BAIT INSECTICIDES AND HOT WATER DRENCHES AGAINST THE LITTLE FIRE ANT, *WASMANNIA AUROPUNCTATA* (HYMENOPTERA: FORMICIDAE), INFESTING CONTAINERIZED NURSERY PLANTS

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ABSTRACT

Broadcasts of hydramethylnon, S-methoprene, and metaflumizone baits, metaflumizone spray, and hot water drenching were evaluated for their efficacy against the little fire ant, *Wasmannia auropunctata* (Roger), in potted nursery plants. Two applications one month apart of either a bait containing 0.365% hydramethylnon and 0.25% S-methoprene (Extinguish Plus) or a spray containing 24% metaflumizone (BAS 320 I 240 SC) reduced foraging worker numbers by >97% as compared with untreated controls. The persistence of weather-exposed metaflumizone (0.063%) bait was also evaluated: bait applied fresh or after 7 days of weather exposure resulted in >98% ant mortality; bait exposed to weather for 14 d before application still achieved >90% mortality. *In vitro* submersion of *W. auropunctata* in 45°C water for 10 min resulted in >99% mortality as compared with 28.9% mortality from submersion in ambient temperature water (26°C) for 12 min. When *W. auropunctata* -infested potted plants were drenched with hot water (45.6°C) for 11 min, the number of live ants were reduced by 99.3 and 89.3% in rhapis and fishtail palm, respectively, as compared with control plants drenched with ambient temperature water. The presence of a single *W. auropunctata* worker in a potted plant would fail to meet export requirements; therefore, the chemical baits and sprays and hot water treatment (45.6°C for 11 min) as evaluated in this study would not individually be a sufficient quarantine protocol against little fire ants in containerized nursery plants.

Key Words: ant control, hydramethylnon, metaflumizone, S-methoprene, hot water

RESUMEN

La diseminación de cebos de hidrametilnon, S-metopreno y metaflumizona, el aerosol de metaflumizona y el empapamiento con agua caliente fueron evaluados por su eficacia contra la pequeña hormiga de fuego, *Wasmannia auropunctata* (Roger), en plantas de maceta. Dos aplicaciones de un mes de diferencia ya sea de un cebo que contiene 0.365% y 0.25% hidrametilnon S-metopreno (Apague Plus) o de un aerosol que contiene 24% metaflumizona (BAS 320 E 240 SC) redujó el número de trabajadores forrajeros por> 97% en comparación con los controles no tratados. La persistencia del cebo con metaflumizona (0,063%) expuestos al ambiente se evaluó también: cebo aplicado fresco o después de 7 días de exposición al ambiente resultó en una mortalidad de > 98% de las hormigas; cebos expuestos al ambiente por 14 días antes de su aplicación aún alcanzó la mortalidad del 90% de las hormigas. La inmersión *en vitro* de *W. auropunctata* en agua de 45°C por 10 minutos resultó en una mortalidad de 99% en comparación con una mortalidad de 28.9% por inmersión en agua a la temperatura ambiental (26°C) por 12 min. Cuando las plantas infestadas con *W. auropunctata* en las macetas fueron empapados con agua caliente (45,6°C) por 11 minutos, el número de hormigas vivas se redujeron en un 99,3 y un 89,3% en palmas de *Rhapis* y de cola de pescado, respectivamente, en comparación con las plantas de control empapado con agua a la temperatura ambiental. La presencia de un solo trabajador de *W. auropunctata* en una planta en maceta no cumplirá con los requisitos de exportación, por lo tanto, los cebos químicos y aerosoles y el tratamiento de agua caliente (45,6°C por 11 min) evaluados en este estudio no individualmente sería un protocolo suficiente para la cuarentena contra la pequeña hormiga de fuego en plantas de vivero en recipientes.

The little fire ant *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae) is among the top 100 of the world's worst alien species (Lowe et al. 2000). Native to South America, this tramp ant first increased its range to the warm subtropical regions of Argentina to Mexico, and through much of the Caribbean (Wetterer & Porter 2003). During the past century, invasive populations of *W. auropunctata* have become established in many other areas, including the Galapagos Islands, Africa, Middle East (Israel), Melanesia (New Caledonia, Solomon Islands, Vanuatu), Polynesia (Wallis, Futuna, Tahiti, Tuvalu, Hawai'i), Australia (Cairns), the continental United States (Florida), and Atlantic islands (the Bahamas and Bermuda) (Krushelnycky et al. 2005; Lubin 1984; Mikheyev et al. 2009; Vanderwoude 2007; Vonshak et al. 2010; Wetterer et al. 1999; Wetterer & Porter 2003). The latitudinal range of known outdoor populations of *W. auropunctata* extends from 32°40'S in Argentina to 32° 20'N in Bermuda. *W. auropunctata* is also a greenhouse pest in more temperate regions, such as England and Canada (Wetterer & Porter 2003). More recently, *W. auropunctata* breached the quarantine barrier between Solomon Islands and Papua New Guinea and is causing severe hardship to forest villages in East Sepik Province in Papua New Guinea where it is responsible for productivity declines in subsistence tree crops planted under the rainforest canopy, driving away game and other wildlife and substantially reducing invertebrate richness and abundance (Vanderwoude 2008). *W. auropunctata* was first detected in Hawai'i in 1999 in the Puna district on the island of Hawai'i (Conant & Hirayama 2000). Hawai'i Department of Agriculture attempted to contain the initial infestation; however, the ant had already been inadvertently dispersed by movement of infested potted plants from nurseries. Currently, *W. auropunctata* is widespread in East Hawai'i island (Hawai'i Ant Group 2010), has been confirmed in the Kona district in West Hawai'i island, and has been reported on the islands of Kaua'i and Maui.

