Palatability of baits containing (S)-methoprene to *Wasmannia auropunctata* (Hymenoptera: Formicidae)

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Abstract

Wasmannia auropunctata Roger (Hymenoptera: Formicidae), little fire ant, is recognized as a serious pest ant species that affects agriculture, homes, gardens, and natural ecosystems in Hawaii, USA, and elsewhere. Anecdotal evidence suggests that insecticidal baits containing (S)-methoprene are not effective against this species. We examined whether *W. auropunctata* is repelled by bait formulations containing this compound and whether the addition of torula yeast (*Candida utilis* Lodder; Saccharomycetales: Saccharomycetaceae) increased palatability of these baits. *Wasmannia auropunctata* was found to be repelled by (S)-methoprene concentrations as low as 0.25% regardless of formulation. The addition of torula yeast (3% by weight) significantly increased worker recruitment to baits with and without (S)-methoprene. Our results indicate bait formulations using (S)-methoprene are likely to offer poor efficacy against *Wasmannia auropunctata* without the addition of a feeding stimulant such as torula yeast due to repellency of the active ingredient

Key Words: little fire ant; Tango™; protein adjuvant; recruitment

Resumen

Wasmannia auropunctata Roger (Hymenoptera: Formicidae), hormiga pequeña de fuego, se reconoce como una especie plaga de hormiga plaga que afecta seria a la agricultura, los hogares, los jardines y los ecosistemas naturales en Hawai y en otras partes. La evidencia anecdótica sugiere que los cebos insecticidas que contienen metopreno-(S) no son eficaces contra esta especie. Examinamos si *W. auropunctata* es repelido por formulaciones de cebo que contienen este compuesto y si la adición de levadura de torula (*Candida utilis* Lodder; Saccharomycetales: Saccharomycetaceae) aumenta la palatabilidad de estos cebos. Se encontró que *Wasmannia auropunctata* es repelido por concentraciones tan bajas como 0.25% de metopreno-(S) independientemente de la formulación. La adición de la levadura de torula (3% en peso) aumentó significativamente el reclutamiento trabajadores para cebos con y sin metopreno-(S). Nuestros resultados indican que las formulaciones de cebo usando metopreno-(S) son propensos a ofrecer una pobre eficacia contra *Wasmannia auropunctata,* si no se le adiciona de un estimulante de la alimentación tales como la levadura de torula debido a la repelencia del ingrediente activo.

Palabras Clave: hormiga de fuego pequeña; Tango ™; adyuvantes de proteínas; reclutamiento

Wasmannia auropunctata Roger (Hymenoptera: Formicidae) is among 5 of the most damaging invasive ant species in the world and is widespread throughout the tropics and subtropics, especially throughout the Pacific (Lowe et al. 2000; Holway et al. 2002; Wetterer & Porter 2003). The workers are small, measuring only 1.5 mm in length. However, the sting from this tiny pest causes severe burning sensations, itchy welts, and other symptoms often lasting a week or longer (Spencer 1941; Fabres & Brown Jr 1978; Wetterer & Porter 2003; Taniguchi 2008). The ants infest a wide range of habitats from urban structures to agriculture and forest ecosystems. Unlike many other pest ant species, *W. auropunctata* does not build noticeable nest mounds but creates shallow, sprawling, 3-dimensional "supercolonies" within the leaf litter, vegetation, and tree canopies (Spencer 1941; de Souza et al. 1998; Wetterer & Porter 2003; Le Breton et al. 2004; Vanderwoude & Nadeau 2009). Conservative estimates suggest population densities can be as high as 20,000 workers per square meter in Hawaii (Souza et al. 2008). Worker-to-queen ratios of 385–549: 1 have been recorded (Ulloa-Chacon & Cherix 1990), suggesting that 37–52 queens may be present per square meter. These extraordinary population densities partly explain the difficulties associated with controlling this species.

