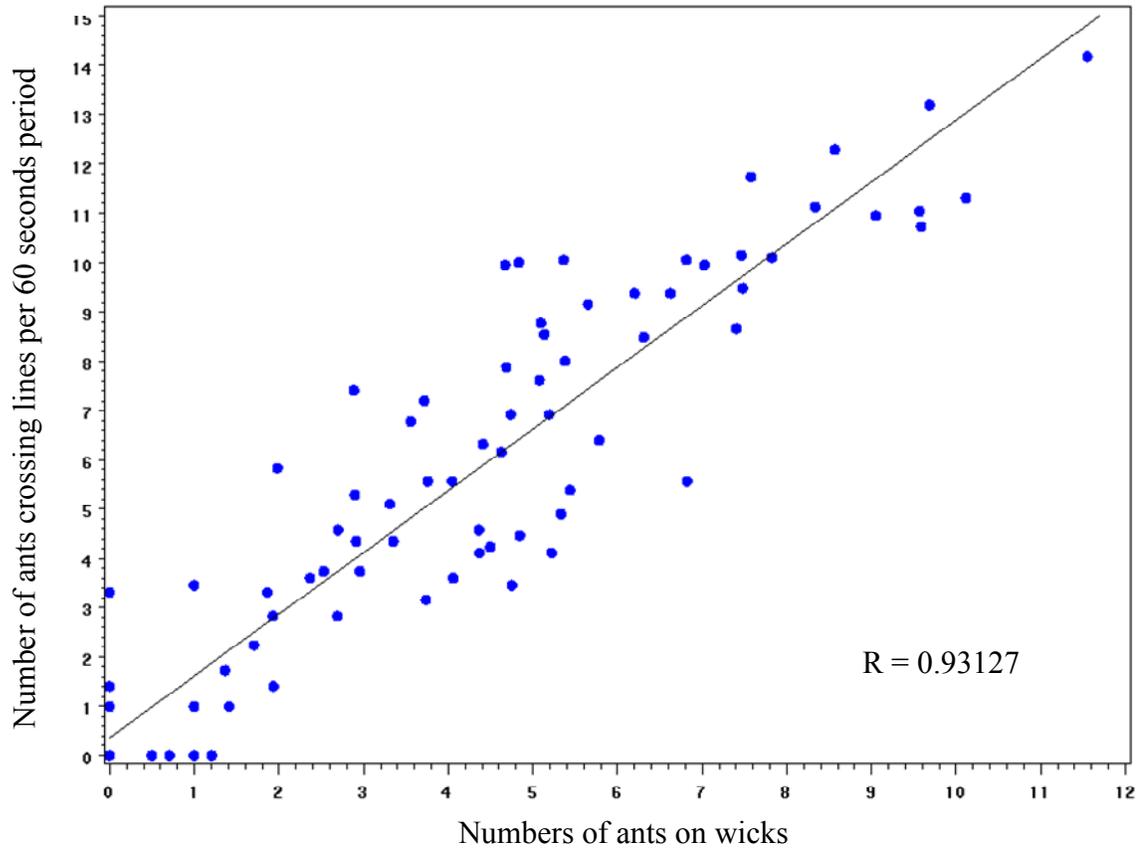


Figure 35. Correlation trailing frequency with numbers of ants on wicks of vials. Means are square root transformed.

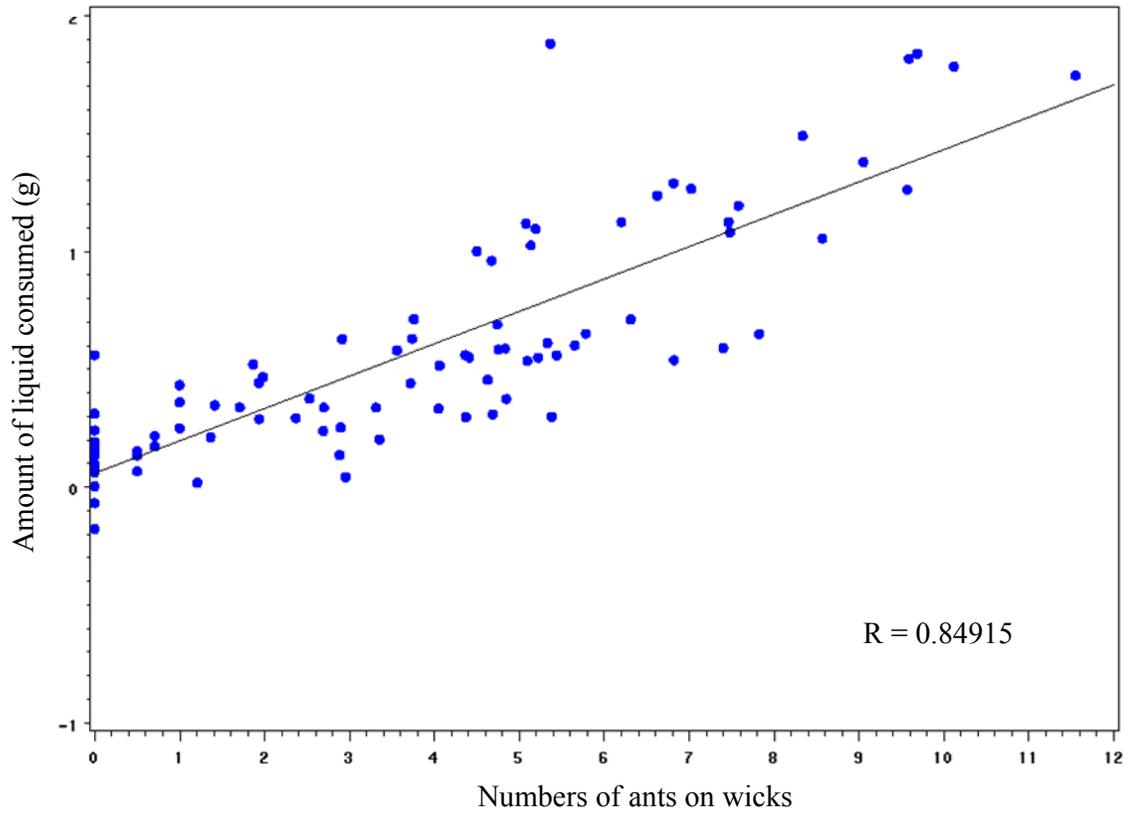


Figure 36. Correlation of net amount of bait consumed by ants with counts of ants on wicks of vials. Means are square root transformed.

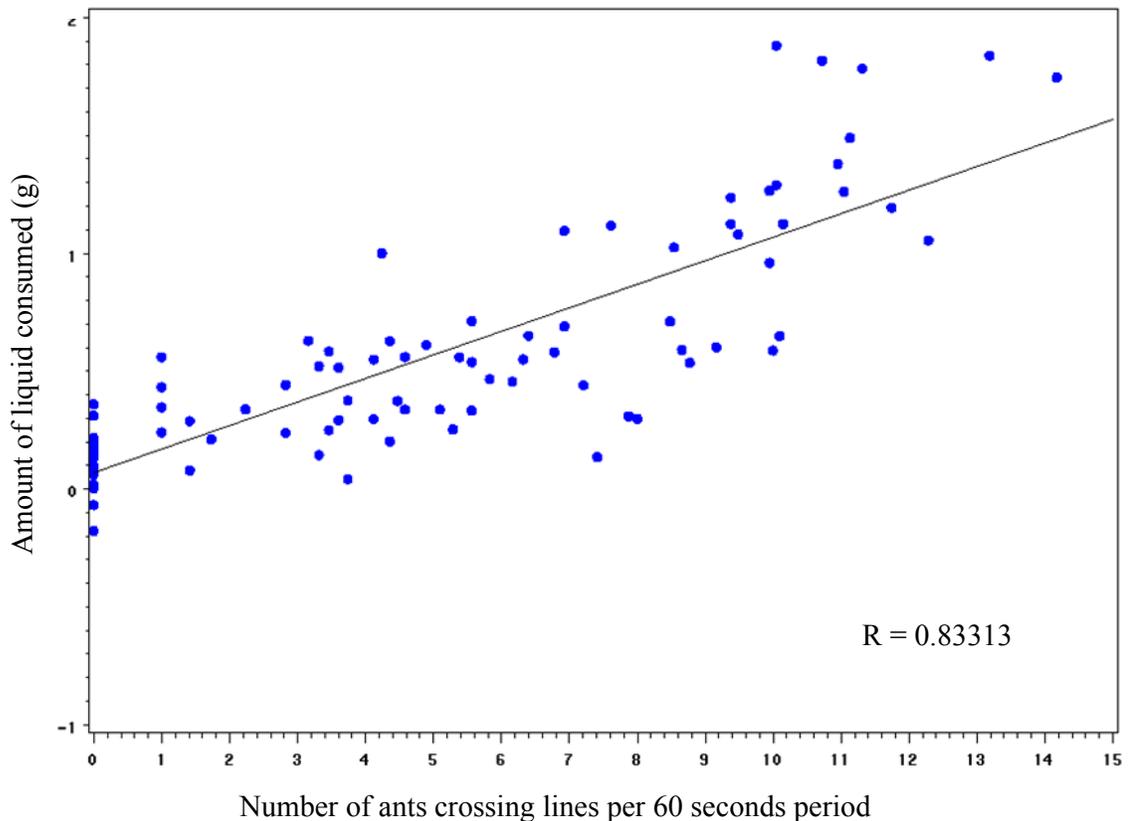


Figure 37. Correlation of net amount of bait consumed by ants with trailing frequency. Means are square root transformed.