The little fire ant has many traits shared with other highly successful and destructive invasive ant species, which include: 1) generalist feeding and nesting habits, 2) superficial nests in manmade and natural cavities not limited to underground, 3) high colony mobility, 4) polygyny (multiple queens in a colony), 5) colony budding, 6) low intraspecific aggression, 7) high interspecific aggression, 8) small physical size, and 9) tending of extrafloral nectaries and homopterans (Brandão & Paiva 1994; Passera 1994). *W. auropunctata*, therefore, has been able to invade diverse habitats, ranging from urban (Fernald 1947) to forest ecosystems, developing 3-dimensional super-colonies that occupy both the ground and arboreal strata (Le Breton et al. 2004).

Ecologically, displaced *W. auropunctata* have disrupted native populations of arthropods by competition, exclusion, or predation (Jourdan 1997; Armbreght 2003; Le Breton et al. 2003; Walsh et al. 2004;Walker 2006). *W. auropunctata* impacts wildlife populations (Jourdan 1997,; Le Breton et al. 2003; Walker 2006) as well as domestic animals including livestock (Theron 2005). Considerable evidence suggests that stings to the eye causes keratopathy (Theron 2005), which inevitably leads to blindness. *W. auropunctata* is a serious agricultural pest (Spencer 1941; Delabie & Cazorla 1991; de Souza et al. 1998, Souza et al. 2008) not only by enhancing hemipterous populations and interfering with beneficial insects introduced for biological control (Fabres & Brown, Jr. 1978) but also posing human health and safety risks (stings, allergic reactions, and burning that may not be immediately attributed to *W. auropunctata*, which due to its small size, often goes undetected) to agricultural workers (Spencer 1941; Conant & Hirayama 2000), impacting employment conditions and available labor force (P. Conant, C. Hirayama, personal communication). Moreover, *W. auropunctata* is a quarantine pest, which hinders exportation of agricultural and floricultural products from Hawai'i and other infested Pacific islands to other states and countries. Additionally, because the little fire ant has a limited distribution in Hawai'i (islands of Hawai'i, Kaua'i, and Maui), intrastate quarantine restricts the movement of plants from infested islands to non-infested islands.

Once established, *W. auropunctata* is extremely difficult to control in all but arid, two-dimensional (ground only) simple ecosystems; *W. auropunctata* was eradicated from ca. 21 ha on Marchena Island in a dryland forest area with an eight-month season of little or no rain on the Galápagos Archipelago using Amdro® (0.88% hydramethylnon) (Causton et al. 2005). As with the red imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae), the most economically feasible and environmentally least damaging treatment for *W. auropunctata* over large areas is the use of the bait toxicant system to minimize quantity of pesticide needed and provide effective colony-level control (Williams 1983). An important characteristic of an effective bait formulation for ants in tropical environments is its ability to persist and remain attractive to ants under wet, humid conditions. Ant baits presently developed for *S. invicta*, such as Amdro®, are formulated as a dry granule, which decomposes quickly (Vander Meer et al. 1982) and cannot be applied immediately prior, during or soon after rainfall. This is problematic in tropical regions, including East Hawai'i, where rainfall averages 300 cm per yr (Souza et al. 2008). Another reason for failed control of *W. auropunctata* in Hawai'i using commercially available ant bait products is due to this tramp ant forming nests in both the arboreal and ground strata; ground-applied baits do not appear to be effective on the arboreal component (Souza et al. 2008). After prolonged (13-17 wks) weekly or bi-weekly applications of granular baits, hydramethylnon (Amdro®) in bait stations and pyriproxyfen (Esteem) by broadcast, ant activity recovered to pre-treatment levels approximately 9 wk after treatments ceased (Souza et al. 2008).

Heat treatments have been effectively used against invertebrate pests including arthropods, gastropods, and platyhelminths (Lurie 1998, Follett & Neven 2006), and more recently a vertebrate, the coqui frog *Eleutherodactylus coqui* (Hara et al. 2010). Previous studies have found temperatures of 40-50°C sufficient to kill target invertebrate, fungal and bacterial organisms without excessively damaging the commodity (Hara et al.1993, Lurie 1998, Gould & McGuire 2000, Tsang et al. 2004). Showering potted plants with hot water at 45°C for 5 min is sufficient to achieve 100% mortality of *E. coqui* adults and eggs (Hara et al. 2010) required to meet quarantine regulations for exportation from Hawai'i, and this heat treatment may be adequate for other quarantine pests, such as *W. auropunctata*.

Two trials were conducted to evaluate bait insecticides other than Amdro® to control *W. auropunctata* within plant nurseries: 1) broadcasts of hydramethylnon, S-methoprene, and metaflumizone baits, and metaflumizone spray were compared for control in potted plants, and 2) the effect of weather-exposure on the efficacy of metaflumizone bait was compared to fresh bait. In a third trial, *W. auropunctata*'s susceptibility to hot water immersion was determined, and efficacy of hot water drenching was evaluated as a disinfestation treatment for potted plants before transport.