Humans are the primary mode of dispersal of *W. auropunctata*, a "tramp ant" species, through transportation to new locations in infested potted plants, plant material, produce, or soil for agricultural, industrial, or domestic purposes. Due to the small size and cryptic nature of these ants, home and property owners are often unaware of infestations until ant populations have grown and spread throughout an entire property. Although primarily an outdoor pest, *W. auropunctata* will infest structures and houses when no suitable outdoor habitat is available or if infested plants and materials are brought indoors. Private property owners often feel overwhelmed when managing *W. auropunctata* using current control methods (M. Montgomery, pers. obs.)

Control of invasive ants often involves the use of persistent insecticide sprays, barriers, mound treatments, insecticidal baits (Williams 1994), or a combination of these methods. Baits offer significant advantages over broadcast applications of persistent insecticides (Wil-

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liams et al. 2001), including lower overall insecticide use and reduced non-target impacts (Williams 1983). Baits utilize ant social behaviors of foraging, recruitment, and stomodeal trophallaxis, the mouth-to-mouth transfer of food or excretions, to direct toxicants to nest mates, and most importantly, the queen or queens of a colony. Exploiting the natural behaviors of ants is an efficient management strategy that potentially lowers pesticide and labor costs (Williams 1983; Klotz et al. 2003; Tollerup et al. 2004).

An effective bait formulation is comprised of an attractant (the bait matrix), a toxicant (the active ingredient), and a carrier to facilitate application. Candidate toxicants undergo rigorous testing and must demonstrate specific properties including delayed mortality, non-repellency at high concentrations, and efficacy when diluted by trophallaxis (Williams 1983; Rust et al. 2000; Braness 2002; Tollerup et al. 2004). However, few active ingredients exhibit all of the necessary traits (Levy et al. 1973; Williams 1983).

The 2nd component of an effective ant bait, the attractant, is often species specific, or specific to a sub-group of ants that share feeding preferences. For example, many granular baits marketed for "fire ant control" are formulated using a defatted corn grit carrier impregnated with soya oil as the attractant, and 1 or more active ingredients (Kidd et al. 1985; Williams et al. 2001). Product labels may indicate effective control for a range of ant species, although much of the initial testing may have been conducted using 1 or only a few model ant species.

Assessment of the biological and behavioral characteristics of the target pest is as important in bait development as the physical properties of the formulation. For example, red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), prefer to carry granular baits back to the nest rather than consume liquid baits in situ (Kidd et al. 1985). Kidd et al. (1985) hypothesized that *S. invicta* is likely to store bait granules within the nest. Due to their relatively large size, it may be more efficient for *S. invicta* foragers to bring granules back to the nest where they can feed at leisure instead of spending time feeding while being exposed to predators. Underground storage may prevent photodegradation of the active ingredients in the bait and prolong their effectiveness. In contrast, *W. auropunctata* is substantially smaller than *S. invicta*, and mostly feeds on resources in situ, rarely attempting to bring food items back to the nest (M. Montgomery, pers. obs.).

Control of *W. auropunctata* in agricultural production systems in Hawaii is confounded by 3 factors: frequent rain that renders baits unpalatable, an inability to control colonies that nest in vegetation, and an apparent repellency of proprietary baits registered for use on food crops. Firstly, granular baits quickly degrade and become unpalatable when exposed to light and water (Markin & Hill 1971), leaving a narrow window of opportunity for *W. auropunctata* to find the bait, feed, and return to the colony before the bait is rendered ineffective. In wet climates such as the windward coasts of Hawaii, annual rainfall often exceeds 3,200 mm (http://www.prh.noaa.gov/hnl/climate/phto_clim.php), and the window of opportunity may be as short as 42 min (Mallipudi et al. 1986). Any bait applied for control of *W. auropunctata* must therefore be highly attractive to stimulate rapid recruitment and uptake before outdoor exposure renders the bait ineffective or unpalatable.