For the most part, the following comparisons use Figs. 7, 15, and 28, the smoothed line, least squared graphs of ants foraging on wicks for the 2002-2004 bioassays respectively, because these graphs of the data minimize daily variances and give a good overall view of test results.

Although the ingredients of the bait matrices for thiamethoxam in the 2004 bioassay, and for imidacloprid in the 2002 and 2004 bioassays, were not known, I assume they contain sugars, since WFA prefer sweet baits, and other ingredients such as preservatives. In 2004, SS was often preferred over TX, (Figs. 30 and 33), which led me to believe TX bait matrix contained some repellent ingredient(s). Warner and Scheffrahn (2003) found that when thiamethoxam was mixed in a 25% sucrose solution, it effectively caused significant mortality to laboratory colonies of WFA that also had

access to a 25% sucrose solution without active ingredient. The 2004 data indicate that IM was less repellent than TX because SS was only preferred over IM on two occasions (Fig. 31 and 34) and on one occasion (28 DAE) IM was preferred over SS (Fig. 34).

Thiamethoxam was added to NecDew in the 2002 and 2003 bioassays (Chapters 2 and 3). In those tests, the control solution was 40% sucrose, while in 2004, it was 25% sucrose. The toxic baits were available to the WFA populations for >200, 42, and 32 days, respectively during the 2002, 2003, and 2004 tests. Thiamethoxam in each case effectively reduced the numbers of foragers at ~20 to 40 days, but in 2003 and 2004, the numbers of foragers tended to increase again more rapidly than they did after feeding on imidacloprid formulations. In 2002, there was no such population recovery because the thiamethoxam bait was provided for a much longer period. A further test comparing efficacy of NecDew with thiamethoxam vs. the 2004 thiamethoxam proprietary formulation is suggested.

In the 2003 bioassay, NecDew with imidacloprid reduced the foraging population more than the other products tested followed by minimal population recovery. In 2002 and 2004, imidacloprid was tested as a commercial formulation (Pre-Empt). In 2002, compared to NecDew with thiamethoxam and 40% sucrose, imidacloprid was intermediate in its effectiveness. In both 2003 and 2004 bioassays, the numbers of ants on thiamethoxam and imidacloprid-treated palms diverge when the toxicants are withdrawn, with WFA foraging populations on the thiamethoxam treated palms tending to increase, while those of the imidacloprid-treated palms remained low.

The smallest foraging populations of the three years of data are NecDew with thiamethoxam, NecDew with imidacloprid and the commercial imidacloprid formulation

(Pre-Empt) (Figs. 7, 15, and 28). NecDew was not included in the 2004 testing, but in 2002, feeding on the NecDew with thiamethoxam formulation resulted in lower counts than Pre-Empt. NecDew-based formulations were the most efficient at reducing WFA populations, usually causing noticeable reductions of foragers by 20 to 40 days.

Surface treatments (bifenthrin and fipronil) were included in the 2002 and 2003 bioassays. During the 2002 bioassay one surface treatment, bifenthrin, resulted in a forager population reduction similar to that of imidacloprid (Pre-Empt), but ~120 DAE, the results were different, with that of bifenthrin being less effective than imidacloprid. Considering that the surface treatments were only applied once and the liquid baits were continually supplied during this test, the population reduction of bifenthrin for >100 days warranted further investigation. In the 2003 bioassay, bifenthrin was applied to a larger surface area than in the 2002 bioassay, but results were similar previous to the additional 2003 application that was made to the crowns, which caused a population reduction (Fig. 15).

In summary, baits made with a preferred liquid matrix such as NecDew and a water soluble, nonrepellent toxicant can effectively reduce the WFA forager populations more effectively than surface sprays. Testing combinations of sprays and liquid baits might be a next step in developing improved WFA control in residential properties.

## CHAPTER 5 RUBIDIUM CHLORIDE MARKING OF WHITE-FOOTED ANTS

### **Introduction**

The marking of insects to facilitate behavioral and ecological studies is not new. Berry et al. (1972) suggested the use of trace element markers, and many studies have used elemental markers, such as rubidium (Rb), strontium, cesium, manganese, hafnium, iridium, lanthanum, samarium, europium, dysprosium, and cerium (Corbett et al. 1996, Fleischer et al. 1989, Hagler and Jackson 2001, Hayes 1989, Hougardy et al. 2003, Jackson et al. 1988, Knight et al. 1989, Prasifka et al. 2001, Qureshi et al. 2004, Stimmann 1974, and Woods and Streett 1996). Berry et al. (1972) add that Rb replaces potassium in biological tissues because they are in the same chemical group.

Many methods of marking insects have been used, including mutilation, use of paint, ink, banding, labeling, colored thread, thin wire, dust, dye, pollen, enzymes, use of radioactive material, and genetic marking (Hagler and Jackson 2001). Akey and Burns (1991) state that the most widely used method for the detection of elemental markers for arthropods is currently Rb with analysis of samples by atomic absorption spectroscopy. Rubidium, as a chloride salt (RbCl) is the marker most frequently used for insects (Hagler and Jackson 2001). Some of the advantages for using RbCl as a marker include that it is not radioactive so special apparatus and precautions are not necessary, it has low background levels in most environments so it is easily detected in minute quantities, and it is inexpensive. In addition, RbCl is water soluble, thus it can be dissolved in various food matrices and fed to target insect populations, or applied to plants where it is

systemic and will mark phytophagous insects that feed on the plant (Hagler and Jackson 2001).

Rubidium chloride has often been used as a marker in studies with lepidopterous insects. Knight et al. (1989) determined that 3,000 mg Rb/l of an artificial diet fed to tufted apple bud moth larvae, *Platynota idaeusalis* (Walker) (Tortricidae), was a good concentration for later detection in emerging adults without causing behavioral or biological effects. Hayes (1989) found that tobacco budworm (*Heliothis virescens* (F.) (Noctuidae) larvae reared on 1,000 ppm Rb passed detectable quantities of Rb on to their next generation and other adults that emerged from these larvae that were killed and analyzed had detectable levels in their whole bodies, wings and head capsules. Qureshi et al. (2003, 2004) found that RbCl fed to larvae of the southwestern corn borer *Diatraea grandiosella* Dyar (Pyralidae) at 1,000µg/g diet had little effect on their behavior and development and emerging adults contained detectable Rb levels and Rb could be detected in larvae after feeding on corn plants sprayed at 1,000 ppm. Stimmann (1974) sprayed collard [*Brassica oleracea* (L.)] fields with 0.27g RbCl/ m<sup>2</sup> in aqueous solution with 0.1% Triton X-114 as a spreader-sticker and was able to detect Rb in *Pieris rapae* (L.) (Pieridae) that fed on the plants, and no phytotoxic effects were observed. Prasifka et al. 2001 say that an insect is considered marked if its Rb level exceeds the mean background plus three standard deviations, as made customary by Stimmann (1974).

Woods and Streett (1996), fed an oil-based bait containing RbCl to two species of grasshoppers, and found that 21% of the insects retained the Rb mark for as long as 16 d after application. Cabrera and Rust, 1999, used Rb as a marker to examine feeding and trophallaxis between castes of the western drywood termite, *Incisitermes minor* (Hagen),

and found that ingestion of Rb at concentrations of 1000 to 5000 ppm caused no mortality to nymphs and was detectable for up to two weeks.

Marking Hymenoptera with Rb has been done previously (Jackson et al. 1988) who added 1,000 ppm RbCl to the diet of *Lygus hesperus* (Knight) (Hemiptera: Miridae) adults and detected Rb in larvae of *Anaphes ovijentatus* (Crosby and Leonard) (Mymaridae), a minute parasitic wasp, that developed in *L. hesperus*' eggs. Fernandes et al. (1997) made aqueous drench and foliar applications of 2,500-10,000 ppm RbCl to sorghum seedlings [*Sorghum bicolor* (L.) Moench] and found that a parasitoid, *Lysiphlebus testaceipes* (Cresson) (Braconidae) larvae that developed within greenbugs [*Schizaphis graminum* (Rondani) (Hemiptera: Aphididae)], that fed on the sorghum, acquired the Rb.

Corbett et al. (1996) detected RbCl in another parasitoid, *Anagrus epos* (Hymenoptera: Mymaridae) after they emerged from eggs of the prune leafhopper, *Edwardsiana prunicola* Edwards (Hemiptera: Cicadellidae), after spraying French prune trees with foliar applications of 5,000 ppm solutions of RbCl. Hougardy et al. (2003) used glass tubing to introduce RbCl into the vascular system of spruce trees (*Picea* spp.), and successfully marked *Rhopalicus tutela* (Walker) (Hymenoptera: Pteromalidae), a parasitoid of a small beetle, *Ips typographus* L. (Coleoptera: Scolytidae) that lives concealed under the tree's bark. Hougardy et al. (2003) found that 20% of the females tested lost the Rb mark after 6 days while marked males did not, and said that this difference might be due to a selective incorporation of the marker into the ovaries and eggs and added that other authors (Van Steenwyk et al., 1978; Fleischer et al. 1989; and

Knight et al., 1989) have also observed high levels of Rb uptake in females of other species.

