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Repellency and Toxicity of Mint Oil Granules to Red Imported Fire Ants (Hymenoptera: Formicidae)

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ABSTRACT Repellency and toxicity of 2% mint oil granules were evaluated against worker red imported fire ants, Solenopsis invicta Buren, in a series of laboratory and field experiments. In continuous exposure experiments, LT₅₀ values ranged from 1.2 h with 164.8 mg/cm² of 2% mint oil granules to 15.3 h with 1.65 mg/cm² of granules. LT₅₀ values declined exponentially with increasing rate of mint oil granules. Limited exposure to 164.8 mg/cm² mint oil granules resulted in 50% knock down (KD) after 30 min; however, there was no KD at 15 min. Twenty-four hour mortality increased linearly with increasing exposure time. Mean repellency of worker red imported fire ants ranged from 49.2% for 0 mg/cm² (untreated control) of mint oil granules at 30 min to 100% for 147.8 mg/cm² of mint oil granules at 3 h. Repellency increased with increasing milligrams per square centimeter of mint oil granules and exposure time. In field tests, 100% of mounds opened and treated with mint oil granules were abandoned 5 d after treatment and ants had relocated or formed satellite mounds by 2 d after treatment. Unopened mounds treated topically with mint oil granules were not abandoned, but ants formed satellite mounds 2 d after treatment. Mint oil granules could provide another tool for red imported fire ant integrated pest management, particularly in situations in which conventional insecticides would be inappropriate.

KEY WORDS Solenopsis invicta, mint oil, essential oil, repellent

The red imported fire ant, Solenopsis invicta Buren, is an important agricultural, medical, and urban pest throughout most of the southeastern United States as well as in parts of several western states, Mexico, and Puerto Rico (Vinson 1997, Taber 2000). This invasive species has outcompeted and eliminated many native ant species in rural, urban, and agricultural areas (Whitcomb et al. 1972). Management of red imported fire ants is usually accomplished with broadcast or individual mound treatments by using baits or traditional contact insecticides. Individual mound treatments generally provide the most rapid control of colonies (Vogt and Appel 1996); however, mound relocations frequently occur (Francke 1983, Lemke and Kissam 1987). Even though many insecticide products are available for fire ant control, most contain synthetic organic insecticides. Homeowners are increasingly concerned about traditional insecticides and therefore are becoming interested in the use of less toxic or “natural” materials for fire ant control (Drees and Lennon 1998).

Naturally occurring insecticides have been used in pest control for centuries (Ebeling 1971, Coats 1994). Many of these compounds are secondary plant substances (Raven et al. 1992), including alkaloids, quinones, essential oils (such as terpenoids), glycosides, and flavonoids. Monoterpenoids such as d-limonene in citrus and l-menthol and menthone in mint add distinctive aromatic characteristics to plants. These compounds are often used in cosmetics, foods, and pharmacological additives where they provide flavors and fragrances. Monoterpenoids induce a variety of responses by insects. For example, several monoterpenoids (Inazuka 1982, Appel et al. 2001) and cedar oils (Appel and Mack 1989) are repellent to American, Periplaneta americana (L.), and German, Blattella germanica (L.), cockroaches, affect insect growth and development (Karr and Coats 1992, Hink and Fee 1986) or are acutely toxic to insects (Smith 1965, Coyne and Lott 1976, Coats et al. 1991, Rice and Coats 1994, Appel et al. 2001). In mound drench tests with red imported fire ants, citrus oil formulations containing d-limonene were as effective as a conventional insecticide (Vogt et al. 2002). Monoterpenoids are considered neurotoxic because of their speed of action and their effects on neurotransmission (Coats et al. 1991).

The purpose of this study was to determine the repellency and toxicity of mint oil granules against worker red imported fire ants. Toxicity was determined using continuous and limited exposure methods. Repellency was determined using a petri dish choice test.

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Materials and Methods

Insects. Worker red imported fire ants were collected from three monogyne colonies on the Auburn University campus in Lee County, Alabama. Workers and nest material were placed into Teflon emulsion-lined plastic trays (40 by 52 by 12 cm) and held at 25–28°C and 50–65% RH, exposed to a photoperiod of 12:12 (L:D) h, and supplied water, a 10% sucrose solution, and dead crickets ad libitum. Ants were used for the laboratory experiments within 48 h of collection. Ants were transferred from the plastic trays to cups and petri dishes by using brushes and index cards; no anesthesia was used to facilitate handling.

Continuous and Limited Exposure Toxicity Tests. Peanut shell granules containing 2% (wt:wt) mint oil (Earth Care Naturals, Insect Killer Granules, Spectrum Group, St. Louis, MO) were used in the toxicity and repellency experiments. Plastic cups (5.5 oz, Dixie Foodservice, GA-Pacific Corp., Atlanta, GA) with 5.2-cm-diameter bottoms were used for both the continuous and limited exposure toxicity tests. The entire inside wall of each plastic cup was coated with Teßon emulsion to prevent the ants from escaping and to force the ants onto granules placed on the bottom. The emulsion was applied to the plastic cups with a sponge brush and allowed to dry for 24 h under a laboratory hood. For the continuous exposure tests, 10 worker fire ants, of a range of sizes, were transferred from the plastic trays to cups and petri dishes by using brushes and index cards; no anesthesia was used to facilitate handling.

Repeatability Tests. Repellency of mint oil granules was determined in 9-cm-diameter, deep glass petri dishes. The inside vertical wall of each petri dish was coated with Teßon emulsion, contained a short (1.5-cm) length of dental wick wetted with a 10% sucrose solution. Mortality was assessed after 24 h.