Materials and Methods

Efficacy of Several Bait Insecticides on *W. auropunctata*

This study was conducted at a nursery in Kea'au, HI (island of Hawai'i) from February to April 2008 using seedling fishtail palms (15.2 to 30.5 cm tall) growing in 10.2-cm diam. containers. Plants were randomly assigned to one of six treatments: 1) 0.73% hydramethylnon granular bait (Amdro® Pro Fire Ant Bait, BASF Corp., Research Triangle Park, NC), 2) 0.365% hydramethylnon and 0.25% S-methoprene granular bait (Extinguish Plus, Wellmark International, Schaumberg, IL), 3) 0.063% metaflumizone fire ant granular bait (BAS 320 I Fire Ant Bait, BASF Corp.), 4) 0.063% metaflumizone sweet granular bait (BAS 320 I Nuisance Sweet Bait, BASF Corp.), 5) 24% metaflumizone soluble concentrate (BAS 320 I 240 SC, BASF Corp.) applied as a spray, and 6) untreated control. Plants were placed on a table (0.74 m^2 surface area) standing in soapy water to prevent escape or immigration of new ant colonies, and allowed to acclimate for several days before taking pre-treatment ant counts. Treatment replicates (12 plants) were isolated on separate tables; each pot contained a separate colony. Preliminary observations indicated that ants of different colonies foraged together on the table surface but returned to their respective colonies (pots). Sprays were applied to the foliage and table surface with a handheld sprayer at 1870.8 liters/ha output; bait granules were evenly dispersed over the table surface. Applications were at label rates for all granular baits (1.7 kg per ha) and at 2.4x label rate for spray application of metaflumizone (3 ml per liter). Treatments were applied twice one month apart. All treatments were replicated four times. Foraging workers were counted in the plants and on the table surface at 2, 7, 10, 14, 21, and 28 d after the first application, and 2, 7, 10, 14, 17 and 21 d after the second application. Plants were then destructively dissected to determine total number of *W. auropunctata* alive. Based on quarantine standards, a plant was considered infested if at least one live ant was present inside the pot or plant root zone. Data of foraging ants and ants remaining post treatment were log 10 (n+1) transformed; infested plant percentages were arcsine sqrt (p) transformed prior to ANOVA (Minitab 2010).

Effects of Weather-Exposure on Efficacy of Bait Insecticides on *W. auropunctata*

A trial was conducted at the University of Hawai'i at Hilo, College of Agriculture, Forestry and Natural Resource Management (CAFNRM) Agricultural Farm Laboratory near Hilo, HI to determine the effects of weather exposure on the persistence of a granular bait (BAS 320 I Fire Ant Bait, BASF Corp.) with 0.063% metaflumizone against *W. auropunctata*. Laboratory colonies of *W. auropunctata* were initiated in March 2009 from queens, brood, and worker ants collected in bait stations with peanut butter lure at the farm site and by extraction from leaf litter from infested areas of the farm using a Berlese funnel. Colonies were maintained for four months to allow establishment, production of additional reproductive brood, and to ensure no mortality by possible exposure to insecticides before field collection. Colonies were housed in 20.5 W × 34.5 L × 11.0 H cm plastic containers (Iris U.S.A Inc. #050513); inner walls of the container were coated with 1:1 water: fluoropolymer (Insect-A-Slip, Bioquip Inc., Rancho Dominguez, CA) solution, and a thin layer of Tanglefoot Tangle-trap paste (The Tanglefoot Company, Grand Rapids, MI) was applied along the top edges to prevent ants from escaping. A polystyrene petri dish (60 × 15 mm) (Falcon #35-1007, Becton, Dickinson, and Co., Franklin Lakes, NJ) containing a cotton ball moistened with tap water was covered with a lid blackened with a felt marker to provide a dark, humid nesting site. The colonies received a diet of sugar water (1:1), peanut butter, and live grasshoppers. Before and during the trials, the colonies were kept in a room at ambient temperature and relative humidity (average 22.7°C, 79% R.H.) with natural light. Forty-eight hours before the start of the trial, the ants were transferred from laboratory colonies into clean plastic containers prepared to prevent escape, provided the same diet and nesting sites, and were held under the same environmental conditions as previously described. Each treatment consisted of four replicates of colonies containing at least 100 workers, one queen, and a small portion of brood (approximately 0.001g of eggs and pupae). Under field conditions, *W. auropunctata* colonies contain multiple queens; therefore, at least one replicate within each treatment had multiple queens. Food was withheld from 4 d before the introduction of the baits, but water was available throughout the trial. Baits were available to the colonies throughout the entire trial.

Treatments consisted of metaflumizone fire ant granular bait that was 1) fresh deposited, 2) weathered 7 days, or 3) weathered 14 days, 4) fresh deposit of hydramethylnon granular bait (Amdro® Pro Fire Ant Bait) as a bait standard, and 5) an untreated check. Untreated control replicates received peanut butter, cooked egg, honey, grasshoppers, and water. Both weather-exposed and fresh baits were measured volumetrically (20 ml) (approximately 1/3 of the maximum recommended label rate for individual red imported fire ant mounds), into aluminum weigh dishes (50 × 13 mm) placed into each colony. Weather-exposure of metaflumizone bait was achieved by placing bait (7 g per rep) in a single layer on a wire screen (1 × 1 mm mesh) over a 34.3 L × 34.3 W × 7.6 H cm wooden box containing potting media (a layer of potting soil, perlite, and vermiculite mix over a layer of cinder) exposed to the elements on a nursery bench for the prescribed time period. During the 7 d exposure, total rainfall was 6.7 cm with 81% R.H. and max/min temperatures of 27.8/17.8°C (average temperature 22.8°C). During the 14 d exposure, total rainfall was 15.5 cm with 77% R.H. and max/min temperatures of 26.7/18.3°C (average temperature 22.5°C). Intermittent sunshine alternated with full cloud cover during the 2-wk exposure period.

Observations for mortality were recorded daily for the first three days, then at 3-d intervals over 3 wk. Digital photographs were taken of each container between 0800 and 0900 h on the days of observation, and ant counts were conducted on these photos enlarged on a computer monitor. Percent mortality of queens and workers relative to initial populations were corrected using Abbott's formula, arcsine transformed, and then compared using ANOVA and Tukey's HSD (Minitab 2010).