Only one previous study was conducted involving the use of a Rb marker with ants. Weeks et al. (2004) investigated the flow of foods among polygyne colonies of the red imported fire ant *Solenopsis invicta* Buren, using Rb and five other rare earth elements mixed with protein, lipid and carbohydrate foods. Analysis of colony members using neutron activation analysis (NAA), having a Rb detection limit > 0.1 microgram performed after 12 hours of feeding, detected varying amounts of these elements in ant tissues and found that food flow patterns among neighboring colonies was mostly influenced by distance factors between colonies and food sources and by the type of food.

Although sharing of food via trophallaxis is the norm with most Formicidae species as was previously mentioned, it is not so with *T. albipes*. Research performed by Yamauchi et al. (1991) found, after many hours of observation using video cameras, and also via experimental use of dyed foods given to adults, that there were no observed trophallactic behaviors and no traces of the dye in larval tissues. Yamauchi et al. (1991) concluded that there is no trophallaxis among WFA adults and that nutrient transfer from adults to other colony members is achieved exclusively via trophic eggs.

The present research was to determine 1) whether RbCl is repellent or toxic to WFA, 2) the concentration of RbCl that can be detected in WFA, and 3) background RbCl levels in WFA tissue, 4) the foraging distance of WFA in a field experiment, testing the hypothesis that RbCl can be detected in WFA that have fed on a bait containing RbCl (15,000 ppm) in 40% sucrose solution and have then foraged to other areas.

## **Rubidium Repellency**

### **Materials and Methods**

Work was initiated with Rb on WFA in November 2003 to determine if RbCl dissolved in 40% sucrose solution was repellent or toxic to WFA. Work was also initiated to determine the RbCl concentration required to be detectable in individual ants that fed on it, and the amount of Rb lost by WFA over time.

The solution fed to WFA contained RbCl, which has a molecular weight of 120.920, 70% of which is Rb (Rb mol. wt. = 85.467 and Cl = 35.453). Determinations of Rb concentration reported by EPD are in mg of Rb per kg of dry body weight of WFA. Ant samples were digested in nitric acid at 95° C for 2.5 h previous to analysis by Inductively Coupled Plasma (ICP) Optical Emission Spectroscopy, using the primary wavelength of Rb (779.961 nm).

To determine whether RbCl was repellent to WFA when in sucrose solution, a plastic spot plate having 3 indentations large enough to hold one to two drops of liquid each, was set out near an active WFA trail in the laboratory (Fig. 38). The spots contained 5,000 ppm and 15,000 ppm RbCl in 40% aqueous sucrose solution, and 40% sucrose solution. There were no replications.

### **Results**

WFA fed on all three solutions in the spot plate. The 15,000 ppm concentration was selected for further testing because a higher concentration was more likely to be detected in individual ants.

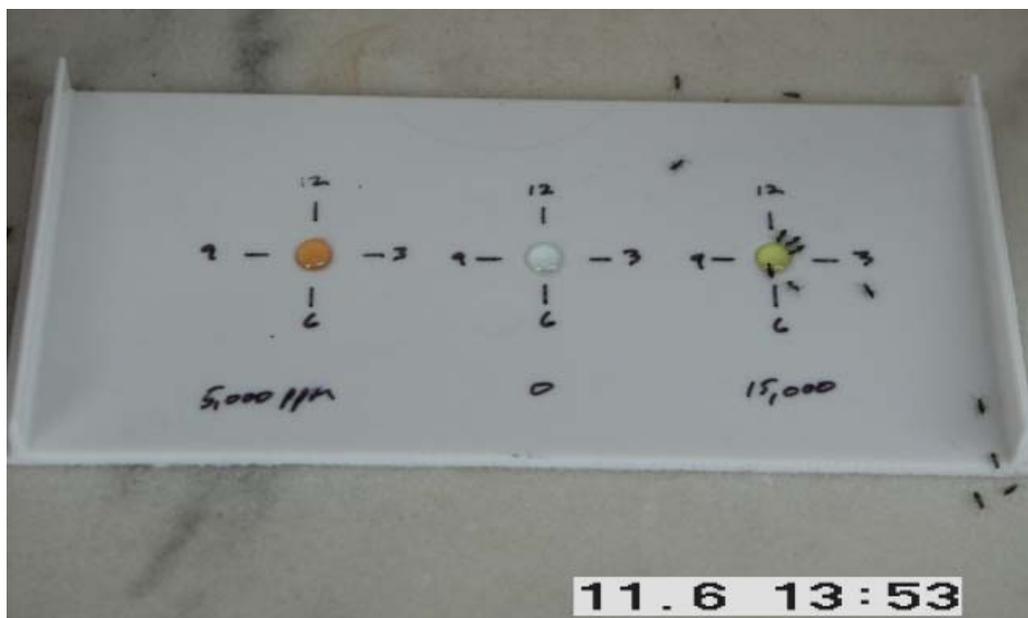


Figure 38. White-footed ants feeding on 40% sugar water containing RbCl. Concentrations of RbCl: 5,000 ppm (left), 0 ppm (center), and 15,000 ppm (right).

### Rubidium toxicity

#### Materials and Methods

To determine if RbCl in 40% sucrose solution was toxic to WFA, a laboratory test compared two concentrations of RbCl (15,000 ppm, and 0 ppm) in 40% sucrose solution for mortality to WFA. Lab-Tek™ reusable plastic Petri dishes (140 x 25 mm, Fisher Scientific) served as test containers for WFA sub-colonies. The lids were fitted with four 1 mm holes covered with a fine mesh for aeration and a thin film of Vaseline® along the inner edge to retard ant escape. Plastic weighing dishes (41 x 41 x 8 mm, Fisher Scientific) contained ¼ of a 45 mm Gelman filter pad that was saturated in the respective treatment solution. After 3 days, additional drops of the solutions were added to keep the pads saturated. A cotton dental wick (38.1 x 9.53 mm, no. 2 medium cotton roll, Crosstex International, Hauppauge, NY) saturated in water was placed inside each Petri dish as a moisture source. There were three replications.

Mean percent mortalities were analyzed by ANOVA and general linear model (SAS Institute 1989) and means separated using Student-Newman-Keuls test at  $\alpha = 0.05$ .

## Results

Table 7 shows the mortalities obtained in this test. The mortality caused by 15,000 ppm RbCl in 40% aqueous sucrose solution was not significantly different than the control solution (0 ppm RbCl).

Table 7. Mean percent mortality ( $\pm$ SD)<sup>1</sup> to WFA exposed to 2 concentrations of RbCl in 40% sucrose solution, 12 DAE.

Treatment ppm RbCl	Mean % mortality	
0	8.095 $\pm$ 11.635	a
15,000	3.077 $\pm$ 3.363	a
Treatment Effects Statistics		
F	0.520	
df	1,6	
P	0.513	

<sup>1</sup>Means of 3 replicates. Means within a column followed by the same letter are not significantly different (Student-Newman-Keuls test) at  $\alpha = 0.05$ .

## Determining the Amount of Rubidium Detectible in White-Footed Ants

### Materials and Methods

The dry weight of individual ants was determined by collecting 100 WFA, drying them in an oven at 60° C for one hour, then weighing them, and dividing by 100. This sample was also used to determine the background Rb level.

Two tests were done using 25 WFA. For the first test, 25 WFA were collected after they had fed on 15,000 ppm RbCl in 40% sucrose solution for >30 seconds. The ants were oven dried and weighed and then sent to EPD together with the sample of 100 ants previously mentioned for analysis by Inductively Coupled Plasma Spectroscopy (ICP), which, according to EPD, “is capable of running rubidium at sensitivities of 1-10 ppb” (N. Gassman, personal comm. 2003). A second sample of 25 individual ants was

collected after they had fed on 15,000 ppm RbCl in 40% sucrose solution for >30 seconds. These ants were sent to EPD in individual vials for analysis to obtain their individual Rb content.

### **Results and Discussion**

The mean dry weight of a WFA adult forager was 0.19 mg. The background Rb level in the 100 WFA sample was 0.059 ppm or 0.0006 ppm (= 0.6 ppb) per ant. Based on similar analyses involving solid matrices, this value would be most likely below an established detection limit value of 1 to 10 ppb (EPD, personal comm.). For the 25 WFA fed 15,000 ppm RbCl, ~5,000 ppm or 200 ppm per individual ant, was detected, which is  $>3 \times 10^5$  times greater than the background level for WFA. Stimmann (1974) set the accepted standard of determining whether or not an insect should be considered to be marked by Rb when its Rb dry weight concentration exceeds the mean plus 3 standard deviations of the level of the untreated insects. Because background level was determined using a sample of 100 WFA rather than individually, a standard deviation was not obtained. However, because the background level here is obviously greater than the required standard, the ants were considered to be marked by Rb.

In the second test (Table 8), the mean Rb content (6823.98 ppm) was 34 times higher than the previous test of 25 ants (200 ppm), probably due to experimental error and individual variations in ant feeding. The correlation between the time ants spent feeding on the solution and the Rb content found in each ant by later analysis was low ( $R= 0.3318$ , Spearman Rank Order Correlation ( $\alpha >0.050$ ), SigmaStat). The 25 WFA tested ingested 88.30 mg Rb/kg (dry weight) per second (approx. mean).

## **Determining the Amount of Rubidium Lost over Time**

### **Materials and Methods**

An experiment was done to determine the amount of Rb lost over time.

Approximately 200 WFA in a lab box were starved for 24 h, then provided with a vial of 15,000 ppm RbCl in 40% sucrose solution with preservatives for 24 h. The RbCl solution was then replaced with 40% sugar water. Approximately 10 ants were removed each day, and oven dried. After 11 samples were collected, they were sent to EPD for analysis.