The 2nd complicating factor is that *W. auropunctata* nests in the upper and mid-story vegetation as well as the ground layer. Typically, ant control using baits involves broadcasting granular baits along the ground, potentially leaving arboreal colonies unaffected (Vanderwoude 2007; Souza et al. 2008; Vanderwoude & Nadeau 2009; Vanderwoude et al. 2010). Souza et al. (2008) observed rapid recolonization within 9 wk of cessation of ground treatments indicating that treatment of arboreal colonies is essential for effective long-term control.

The 3rd issue confounding *W. auropunctata* control is that this species appears to be repelled by certain proprietary "fire ant" baits (Cabral et al. 2012; M. Montgomery, pers. obs.), especially those containing the insect growth regulators pyriproxyfen or (S)-methoprene. Proprietary baits containing these active ingredients are the only formulations permitted for use on many food crops in Hawaii and the continental United States and are therefore vital to agricultural producers. An additional bait formulation therefore is needed for effective control that is not repellent to little fire ants.

Wasmannia auropunctata is a serious pest in Hawaii and presents unique control challenges. This study investigated the utility of a novel gel formulation containing the active ingredient (S)-methoprene (Tango™, Wellmark International, West Schaumburg, Illinois, USA; EPA reg 2724-420), the apparent repellency to *W. auropunctata* of this compound, and potential additives to enhance bait palatability.

Materials and Methods

We investigated the reported repellency of baits containing (S)-methoprene to *W. auropunctata* by observing changes in recruitment over time, as a surrogate measure for bait attractiveness and palatability, to a standard bait matrix mixed with various concentrations of (S)-methoprene. We also tested the effect of adding torula yeast (*C. utilis* Lodder; Saccharomycetales: Saccharomycetaceae) on overall bait recruitment. Both experiments were conducted in a forested area heavily infested with *W. auropunctata* near Hilo on the Island of Hawaii (19°40'N, 155°6'W) during the summer of 2012.

DOSE RESPONSE TO (S)-METHOPRENE

A standard Hawaii Ant Lab (HAL) bait matrix was used for all treatments. The HAL matrix comprised the following ingredients: 1) refined and dewaxed corn oil, (Superb™, Stratas Foods LLC, Memphis, Tennessee, USA) at 350 g/kg; 2) Ziboxan "RD" Rapid Dispersal Xanthan Gum (Deosen Biochemical Ltd, Shandong, China) at 8g/kg; and 3) water at 642 g/kg.

Tango™ (Wellmark International, EPA reg 2724-420; 4.9% [S]-methoprene) was added to this matrix to produce 4 gel formulations with concentrations as follows: 1) control, 0.0%; 2) low dose, 0.25%; 3) medium dose, 0.5%; and 4) high dose, 2.5% (S)-methoprene active ingredient (a.i.) (Table 1). A 5th treatment consisting of a proprietary granular bait that is an effective control product for *S. invicta* and registered for use in and around edible crops in Hawaii, Extinguish® Professional™ (Wellmark International, EPA reg 2724-475) containing 0.5% (S)-methoprene, was included as a standard for comparison.

A randomized block experimental design was chosen for the 1st experiment. Treatments within each block were placed 5 m apart to establish independence and replicated 8 times. Bait stations each consisted of a 4.5 × 4.5 cm laminated card with treatments randomly assigned to bait stations within each block. Recruitment to the bait was measured via high-resolution digital photographs of each plot taken

Table 1. Treatments and concentrations of the active ingredient (S-methoprene used in experiment 1.

Treatment	% (S)-methoprene	Product
1	0	Experimental bait matrix
2	0.25	Experimental bait matrix
3	0.50	Experimental bait matrix
4	2.50	Experimental bait matrix
5	0.50	Extinguish™ Pro™

every 30 min over the course of 3 h. Photographs were examined in the laboratory and ants on bait cards were counted and recorded.