In vitro Hot Water Tolerance of *W. auropunctata*

An *in vitro* study was conducted at Waiakea Agricultural Research Station in Hilo, HI to determine the lowest hot water temperature and shortest treatment duration that would be lethal to *W. auropunctata* using a hot water immersion system previously described by Hara et al. (1993). Worker ants were placed into modified polystyrene petri dishes (150 × 20 mm) sealed along the circumference with masking tape (Shurtape Technologies, Inc., Hickory, NC). The cover and bottom of each dish was modified with a 9-cm diam. hole at the center, which were covered with silk organza (74 µm pore size) sealed with hot glue. This modification allowed the exchange of hot water through the petri dish while preventing ant escape. The dishes were submerged in hot water (45°C) for either 5 min or 10 min, followed by 2 min dip in ambient temperature water (26°C). Treated control replicates were submerged in ambient temperature water for 12 min (equivalent to the duration of the longest hot water treatment evaluated). There were four or five replicates (300 to 700 worker ants each) per treatment; the trial was repeated twice. After treatment, the petri dishes were immediately blotted with paper towels and left to dry overnight. Ant counts were taken 24 h after treatment with the aid of a dissecting microscope. Dead and alive ant counts after hot water (45°C for 5 or 10 min) or ambient temperature immersion (26°C for 12 min) were subjected to chi-square analysis (Minitab 2010).

Efficacy of Hot Water Drenching to Disinfest Potted Plants of *W. auropunctata*

Hot water drench as a *W. auropunctata* disinfestation treatment of potted plants was evaluated by using naturally-infested fishtail palm *Caryota mitis* Lour. and rhapis palm *Rhapis excelsa* (Thunb.) in plastic pots (7.6 liter capacity, 15.2 cm diameter) in media composed of potting soil and volcanic cinder. Each pot was placed on top of an overturned saucer (15.2 cm diameter, 5.1 cm deep) inside a larger saucer (25.4 cm diameter, 5.1 cm deep) creating a moat that was filled with soapy water to prevent escape or immigration of new ant colonies. Infested palms were randomly assigned (3 replicates per species per treatment) to one of two treatments: 1) hot water drench at 45.6°C for 11 min followed by ambient temperature water drench (26°C) for 2 min, or 2) treated control (ambient temperature water drench for 13 min). Each pot was flooded with water via a drenching system described by Arcinas et al. (2004) allowing water to flow freely through the pot for the prescribed duration. Temperature of the water was monitored with a handheld digital thermometer (Fluke 52 Series II, Fluke Corporation, Everett, WA) at the point of entry into the potting media. Pre-treatment observations indicated that each plant was heavily infested with *W. auropunctata* (queens, workers, brood). At 2, 5, and 9 days after treatment, two wooden disposable chopsticks (each 20.3 cm length, 0.6 cm diameter) lightly smeared with peanut butter were placed in each pot for 45 min, then collected and sealed in plastic bags for counting under a dissecting microscope. At 14 days after treatment, each palm plant was removed from its pot and visually inspected for live ants. Live ant counts at 2, 5, 9, and 14 DAT among treated and untreated palm plants were analyzed by one-way ANOVA (Minitab 2010).

Results and Discussion

Efficacy of Bait Insecticides on *W. auropunctata*

Before treatment, numbers of foraging worker ants (mean ± SE = 41.1 ± 4.1) did not differ between infested plants assigned to treatments. After the initial treatment application, foraging behavior was altered within 2 DAT, and treatment differences were detected by 10 DAT (Table 1). While all treatments reduced the initial number of foraging ants after both the first and second applications, applications of metaflumizone spray or bait containing both hydramethylnon and S-methoprene (Extinguish Plus) resulted in the greatest and most sustained reduction. The metaflumizone fire ant bait reduced ($P < 0.01$) the number of foraging ants 7 to 10 d after the second application as compared with the untreated check, but its efficacy began to subside by 14 DAT. Amdro® Pro bait tended to suppress the average number of live ants throughout the study but levels were lower ($P < 0.01$) than the untreated control only once, at 10 d after the second application. Amdro® Pro's lower efficacy may be due to its higher level of active ingredient (0.73% hydramethylnon) as compared with Extinguish Plus (0.365% hydramethylnon), or the latter's additional active ingredient, S-methoprene. Workers may have detected the higher level of toxicant and avoided recruiting more workers, similar to observations by Drees et al. (1998) of *S. invicta* exposed to bait containing 1% hydramethylnon, a level at which the toxicant is fast-acting and subsequently avoided by workers. Hooper-Bui & Rust (2000) observed a dramatic reduction in number of Argentine ant *Linepithema humile* (Mayr) (Hymenoptera: Formicidae) workers with much lower levels of hydramethylnon: 60 to 100% mortality was achieved by 24-h exposure to 0.025 to 0.1% hydramethylnon or continuous exposure (14-day) to 0.001 to 0.05% hydramethylnon in solution with sugar water.

At the conclusion of the study (7 wk after the first application), only the granular bait with a combination of hydramethylnon and S-methoprene (Extinguish Plus) and metaflumizone applied as a spray (BAS 320I 240 SC) resulted in less ($P < 0.05$) live worker ants and infested plants (%) than the untreated check (Table 2). Nearly all of the plants treated with the metaflumizone sweet bait were infested with *W. auropunctata*, with mean number of ants as high as the untreated check. While more than 90% of plants treated with Amdro® Pro or metaflumizone fire ant bait were "infested" with at least one live worker ant, the average number of ants in the treated plants were reduced by >50% as compared with the untreated check.

As a quarantine treatment, which requires 100% *W. auropunctata* control, two applications of granular bait containing both hydramethylnon and S-methoprene, or metaflumizone applied as a spray, demonstrated potential to be part of a multiple treatment protocol for infested potted plants, as they completely disinfested 63 to 66% of plants treated, while the remaining infested plants averaged only 14 to 29 live worker ants per plant. All of the untreated plants (100%) were infested with >400 live workers and at least one queen. Complete eradication of *W. auropunctata* with insecticidal baits, granules, and sprays may be accomplished by modifying application frequency and/or combining these bait treatments with an effective granular or liquid drench insecticide, such as bifenthrin (K. Onuma, personal communication).