Simultaneously, WFA nesting in an isolated palm tree that had been provided with sugar water were starved for 1 day and then provided with 15,000 ppm RbCl in 40% sucrose solution with preservatives for 1 day and then switched back to plain sugar water.

Approximately 50 ants were collected each day, dried, and sent to EPD for analysis.

### **Results and Discussion**

The results of the analyses for the laboratory and field tests are in Tables 9 and 10 and Figures 39 and 40. There was a high negative correlation ( $R = -0.85$ ) between number of days and loss of Rb concentration per ant. The field test shows a negative correlation ( $R = -0.62$ ) that is somewhat weaker than the laboratory correlation probably because the WFA population in the tree was far larger and more variable than the laboratory colony. In the field, there was a greater likelihood of collecting ants that had not fed on the RbCl during the 24 h it was available to them.

Based on the data cited above and the review of literature, the use of RbCl is considered to be a valid method of marking WFA. White-footed ants collected within ~15 to 20 days of feeding on 15,000 ppm RbCl should contain greater than background Rb levels.

Table 8. Rubidium content of individual ants that had fed >30 sec on 15,000 ppm RbCl in 40% sugar water. Correlation between feeding time and Rb content was  $R= 0.3318$  (Spearman Rank Order Correlation ( $\alpha >0.05$ ), SigmaStat).

Ant number	Seconds feeding	Rb ppm
1	84	4,372.61
2	111	9,214.29
3	70	7,550.48
4	113	6,572.73
5	139	7,232.17
6	37	4,129.92
7	135	10,795.00
8	56	8,960.31
9	64	4,205.00
10	56	10,030.43
11	57	5,347.04
12	36	2,606.96
13	60	733.24
14	38	8,793.46
15	50	7,855.19
16	222	10,328.89
17	75	4,832.86
18	69	5,509.60
19	112	7,897.62
20	51	2,764.84
21	83	6,157.83
22	68	4,340.42
23	52	10,820.00
24	61	10,337.83
25	33	9,210.71
total	1932	170,599.40
mean	77.28	6,823.98

Table 9. Descriptive statistics of Rb content of 25 ants after feeding on 15,000 ppm RbCl in 40% sugar water.

Rb content mg/kg	
Mean	6,823.98
Median	7,232.17
Standard Deviation	2,853.69
Range	10,086.76
Minimum	733.24
Maximum	10,820.00

Table 10. Field test of WFA that had fed on 15,000 ppm RbCl showing Rb concentrations over time.

Vial	DAE <sup>1</sup>	No. ants	Rb/ant (mg/kg)
1b	1	51	54.4
2b	2	53	7.8
3b	5	49	11.3
4b	6	48	8.0
5b	7	52	14.7
6b	8	51	5.5
7b	12	47	5.1
8b	14	49	3.2
9b	15	50	4.4
10b	16	47	2.5
11b	19	48	2.8
12b	20	50	2.0

<sup>1</sup>DAE= days after exposure.

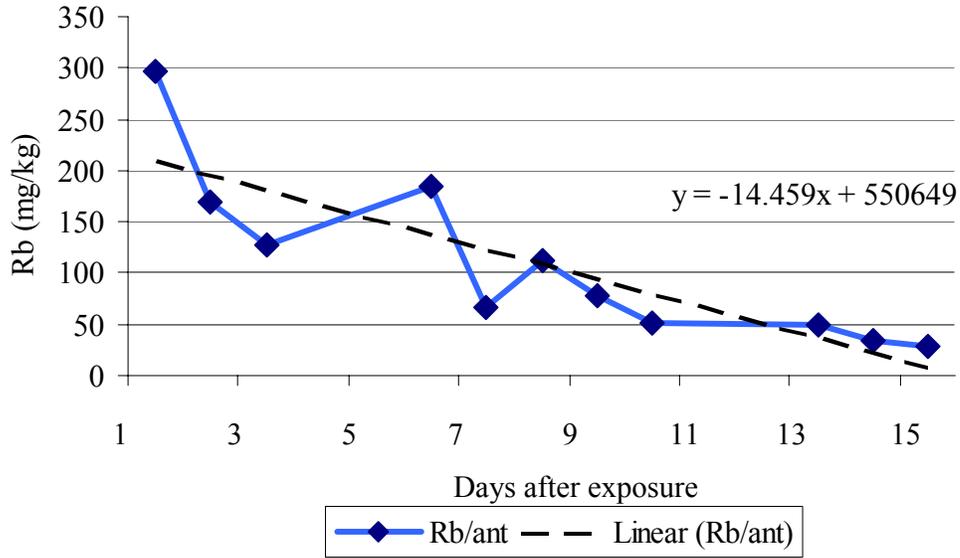


Figure 39. Loss of Rb over time in WFA laboratory colony that had fed on 15,000 ppm RbCl.

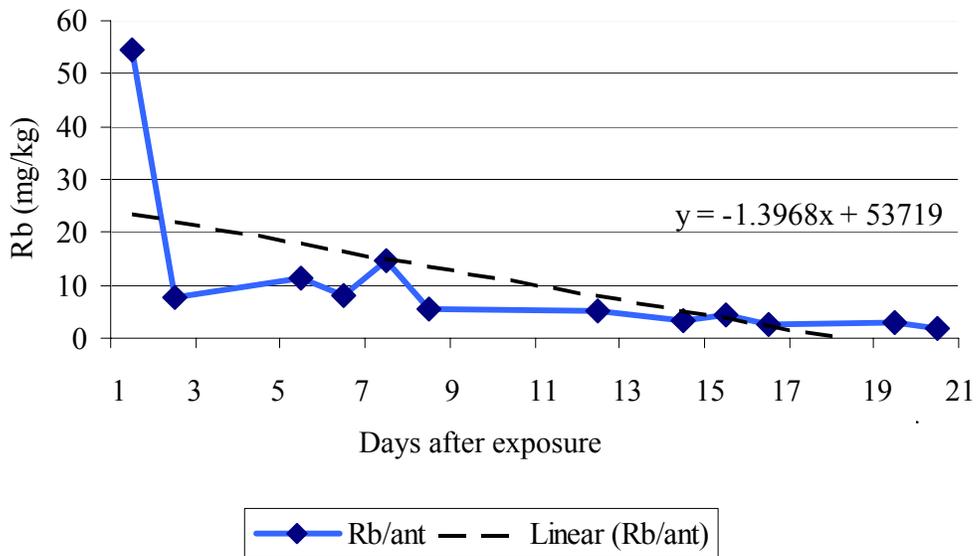


Figure 40. Field test of WFA that had fed on 15,000 ppm RbCl showing loss of Rb over time.

## CHAPTER 6 RUBIDIUM MARKING TEST OF WHITE-FOOTED ANT TRAILING DISTANCES

### **Introduction**

The use of liquid baits or residual sprays for the control of WFA can be improved by a better understanding of the trailing behaviors of these ants. Both the location of established trails and the distance WFA travel from a nest a food source are important considerations for the placement of baits or residual sprays. For a bait to be effective, it must be placed within or in close proximity to their foraging area. Baits placed too far from a target population may have no effect. Likewise, a spray applied directly to active trails, or where they might form trails could significantly impact the population, depending on the efficacy of the product used.

The following experiment examines the distances WFA travel from a food source containing Rb to sampling sites at varying distances.

### **Materials and Methods**

An area around and including the Ant Lab at FLREC that was infested with WFA was selected for studies to estimate their trailing distances. Established WFA trails that had not altered very much over several years were mapped (Fig. 41). Eleven sampling sites were marked within the area. At sites, R01 and R06, a 40% aqueous sucrose solution (SS40), was provided for ant feeding and placed in plastic tubes within suet boxes, for protection against raccoons. SS40 was also provided at R09 outside the door to the Ant Lab, but without the suet box. Baby food was periodically provided at these sites. The mango tree (Fig. 42) was designated as site

R01 because it consistently had the highest ant activity over the last few years and was where the RbCl solution was administered. The foraging distances in Fig. 41 include approximate distances foraging on vertical surfaces.

A pretest sample to determine baseline Rb levels in WFA was collected on 18 May 2005 using a mini-aspirator (Fig. 43), collecting 3 samples of 1 ant each, at each site, R01, R02, R06, R07, R09, R10 and R11. Only 3 samples were collected due to budgetary constraints. Sites R03, R04, R05, and R08 were removed from the study as collection sites due to low numbers of foragers.

On 24 May, 50 g of 15,000 ppm RbCl in 40% aqueous sucrose solution with preservatives was administered in a plastic tube within the suet box on the mango tree (R01) (Figs. 42, 44, and 45). Three ants that appeared to be exiting from the tube were collected by aspiration (1 ant/vial). The following day, 25 May, 3 ant samples (1 ant/vial) were collected at each site, R01, R02, R06, R07, R09, R10 and R11. This procedure was repeated on 26, 27, and 30 May. After the sampling was completed, the RbCl solution was replaced by SS40 on the mango tree.