STATISTICAL ANALYSES

All data were analyzed using Minitab statistical software (Minitab version 17, Minitab Ltd., Pennsylvania, USA). Data from treatments 1 through 4 were first analyzed using non-linear regression analysis to identify any correlation trends between ant recruitment and (S)-methoprene concentration. Next, the data from all 5 treatments were analyzed via 2-way ANOVA and Tukey post-hoc test for multiple comparisons between means.

EFFECT OF TORULA YEAST ADJUVANT

The effect of adding torula yeast to the gel formulations was tested by comparing recruitment to HAL gel baits containing combinations of (S)-methoprene 0.25% a.i. and torula yeast (3% by weight of finished bait) in a factorial design. This experiment was laid out as a randomized block design as in experiment 1, differing only in the number of treatments and replications (Table 2). Recruitment to the bait was measured via high-resolution digital photographs of each plot every 30 min over the course of 2 h. Photographs were examined in the laboratory and ants on bait cards were counted and recorded. Data were analyzed using a 2-way ANOVA and Tukey post-hoc test in Minitab statistical software (Minitab version 17, Minitab Ltd.).

Results

DOSE RESPONSE TO (S)-METHOPRENE

The dose response curve for experiment 1 showed a negative exponential correlation between the proportion of (S)-methoprene in the bait and number of ants recorded at bait (Fig. 1). The trend was corroborated by results from the 2-way ANOVA test and Tukey post-hoc test. A significant correlation between treatment and recruitment rate was detected ($F = 129.78$, $P < 0.001$). Recruitment to treatments 1 (0% [S]-methoprene), 2 (0.25% [S]-methoprene), and 4 (2.50% [S]-methoprene) were statistically significant with the greatest recruitment observed at treatments without (S)-methoprene and lowest recruitment observed at treatments with 2.50% (S)-methoprene. No difference in recruitment was detected between treatments 3 (0.5% [S]-methoprene) and 5 (Extinguish® Professional™ standard, 0.5% [S]-methoprene) (Fig. 2). Ant recruitment rates for all treatments remained relatively constant over the course of the 3 h measurement period with no significant difference between recruitment and exposure time detected. During the entire experiment, the high-dose treatment attracted 79% fewer ants than the medium-dose treatment and was the least attractive treatment (means ranging from 18.88 to 40.63 ants per observation).

Table 2. Treatments and concentrations of the active ingredient and the adjuvant used in experiment 2.

Treatment	% (S)-methoprene	% protein adjuvant
Control	0	0
Protein	0	3
(S)-methoprene	0.25	0
Protein + (S)-methoprene	0.25	3

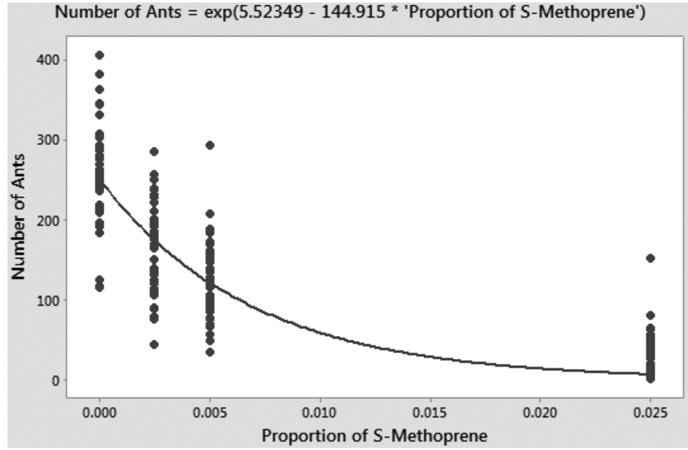

Fig. 1. Non-linear regression curve showing the relationship between ant recruitment and proportion of (S)-methoprene in the Hawaii Ant Lab's novel gel bait.