Effects of Weather-Exposure on Efficacy of Bait Insecticides on *W. auropunctata*

Mortality of worker ants offered fresh or 7 day exposed (DE) metaflumizone fire ant granular bait reached 91.2% and 71.0%, respectively, by 3 days after treatment (DAT), and persisted throughout the trial period, rising to 99.7% and 98.6%, respectively, by 20 DAT (Fig. 1). Mortality of worker ants offered fresh hydramethylnon bait or 14 DE metaflumizone bait rose steadily for the duration of the trial achieving 84.5% and 90.6% by 20 DAT ($P < 0.01$). For all three metaflumizone bait treatments, foraging by workers ceased at 7 DAT. Among the colonies offered fresh hydramethylnon bait, the number of ants observed foraging on the bait declined by 6 DAT; at 1 DAT, queens, pupae, and brood were relocated from the nest, which contained bait granules carried by worker ants, possibly indicating potential bait avoidance. The colony was restored back to the nest a day later, possibly due to reduced potency of hydramethylnon from exposure to sunlight during the initial 24 h of observation (Vander Meer et al. 1982). As with many other ant bait studies, food was withheld for four days to ensure ingestion of each treatment in sufficient quantities to cause mortality; however, this method may not be ideal in determining bait attraction as the level of starvation may have caused consumption of less-preferred baits.

TABLE 1. MEAN NUMBER OF FORAGING *WASMANNIA AUROPUNCTATA* WORKERS OBSERVED FOLLOWING APPLICATION OF EITHER BAIT OR BROADCAST INSECTICIDES.

Treatment/ Formulation	Active ingredient	Rate amount per ha	2 DAT	7 DAT	10 DAT	14 DAT	21 DAT	28 DAT
First application: February 4, 2008								
Amdro®Pro Bait G	0.73% hydramethylnon	1.7 kg	5.8 a	18.4 a	17.7 ab	18.2 ab	15.0 abc	18.8 ab
BAS 320 I Fire Ant Bait G	0.063% metaflumizone	1.7 kg	6.3 a	6.3 a	15.8 ab	15.6 ab	24.6 ab	24.7 ab
BAS 320 I Nuisance Sweet Bait G	0.063% metaflumizone	1.7 kg	21.8 a	25.5 a	27.6 ab	26.2 ab	44.7 a	44.7 a
Extinguish Plus Bait G	0.365% hydramethylnon and 0.25% S-methoprene	1.7 kg	12.3 a	9.6 a	5.2 b	8.0 b	2.5 c	3.4 b
BAS 320 I 240 SC – Spray	24% metaflumizone	300 ml/100 L	2.2 a	5.0 a	5.4 b	5.6 b	5.3 bc	5.2 b
Untreated check	—	—	40.8 a	42.2 a	93.3 a	69.3 a	63.2 a	47.8 a
Second application: March 5, 2008								
Amdro® Pro Bait G	0.73% hydramethylnon	1.7 kg	6.8 abc	18.3 ab	10.1 b	13.6 ab	11.5 ab	10.8 abc
BAS 320 I Fire Ant Bait G	0.063% metaflumizone	1.7 kg	11.2 ab	14.9 bc	10.7 b	19.8 a	31.1 a	29.7 ab
BAS 320 I Nuisance Sweet Bait G	0.063% metaflumizone	1.7 kg	18.5 a	26.2ab	34.4 ab	29.5 a	59.8 a	41.6 a
Extinguish Plus Bait G	0.365% hydramethylnon and 0.25% S-methoprene	1.7 kg	0.4 c	2.2 cd	0.6 c	1.7 bc	2.4 b	3.1 bc
BAS 320 I 240 SC – Spray	24% metaflumizone	300 ml/100 L	0.9 bc	1.9d	0.0 c	0.6 c	1.5 b	0.4 c
Untreated check	—	—	43.9 a	52.2a	50.1 a	55.9 a	65.7 a	74.1 a

For each application date, means within a column followed by the same letter are not significantly different ($P < 0.05$) by Tukey's HSD test.

TABLE 2. NUMBER OF LIVE *W. AUROPUNCTATA* WORKERS REMAINING AND INFESTATION RATES OF POTTED PALMS (21 DAYS AFTER SECOND TREATMENT APPLICATION).

Treatment / Formulation	Active ingredient	Rate amt/ha	Mean ants remaining, N*	Infested plants, %
Amdro® Pro Bait G	0.73% hydramethylnon	1.7 kg	184.8 ab	90.6 a
BAS 320 I Fire Ant Bait G	0.063% metaflumizone	1.7 kg	192.6 ab	93.3 a
BAS 320 I Nuisance Sweet Bait G	0.063% metaflumizone	1.7 kg	388.9 a	99.2 a
Extinguish Plus Bait G	0.365% hydramethylnon and 0.25% S-methoprene	1.7 kg	28.8 bc	36.4 b
BAS 320 I 240 SC—spray	24% metaflumizone	300 ml/100 L	13.5 c	33.4 b
Untreated check	—	—	407.6 a	100.0 a

abc Means in a column followed by different letters were different ($P < 0.01$).