## Results

No Rb was detected in the 3 ants collected during the pretest sampling at each of sites R01, R02, R06, R07, R09, R10 and R11. Table 12 shows the overall statistics for Rb content for samples collected during this period. The three ants collected at site R01 on 24 May as they exited from the RbCl solution-filled feeding tube all contained Rb (Table 12 and Figs. 46 to 53). All three ants collected from R01 contained Rb on all days collected, except for 30 May, when only 2 out of 3 ants were positive for Rb (Fig. 47). Results varied more at all the other sites. Site R02 (6.14 m from R01) (Fig. 48) had 1 ant with Rb on 25 May, 2 on 26 and 27 May, and 1 on 30

May. Site R06 (14.99 m from R01) (Fig. 49) had 1 ant with Rb on 25 and 26 May, 2 on 27 May, and no ants with Rb on 30 May. Site R07 (10.95 m from R01) (Fig. 50) had 3 ants with Rb on 27 May, but none on any other day. Site R09 (14.81 m from R01) (Fig. 51) had 1 ant with Rb on 26 May and 3 ants with Rb on 27 May and 1 on 30 May. Site R10, the furthest from R01 (20.12 m from R01) (Fig. 52), had 1 ant with Rb on 25 and 26 May and 3 on 27 May. Site R11 (inside the ant lab, 15.89 m from R01) (Fig. 53) had 1 ant with Rb on 25 and 27 May. Only ants collected from sites R01 and R02 ever had  $>2,000$  mg/kg Rb (mean amount for ants collected on each day).

### **Discussion**

In the area where this test was performed, the exact location of WFA nests had not been determined. There probably were numerous nesting sites hidden in the roof or ceiling of the ant lab building. The ceiling is made of a material consisting of compressed sugarcane bagasse, which is in a state of slow decomposition producing a constant fall of dust and debris inside the laboratory. Ants have foraged inside the lab, apparently originating from the roof/ceiling area, for years. Ants coming from the mango tree (R01), going along the north face of the building and entering the lab through openings around the windows have also been observed for years, and during this time, many trail routes have deviated only slightly, if at all.

The results show that WFA forage as far as 20 m from a food source, although the distances from nests were not determined. The reduction in Rb concentrations found in ants collected at sites further from the mango tree than R02 indicates a Rb loss due to excretion, although it is possible, that those ants contained less Rb because they consumed less at the RbCl feeding site than those collected at R01 and R02.

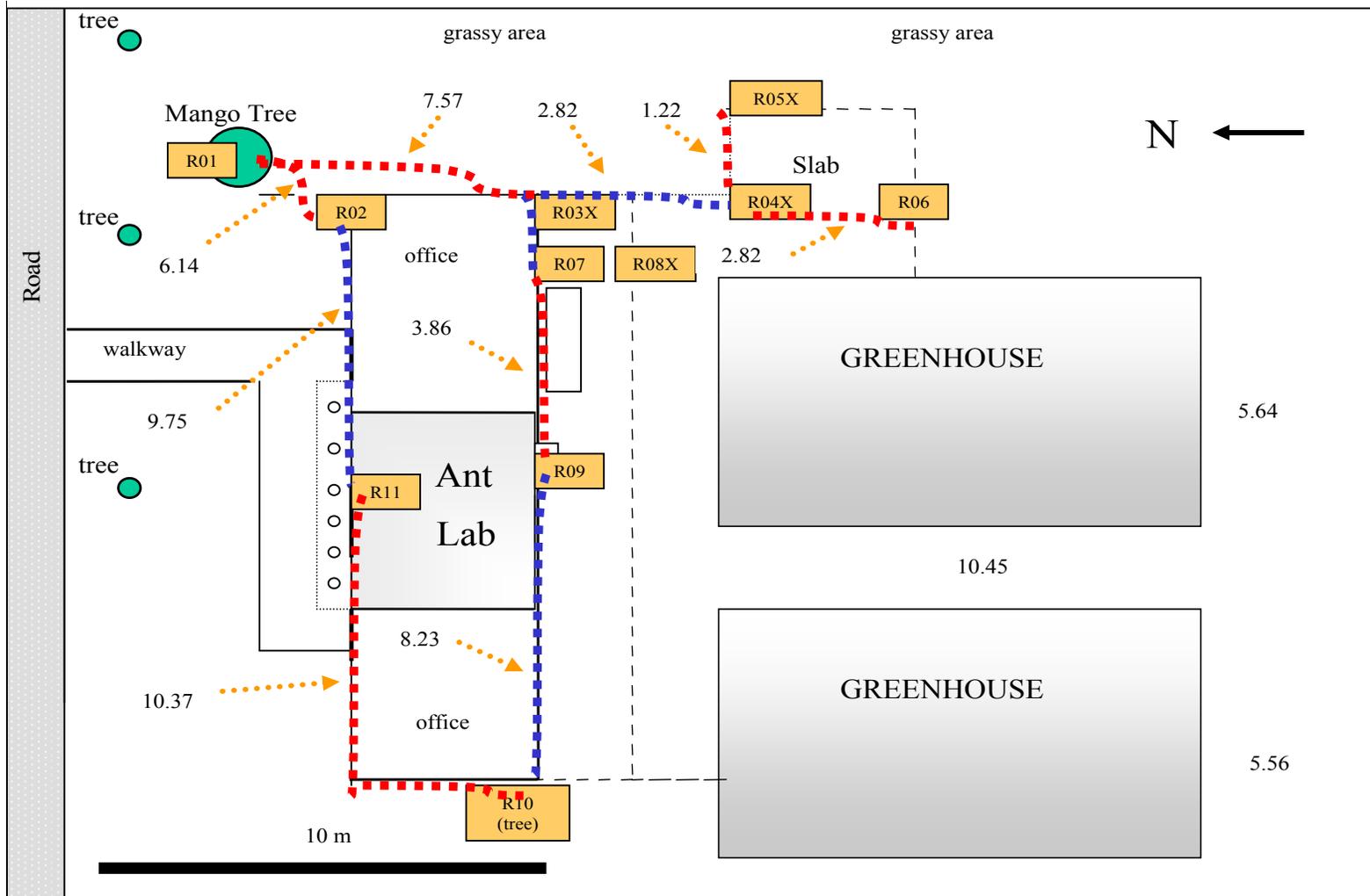


Figure 41. Map showing distances from mango tree rubidium source (R01). Dotted red and blue lines indicate WFA foraging trails. Sampling sites are indicated by R01 – R11. Sites marked X were not used as sampling sites.



Figure 42. Rubidium marking test site. A) Mango tree (R01) where 15,000 ppm RbCl was provided to WFA, B) Ant Lab, North-facing window.

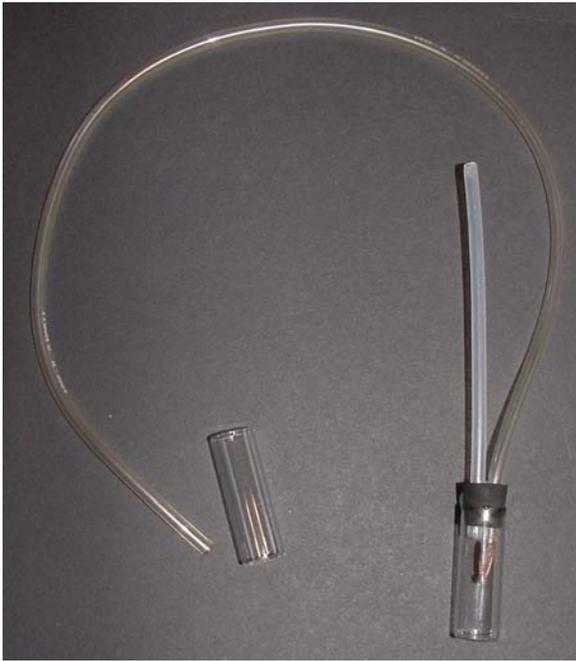


Figure 43. Mini-aspirator with 6 mL glass shell vial.

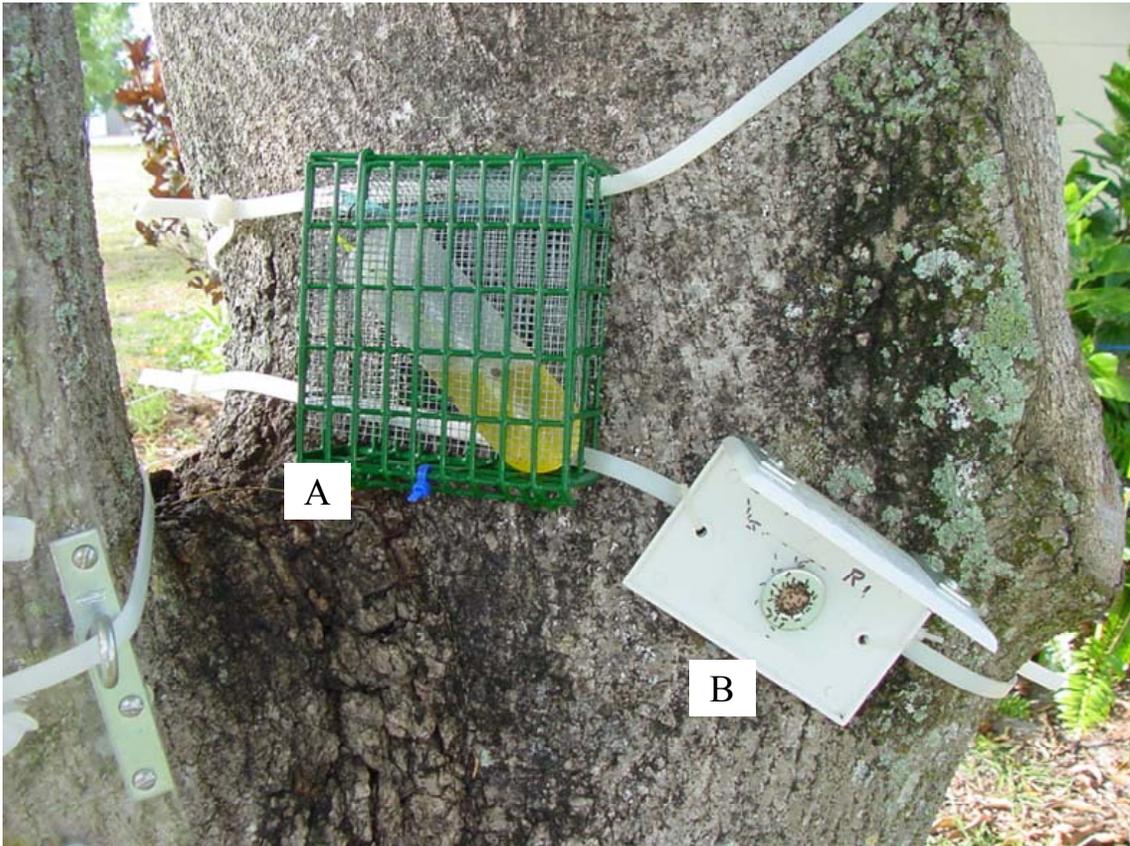


Figure 44. Mango tree near the Ant Lab. A) Suet box holding a plastic tube containing 15,000 ppm RbCl in 40% aqueous sucrose solution. B) Plastic holder with baby food.