EFFECT OF TORULA YEAST

There were significant differences in recruitment between treatments with and without (S)-methoprene as well as with and without the protein adjuvant (Fig. 3). Overall, ant recruitment to baits formulated using the product label rate of 0.25% (a.i.) and without protein adjuvant was significantly lower than to baits containing 0% (S)-methoprene ($F = 9.1037$, $P < 0.01$). However, the addition of torula yeast substantially improved recruitment to baits. Mean recruitment to baits with the protein adjuvant was significantly higher than to baits formulated without adjuvant ($F = 22.1801$, $P < 0.001$). Recruitment to bait with (S)-methoprene plus torula yeast was comparable to that of the control with no difference detected during data analysis (Fig. 4).

Discussion

Control measures for *W. auropunctata* have met with limited success throughout the tropics due to a variety of environmental, biological, and behavioral factors. *Wasmannia auropunctata* are repelled by (S)-methoprene baits at concentrations that do not repel some other ant species, as is evident by significantly lower recruitment rates. This sensitivity to baits with even relatively low concentrations of (S)-methoprene has not been reported previously. Our study revealed that the addition of (S)-methoprene significantly reduced recruitment to baits, which may explain reduced efficacy of certain proprietary ant baits against *W.*

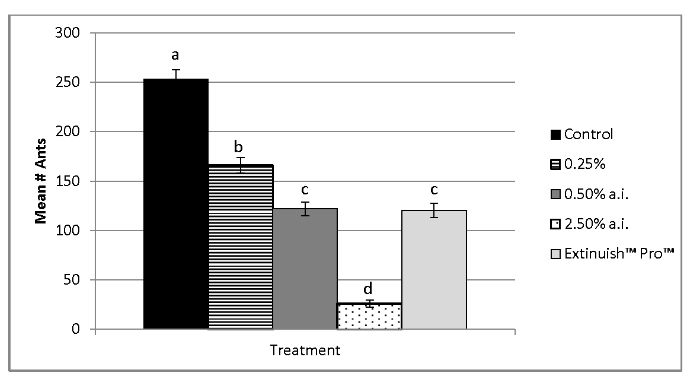

Fig. 2. Mean recruitment of ants to baits containing different concentrations of (S)-methoprene. Treatments with different letters are significantly different ($P < 0.05$).

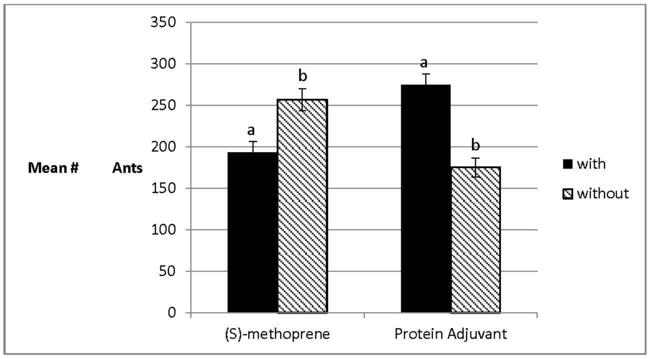

Fig. 3. Differences between all baits with and without (S)-methoprene as well as all baits with and without the adjuvant. Treatments with different letters are significantly different ($P < 0.05$).

auropunctata in Hawaii. Furthermore, we tested a masking agent that effectively increased recruitment to baits containing (S)-methoprene. The proprietary granular bait Extinguish® Professional™ is an effective control product for the red imported fire ant (*S. invicta*) and contains 0.5% (S)-methoprene as the active ingredient. In contrast, the results from our study indicates this rate is moderately repellent to *W. auropunctata* and may explain the reported poor efficacy of Extinguish® Professional™ against *W. auropunctata* in Hawaii. Indeed, the recruitment rate to the standard treatment, Extinguish® Professional™, and that to the medium-dose gel bait treatment containing 0.5% methoprene were comparable, thus corroborating this observation and suggesting that it is the concentration of (S)-methoprene and not the bait formulation that is the repellent factor. Additionally, the labeled concentration for commercially available (S)-methoprene, i.e., Tango™, is 0.25% and even this rate suppressed *W. auropunctata* feeding activity.