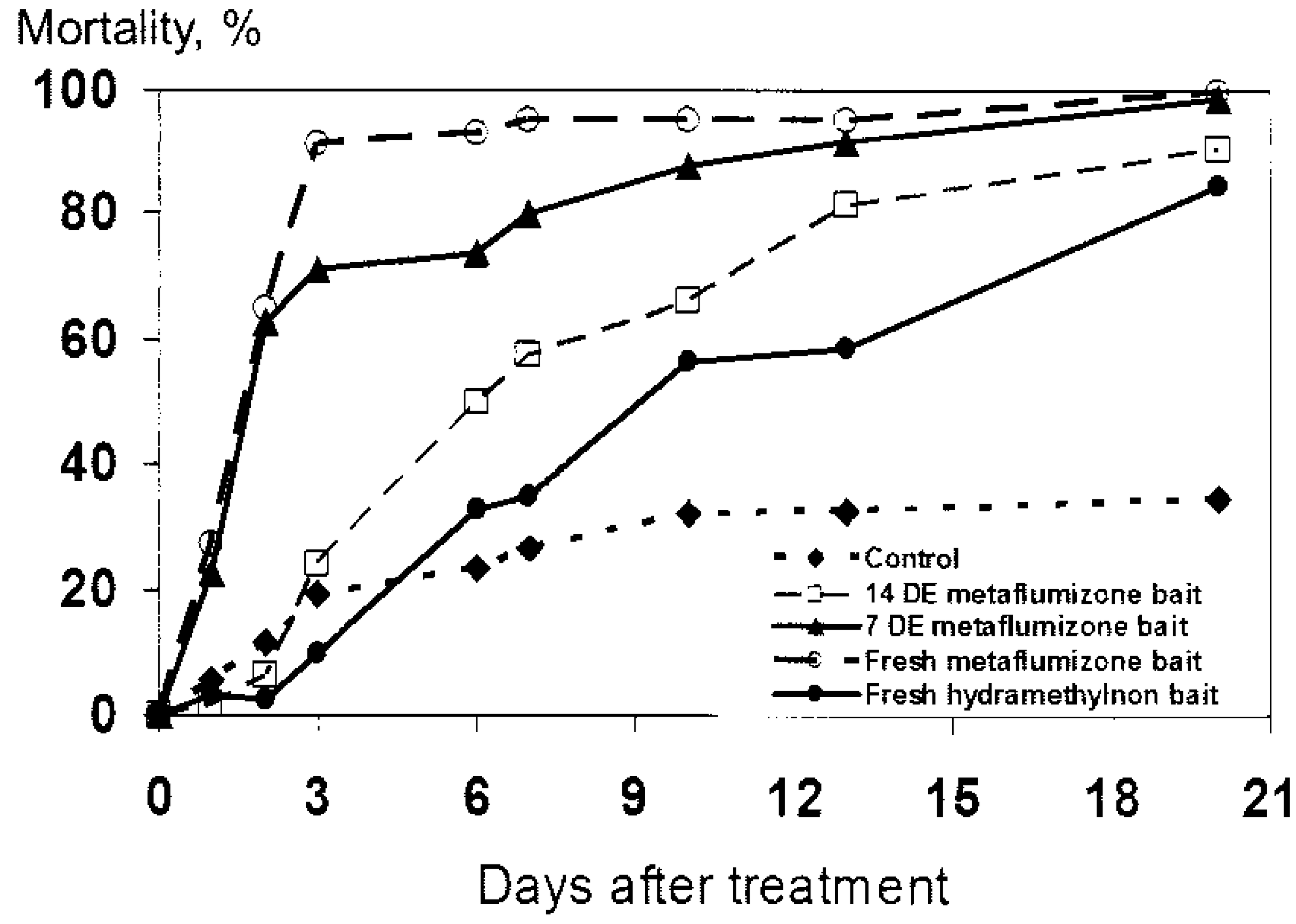

Fig. 1. Efficacy of fresh and weather-exposed bait insecticides on *Wasmannia auropunctata*.

Average mortality rates of queens were higher among colonies offered fresh (77.8%, n = 9) or 7 DE metaflumizone bait (80.0%, n = 5) than other treatments (62.5%, n = 8 and 44.4%, n = 9 for 14 DE metaflumizone and fresh hydramethylnon baits, respectively). Replacement queens were observed among the 14 DE metaflumizone treatment but all died within 20 DAT. Replacement queens observed among the hydramethylnon treatment survived past the 20 DAT observation period, which was consistent with findings reported by Hooper-Bui & Rust (2000) with *L. humile* and southern fire ants *Solenopsis xyloni* (McCook) (Hymenoptera: Formicidae) where lethal doses of hydramethylnon to workers were not transferred to queens in laboratory colonies. There was no queen mortality (n = 5) or replacement queens observed in the control treatment for the duration of the trial. Larger trials with more queens are necessary for a conclusive study on effects of bait insecticides on replacement queens.

In vitro Hot Water Tolerance of *W. auropunctata*

In vitro hot water dipping at 45°C for 5 min killed only 74% of the ants compared with 99.95% mortality for 10 min (Fig. 2); therefore, the shorter treatment duration was not evaluated in Trial 2. In both trials, hot water dipping for 10 min resulted in nearly 100% mortality. Most of the ants (71.2%) in the treated control were able to survive 12 min. under ambient temperature water, indicating that hot water, and not submersion in ambient temperature water alone, was efficacious ($\chi^2(1, N = 5{,}455) = 598.534, p = 0.000$).