Figure 45. Ants at RbCl source. Arrows indicate WFA entering and exiting a plastic tube containing RbCl solution within a suet box strapped to the mango tree near the Ant Lab.

Table 11. Descriptive statistics of Rb content (mg/kg) for 87 ants collected from sites near the FLREC Ant Lab 24 to 30 May 2005. Ants were provided with 15,000 ppm RbCl during this period.

	Rb content mg/kg
Mean	1,599.90
Standard Deviation	3,679.07
Range	18,944.00
Minimum	0.00
Maximum	18,944.00
Sum	139,191.50
Count	87

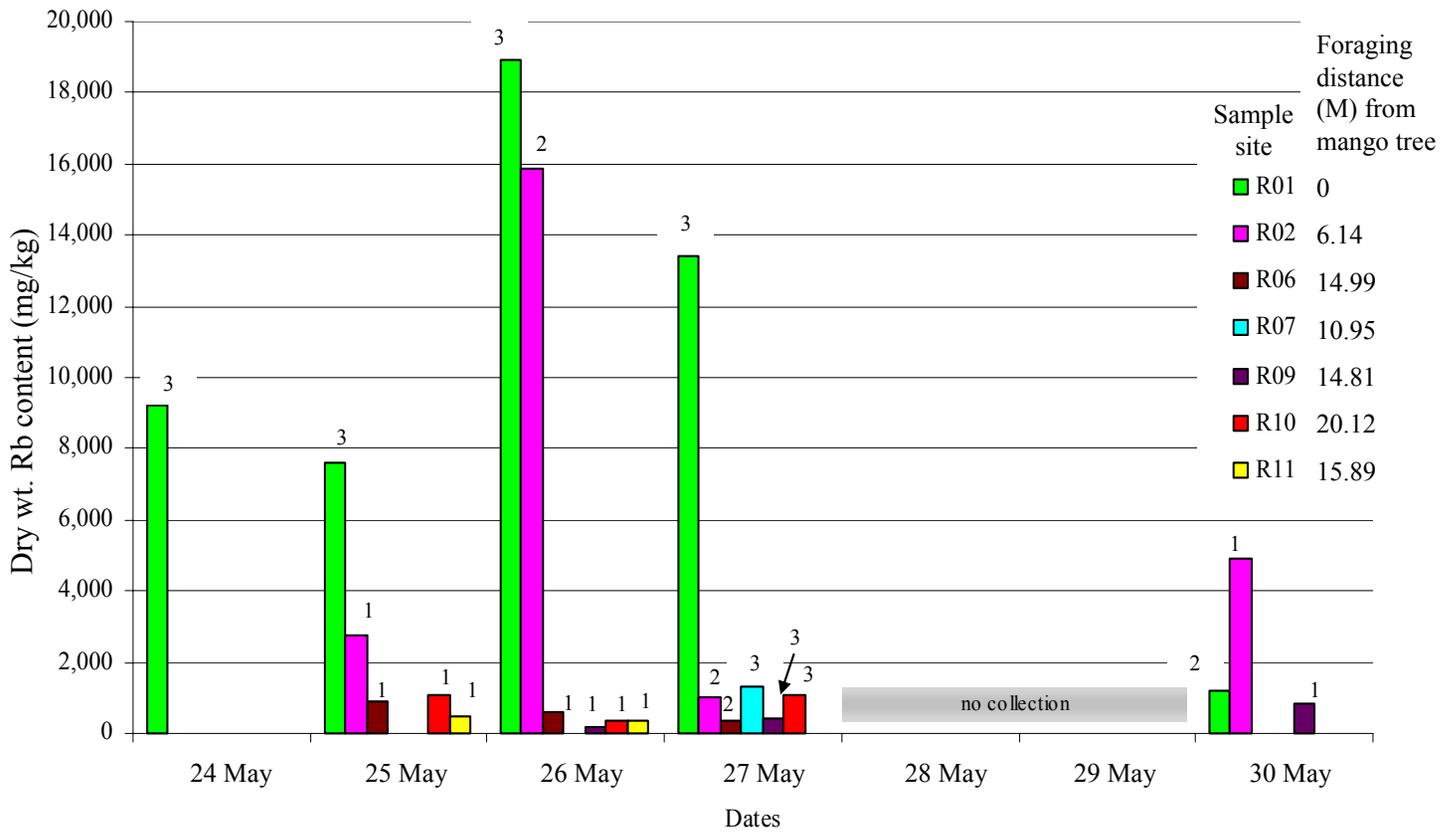


Figure 46. Daily maximum Rb content of 3 ants collected at each site, 24 to 30 May 2005 after feeding on 15,000 ppm RbCl; numbers above graph bars indicate number of ants collected that contained Rb, out of 3 ants collected at each site. Only site R01 was collected on 24 May.

Table 12. Ants collected 24 to 30 May 2005 after feeding on 15,000 ppm RbCl. R = number of sample site; 3 ants were collected at each site. Only site R01 was collected on 24 May. N=87.

2005 date	Site	No. of ants with Rb	Rb mg/kg min.	Rb mg/kg max.	Rb mg/kg mean	Distance from mango tree (m)
24-May	R01	3	7,816	9,214	8,703	0
25-May	R01	1	1,273	7,633	5,325	0
	R02	1	0	2,737	912	6.14
	R06	1	0	927	309	14.99
	R07	0	0	0	0	10.95
	R09	0	0	0	0	14.81
	R10	1	0	1,072	357	20.12
	R11	1	0	453	151	15.89
26-May	R01	3	2,075	18,944	9,914	0
	R02	2	0	15,890	6,680	6.14
	R06	1	0	602	201	14.99
	R07	0	0	0	0	10.95
	R09	1	0	161	54	14.81
	R10	1	0	385	128	20.12
	R11	1	0	336	112	15.89
27-May	R01	3	3,030	13,394	8,947	0
	R02	2	512	1,007	506	6.14
	R06	2	0	342	162	14.99
	R07	3	13	1,301	480	10.95
	R09	3	69	404	224	14.81
	R10	3	316	1,048	576	20.12
	R11	0	0	0	0	15.89
30-May	R01	2	0	1,179	734	0
	R02	1	0	4,929	1,643	6.14
	R06	0	0	0	0	14.99
	R07	0	0	0	0	10.95
	R09	0	1	844	281	14.81
	R10	0	0	0	0	20.12
	R11	0	0	0	0	15.89
total		36			1,599.91	11

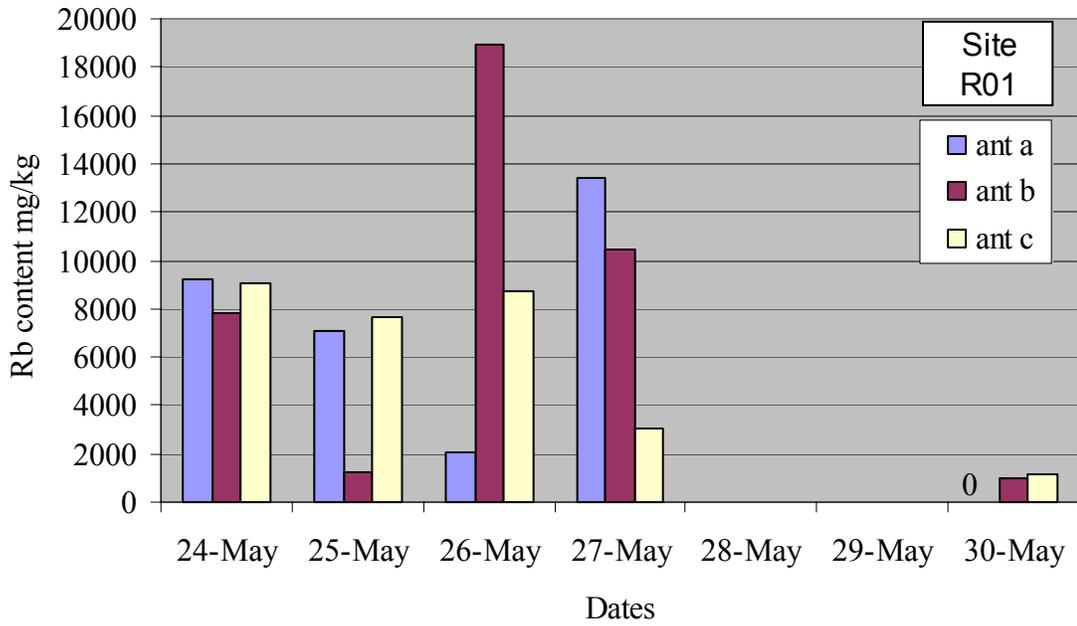


Figure 47. Site R01. Rb content for each of three ants (a, b and c) sampled daily 24 to 30 May 2005 after feeding on 15,000 ppm RbCl. No samples were collected 28 and 29 May.