Bait efficacy relies on bait consumption and sharing among nestmates via trophallaxis. Therefore efficacy improves with increased recruitment to baits, as it leads to greater consumption by the target species. This is especially so for ants because bait sharing through trophallaxis dilutes the concentration of the active ingredient through the colony. In the case of insect growth regulators, accumulation of the active ingredient in the queen(s) and larval stages is essential to obtain optimum physiological effects. Any bait must therefore be highly attractive and palatable to ensure maximum consumption and effectiveness.

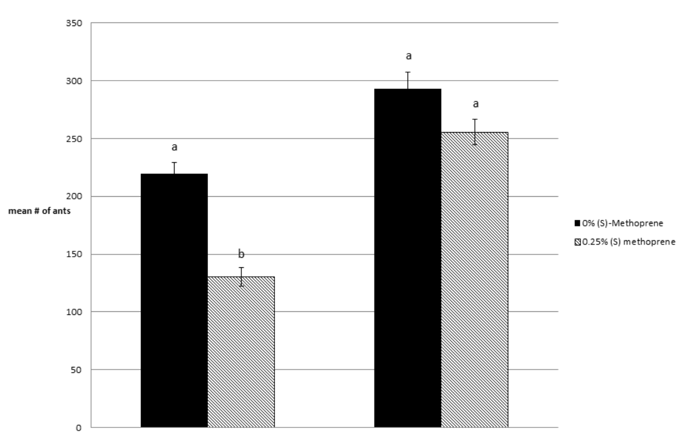

Fig. 4. Effects of addition of 0.25% (S)-methoprene and increase in attractiveness of (S)-methoprene baits by the addition of an adjuvant as a masking agent. Treatments with different letters are significantly different (P$p < 0.05$).

Although *W. auropunctata* did recruit to bait containing the label-recommended dose of 0.25% (S)-methoprene, our results demonstrated overall recruitment was significantly improved with the addition of torula yeast. Our study also demonstrates that the HAL gel matrix (with the addition of torula yeast and (S)-methoprene) is an effective alternative to other baits. The higher recruitment rate at baits containing the protein adjuvant for an extended period of time suggests greater consumption of bait and, in turn, (S)-methoprene, compared with proprietary baits containing the same active ingredient.

The 2 other impediments to control of *W. auropunctata* in Hawaii are the arboreal nesting habit of some colonies and the high rainfall experienced on the windward coasts of the Hawaiian archipelago. Previous efforts to control this species in Hawaii have been hampered by these factors (Souza et al. 2008). However, gel baits incorporating (S)-methoprene can be applied to vegetation where its gelatinous consistency allows it to adhere to leaves and branches. It also appears to be moderately resistant to removal by rainfall, which along with its palatability extends the window of opportunity of recruitment to the bait (M. Montgomery, pers. obs.). Both attributes are essential for an effective control product for *W. auropunctata* in tropical locations.

We provide here evidence supporting observations that classical ant control measures are not suitable for effective control of *W. auropunctata*. When managing pests through baiting, the baits being used must display the specific characteristics of delayed mortality, efficacy at extremely low concentrations, and non-repellency. Products currently available in Hawaii do not meet these requirements for controlling *W. auropunctata*. Our study has developed a bait showing potential as an alternative to the currently available products. Further research will focus on efficacy of this bait and its potential as a viable control method amid a range of land use types rather than mere palatability as was our focus here. Additionally, the bait matrix described in our study may have the potential to be used with a variety of active ingredients previously thought to be repellent, thus unsuitable, to *W. auropunctata*. Further research in this area is also needed.

Acknowledgments

This work forms part of the Ph.D. research by the corresponding author at the University of Canberra, Australia, and is supported by funding from the Hawaii Invasive Species Council.

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