Efficacy of Hot Water Drenching to Disinfest Potted Plants of *W. auropunctata*

After potted palms were drenched with hot water (45.6°C) for 11 min, no ants were attracted to peanut butter baiting at 2, 5 or 9 DAT as com-

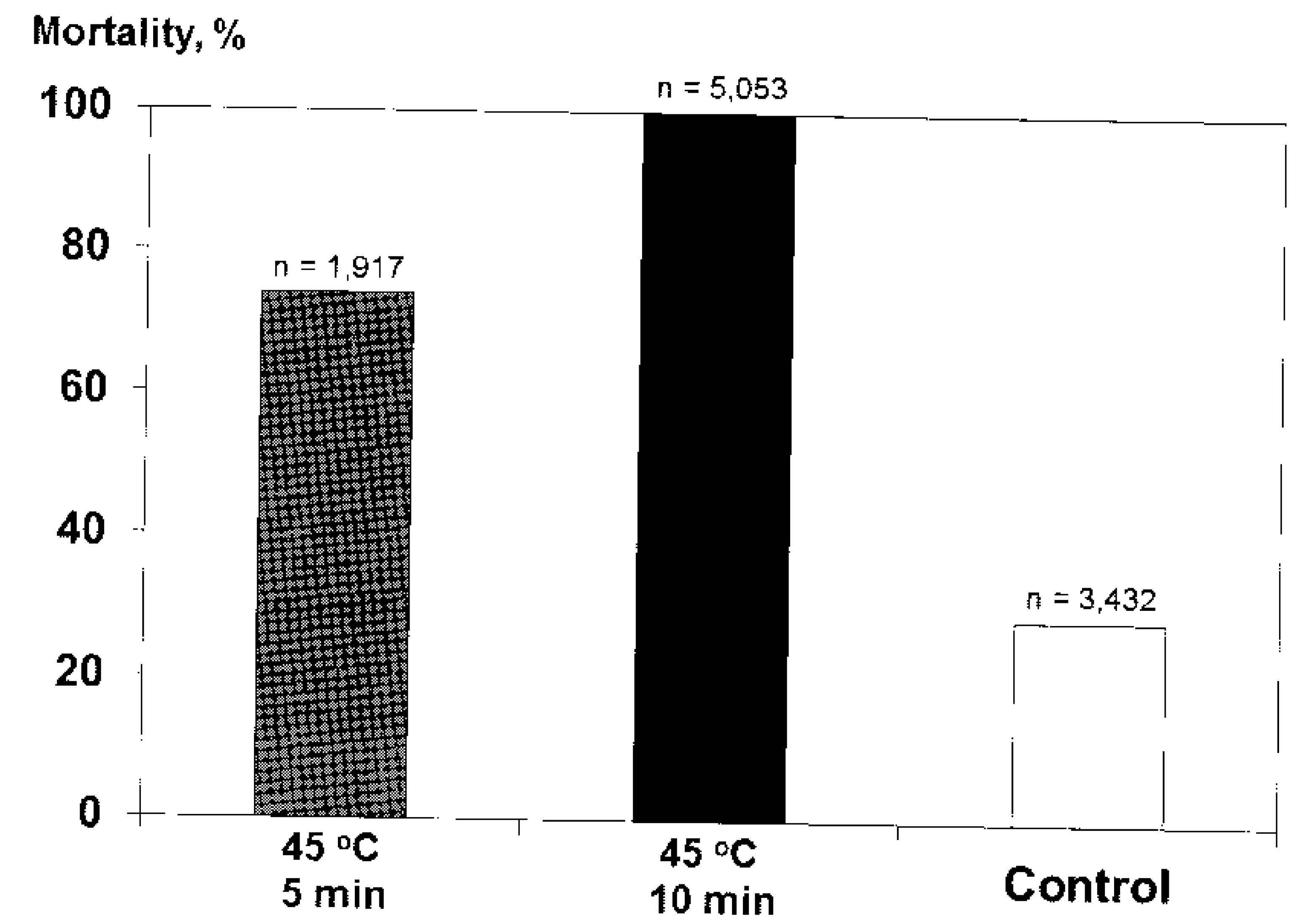

Fig. 2. Efficacy of hot water (45°C) for 5 or 10 minutes on little fire ant workers, *Wasmannia auropunctata*.

pared with an average of 19.8 and 34.6 ants per pot among untreated rhapis and fishtail palms, respectively (Fig. 3). Live worker numbers after hot water drenching were reduced ($P < 0.05$) by 99.3 and 89.3% in rhapis and fishtail palm, respectively, as compared with the control drenched with ambient temperature water, indicating that heat was the lethal factor and not asphyxiation by drowning. At 14 DAT, ant counts were taken by dissecting each plant, resulting in total colony, and therefore, higher, counts; 100% of the untreated palms were infested with *W. auropunctata* (average of 35.3 and 74.3 ants per pot for rhapis and fishtail palms, respectively). All hot water-treated rhapis palms were ant-free; however live *W. auropunctata* workers were recovered from all replicates of hot-water treated fishtail palms (average of 19 ants per pot). When the plants were taken out of their pots for inspection at 14 DAT, the fishtail palms were found to be severely root-bound, which may have affected the extent of heat conduction between hot water, potting media, and ants, possibly accounting for the lower ant mortality rate observed in these palms. In addition, new workers may have hatched during the 14 d observation period.