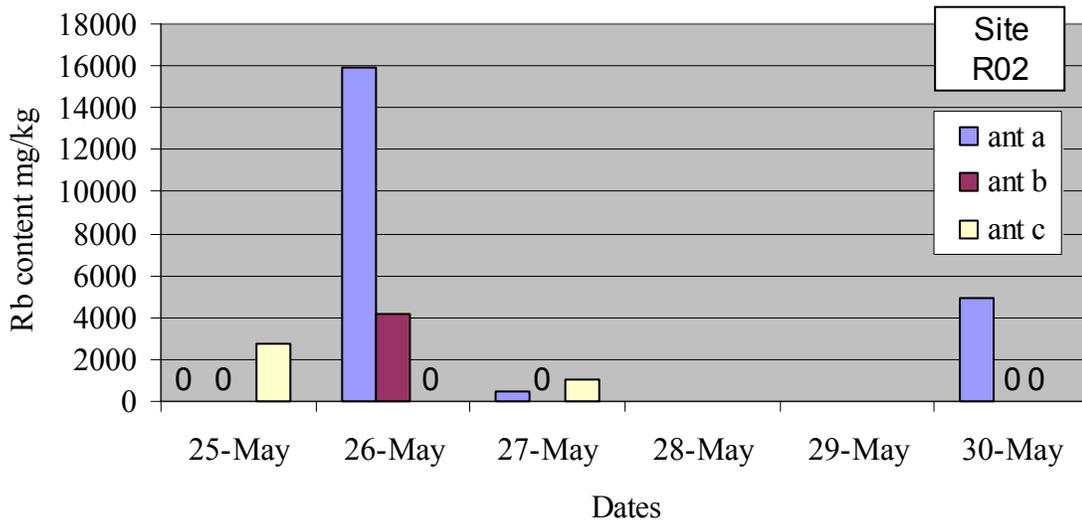


Figure 48. Site R02. Rb content for each of three ants (a, b and c) sampled daily 24 to 30 May 2005 after feeding on 15,000 ppm RbCl. No samples were collected 28 and 29 May.

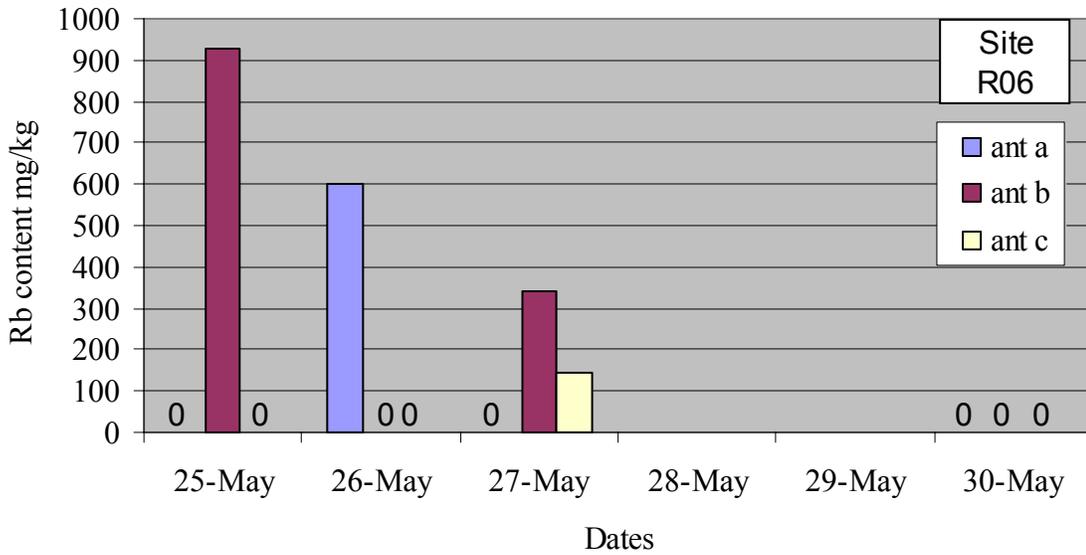


Figure 49. Site R06. Rb content for each of three ants (a, b and c) sampled daily 24 to 30 May 2005 after feeding on 15,000 ppm RbCl. No samples were collected 28 and 29 May.

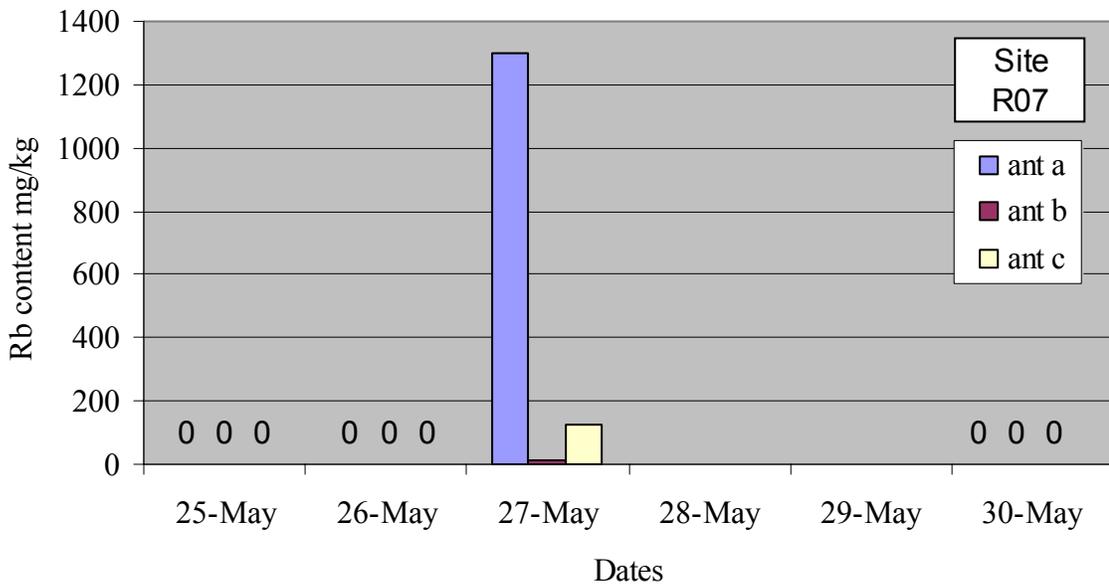


Figure 50. Site R07. Rb content for each of three ants (a, b and c) sampled daily 24 to 30 May 2005 after feeding on 15,000 ppm RbCl. No samples were collected 28 and 29 May.

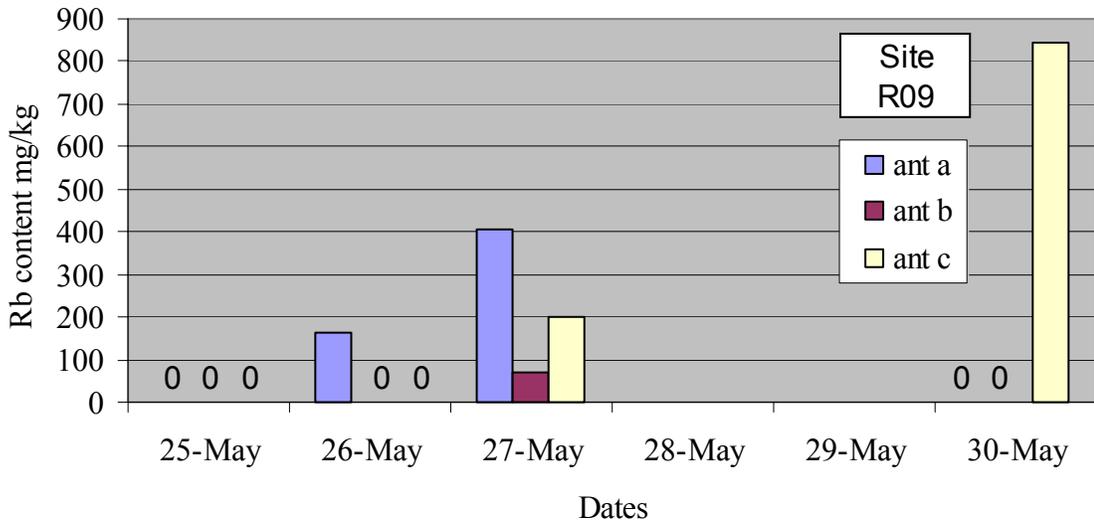


Figure 51. Site R09. Rb content for each of three ants (a, b and c) sampled daily 24 to 30 May 2005 after feeding on 15,000 ppm RbCl. No samples were collected 28 and 29 May.