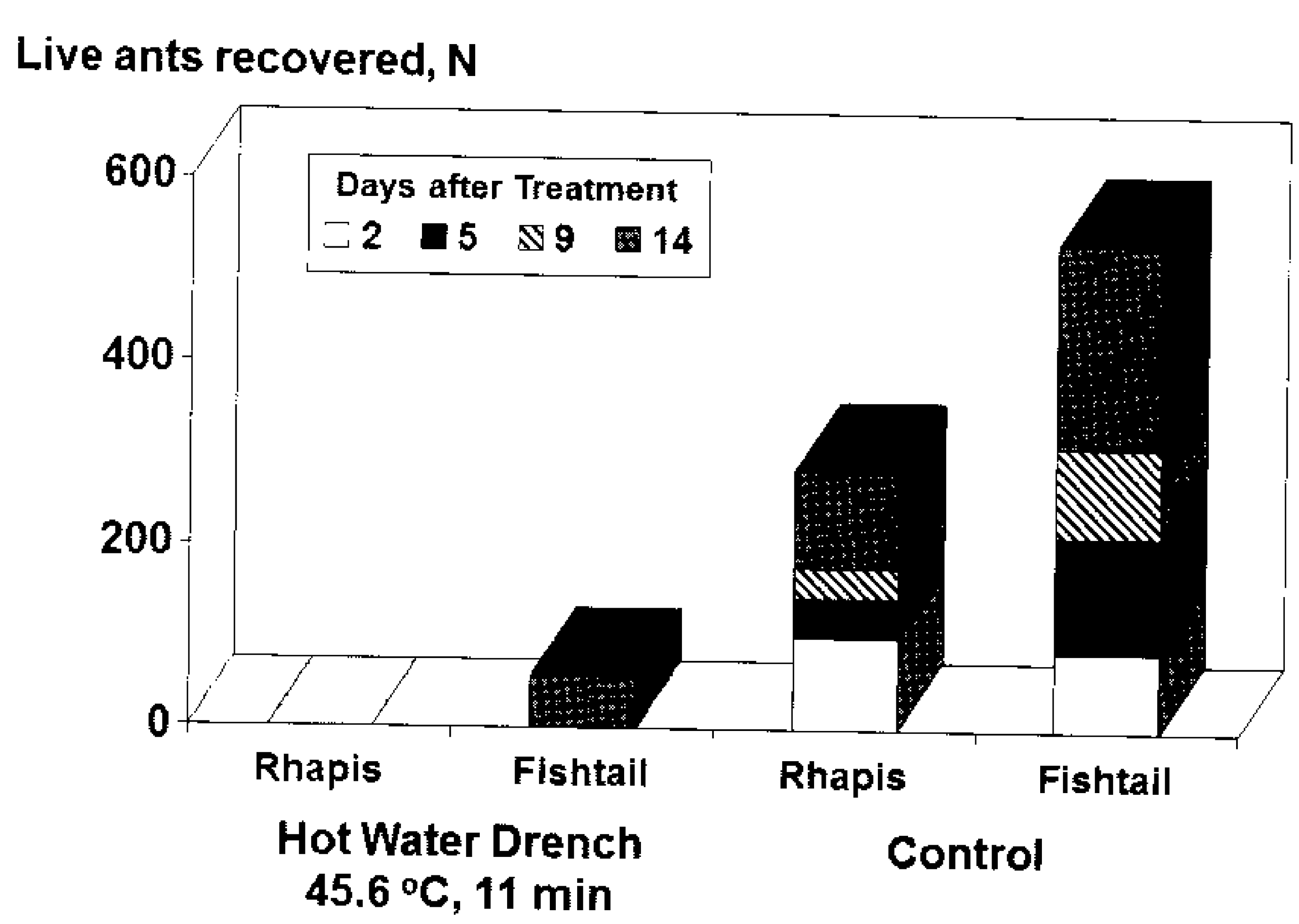

Fig. 3. Efficacy of hot water drenching (45.6°C, 11 min) on *Wasmannia auropunctata* in potted palm plants.

Hot water disinfestation is effective against many vertebrate and invertebrate quarantine pests of nursery plants, and is safe for many heat-tolerant plant species (Hara et al. 2010). Hot water treatment at 45°C for 10 min resulted in >99% mortality of *W. auropunctata* both *in vitro* and in infested potted plants; however, in order to meet quarantine requirements and consistently achieve 100% mortality, drenching at a higher temperature or for longer duration is necessary to adequately treat plants that may be root-bound or in media that does not allow adequate heat conduction. Francke et al. (1985) reported that temperature tolerance (LD95) for four fire ant species, *Solenopsis aurea* Wheeler, *S. geminata* (Fabricus), *S. invicta* Buren, and *S. xyloni* McCook, previously acclimated for 2 wk at 22°C under similar conditions as this study, were reported in the range of 43.8-45.7°C (1 h exposure). Upper lethal temperature limit of desert honey ant workers, *Myrmecocystus* spp., was determined to be between 40-45°C (2 h exposure) (Kay & Whitford 1978). Many ornamental plants, flowers, and foliage can tolerate hot water disinfestation treatments. No detrimental effects are observed when bamboo or Reed palms *Chamaedorea seifrizii* Burret and fishtail palms *Caryota mitis* Lour. are subjected to continuous hot-water drenching of roots and potting media for burrowing nematode *Radopholus similis* at 50°C for up to 20 min followed by immediate cooling in ambient temperature water (Tsang et al. 2003); certain *Anthurium* cultivars exhibit no damage from hot water drenching at 50°C for up to 15 min (Tsang et al. 2004). Treatment of the entire plant by hot water shower for pests that infest plant parts other than roots and potting media is also tolerated by a variety of plant species; *Chrysanthemum* spp., bromeliads *Guzmania* spp. and *Vriesea* spp. can withstand hot water at 47°C for up to 10 min or 45°C for up to 15 min (Hara et al. 2010).

Preventing reinfestation by *W. auropunctata* in nurseries necessitates control of arboreal and terrestrial recolonization on nursery grounds and immediate surroundings. In this study, two applications of granular bait containing both hydramethylnon and S-methoprene (Extinguish Plus), or metaflumizone (24%) applied as a spray one month apart, reduced *W. auropunctata* foraging worker numbers by >97% as compared with untreated controls. Metaflumizone-based (0.063%) fire ant bait achieved >98% mortality for up to 7 days after weather exposure; the active ingredient was still >90% effective after being weather-exposed for 14 d. Development of paste or gel bait formulations and means of application along tree trunks and into tree canopies are needed for *W. auropunctata* in arboreal colonies (Souza et al., 2008, Vanderwoude & Nadeau, 2009).

ACKNOWLEDGMENTS

The authors thank Kris Aoki and Darsen Aoki, University of Hawai'i at Manoa, College of Tropical Agriculture and Human Resources, for their assistance in conducting the trials.

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