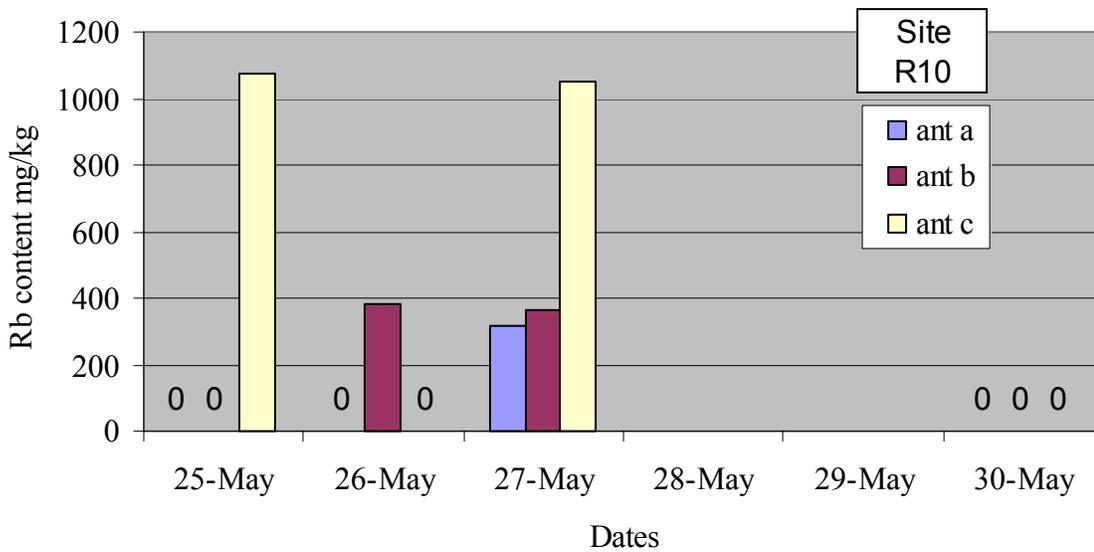


Figure 52. Site R10. Rb content for each of three ants (a, b and c) sampled daily 24 to 30 May 2005 after feeding on 15,000 ppm RbCl. No samples were collected 28 and 29 May.

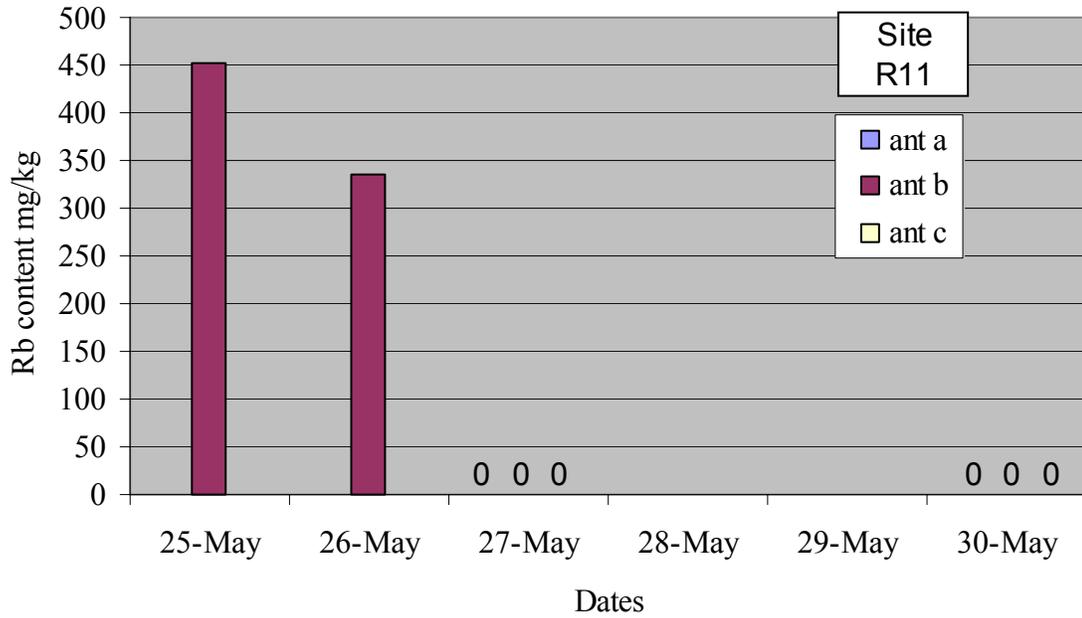


Figure 53. Site R11. Rb content for each of three ants (a, b and c) sampled daily inside the Ant Lab, 24 to 30 May 2005 after feeding on 15,000 ppm RbCl. No samples were collected 28 and 29 May.

This could mean that foragers bringing food back to their nest from a distance greater than ~6 m are not very efficient as foragers because they bring back a reduced quantity of food in their crops. This could also show that the nests involved in this test have foragers returning from nearer, unknown food sources with greater quantities. A larger foraging arena is needed to determine maximum trailing distances.

CHAPTER 7  
COMMENTS AND CONCLUSIONS

**Arboreal Bioassays**

The WFA continues to be a major household pest in south Florida, and its established geographical range is continually expanding throughout Florida and into nearby states. For years I have received a continual flow of desperate communications from affected citizens, not only in Florida, but from a number of countries where the WFA is established, requesting recommendations for WFA treatments. The tests performed over the last few years, described above, give some insight into treatments that should help control this pest species in residential settings, but actually testing the products used in these experiments in such settings in controlled, replicated studies is more difficult and possibly produces less accurate results due to differences in residential structures and surrounding gardens, climatic considerations and homeowner interference. It is therefore suggested that the “arboreal bioassay” procedure outlined in this paper be developed further as a substitute for research involving residential treatments.

Arboreal bioassays in which insect species are isolated in trees by sticky barriers could have further use to permit a deeper understanding of several entomological subjects. That there is a relationship between the Formicidae and honeydew producing Hemiptera has long been known. The use of arboreal bioassays in which species of these two families are isolated in trees could provide valuable information about their interactions. Replicated studies could be performed in which, for example, a systemic insecticide is applied by drench to the tree’s root zone to kill the honeydew producer,

while collecting data to monitor the change in population of the ant species. The use of RbCl as a marker could be added to detect, for example, individual ants that fed from honeydews produced by aphids that ingested Rb in the plant sap.

Arboreal bioassays would not necessarily be limited to tree-nesting ants. Containers containing soil-nesting ant species could be suspended from tree limbs or otherwise attached to trees for research. Other insect families could also be candidates for similar bioassays. Arboreal termites, for example of the genus *Nasutitermes*, might be studied by placing an active nest into a tree and then examining their foraging behaviors, or for control experiments. Although the Asian citrus canker *Xanthomonas axonopodis* pv. *citri* (Xac), does not appear to be spread by insects (Belasque et al. 2005), since the WFA, and numerous other species of ants, especially the Argentine ant, are often encountered foraging in citrus trees, an arboreal bioassay could be done to determine whether ant species are capable of spreading pathogens. This might be tested by moving ants foraging in a canker-infected tree to an uninfected tree using the arboreal bioassay design and then observing whether the disease becomes established.

### **White-Footed Ant Control**

The results of experiments performed both in the laboratory and the field, as described above, pointed toward the efficacy of liquid baits as WFA controls as superior to the use of surface insecticidal applications. The discovery of occult, residual WFA populations after the application of both baits and surface sprays, suggests that further experimentation be done if the desired end result is colony elimination in a treated area. The use of arboreal bioassays could be continued to examine a program integrating surface sprays and baits. One interesting possibility is the development of the NecDew bait, which has been patented by the University of Florida, as an initial population

control, followed by a surface spray application, and then, if any residual ant population is discovered, a return to bait application, as described below.

The rationale for using a bait before a spray comes from many years of field observations of residential WFA infestations. In a severe infestation, I have observed WFA foraging throughout the exterior residential vegetation. They usually have numerous nesting sites that are sometimes within reach of standard insecticidal surface spray applications but often high in trees. In such a case, the use of liquid baits placed in strategic locations in contact with foraging trails coming from all sectors of the property will provide a toxic dose to a high percentage of the overall WFA population and will cause a noticeable decline in foragers within ~14 days, as seen in our experiments. A follow-up inspection at this time will aid in the identification of remaining viable nests. Spot surface treatments directed at these nests and trails will further reduce the population. An additional application of a liquid bait would then serve to maintain the population at a very low level for a time or could eliminate the population completely.

#### **Rubidium as a Marker for White-Footed Ants**

The results have shown that Rb is an effective marker for WFA. Quantities of Rb can be detected in individual ants, no behavioral changes have been observed after ants imbibe the RbCl solution, including being repelled or killed and the element remains detectible for days: long enough to be useful for field tests, such as foraging studies. Further tests are needed to determine the furthest extent of foraging distances, and the flow of nutrients within colonies. Additional experimental work using a Rb-marked toxic bait could examine the flow of a toxic bait throughout the colony to see if it differs with that of a non-toxic bait.

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## BIOGRAPHICAL SKETCH

John Warner was born in New York City, raised and educated on Long Island, left on a 10-speed bicycle in 1971 and headed west until the Pacific Ocean persuaded him to turn south. He stayed in California for 5 years, completing a B.S. degree in Crop Science at California Polytechnic State University at San Luis Obispo. His next stop was Ecuador as a Bahá'í Pioneer, remaining there for nearly 18 years: first as a Peace Corps volunteer, then selling agricultural chemicals, working on shrimp farms, and teaching English and Literature. He moved to Florida with his Ecuadorian family in 1993 and eventually began working in and studying urban entomology. In 2000 he started Shalom Pest Control, Inc. He completed an M.S. in entomology in 2003 at University of Florida's Fort Lauderdale Research and Education Center doing research on the control of *Technomyrmex albipes*. His hobbies include bicycling and karate.