Data Analysis. Mortality (LT_{50}) in the continuous exposure trials was analyzed by probit analysis for correlated data (Throne et al. 1995) at each rate because multiple observations were taken on the same individuals. Significant differences in LT_{50} were based on nonoverlap of the 95% confidence intervals (CIs). A three-parameter exponential decay model of the form \( LT_{50} = a \exp(-bx) + c \) was used to quantify the relationship between LT_{50} and rate of mint oil granules in the continuous exposure tests (SigmaPlot 8.0, SPSS 2002). In this equation, LT_{50} is the estimated time (hours) to reach 50% mortality, \( a \) is the estimated augmentation LT_{50} (hours) of an untreated control population (i.e., a population exposed to 0 mg/cm² of mint oil granules), \( b \) is the rate constant, and \( c \) is the...
asymptotic limit of the minimum LT_{50} for a population exposed to 164.81 mg/cm² of mint oil granules. The numerical value of the LT_{50} at 0 mg/cm² is \( a + c \) and defines the \( y \)-intercept of the model. Biologically, \( a \) represents the natural LT_{50} of an untreated control population, and \( c \) represents the minimum LT_{50} necessary to kill at the maximum rate. Speed of penetration, time required to reach the active site(s), and the mechanism of toxicity all contribute to the value of \( c \). This regression model was selected because a linear model was not significant (\( P > 0.1 \)), an exponential model closely resembled a plot of the data, one- and two-parameter exponential models were not significant (\( P > 0.05 \)) but had increasing \( R^2 \) values, and additional variables did not add significantly to the \( P \) or \( R^2 \) values. Analysis of variance (ANOVA) followed by Tukey’s multiple comparison test (SigmaStat 2.03, Jandel Scientific 1997) was used to compare KD and 24-h mortality among limited exposure periods. Regression was also used to relate KD and 24-h mortality to exposure period (SigmaPlot 8.0, SPSS 2002).

Repellency (percentage of live ants in the untreated side of the petri dish) was analyzed using a two-way repeated measures ANOVA followed by Tukey’s multiple comparison tests (SigmaStat 2.03). Fisher exact tests (Sokal and Rohlf 1995, SigmaStat 2.03) were used to compare mint oil granule-treated mounds with the appropriate control mounds in the field test. A significance level of \( P = 0.05 \) was used for all statistical tests.

### Results

**Toxicity.** In the continuous exposure tests, LT_{50} values for worker red imported fire ants ranged from \( \approx 16 \) h for 1.65 mg/cm² of mint oil granules to 1.36 h for 164.81 mg/cm² (Table 1). Homogeneity of response (slope of the log-time probit relationship) was similar (0.22–0.34) between 1.65 and 47.08 mg/cm² but increased to 1.3 at 164.81 mg/cm². There was no control mortality in the continuous exposure test. LT_{50} values declined exponentially with increasing rates of mint oil granules: LT_{50} = 14.64 (±1.03) \( \exp[-0.024 (±0.005) x] \) + 1.17 (±0.94); \( F = 109.15; df = 2, 4; P = 0.0091; R^2 = 0.982; \) residual mean square error = 0.62. This model indicates that mint oil granules are toxic at even relatively low rates (1.65 mg/cm²) and that toxicity increases with increasing rates of granules. The estimated asymptotic limit of the minimum LT_{50} (\( c \) in the exponential equation) overlaps the LT_{50} value for the maximum amount of mint oil granules used in the test (164.81 mg/cm²); experiments indicated that no additional decline in LT_{50} was achieved using greater densities of granules.

As little as 30 min of exposure to mint oil granules resulted in >50% KD. However, there was no KD after 0- or 15-min exposures (Fig. 1). KD increased exponentially with increasing exposure time: \( %KD = 114.13 (±37.84) [1 - \exp(-0.018 (±0.012) x)] \); \( F = 22.93; df = 1, 4; P = 0.05; R^2 = 0.846; \) residual mean square error = 327.26. A 15-min exposure resulted in significant mortality (37.10 ± 6.54%) at 24 h; double that of control mortality (Fig. 1). Twenty-four hour mortality increased linearly with increasing exposure time: \( %mortality = 0.57 (±0.10) x + 23.73 (±5.98); F = 35.05; df = 1, 4; P = 0.0096; R^2 = 0.895; \) residual mean square error = 84.21.

**Repellency.** Mean repellency of worker red imported fire ants ranged from 49.22 ± 5.43% for 0 mg/cm² of mint oil granules at 30 min to 100% for 14.8 and 147.8 mg/cm² of mint oil granules at 3 h (Fig. 2). In the two-way repeated measures ANOVA, repellency differed significantly among mint oil treatments (\( F = 22.71; df = 6, 105; P < 0.001 \)) and time periods (\( F = 28.42; df = 3, 105; P < 0.001 \)). Control (0 mg/cm² of mint oil granules) repellency over the entire 3-h test had a least square mean of 53.02 ± 2.56% and did not differ significantly among time periods. Combining time periods, control repellency was significantly less than all mint oil treatments. Percentage of repellency increased significantly with exposure period for all mint oil treatments (treatment by time interaction: \( F = 2.05; df = 18, 105; P = 0.013 \)) with maximum repellency after 3 h. There were no differences among mint oil containing treatments at 0.5, 2, and 3 h; how-
ever, at 1-h repellency of the 40.9 mg/cm² mint oil treatment was significantly greater than that of the 0.4 and 147.8 mg/cm² treatments.

**Field Tests.** One day after treatment, fire ants abandoned 67% of mounds opened and treated with mint oil granules, significantly more ($P = 0.005$) than control mounds. One hundred percent of the mounds were abandoned by 5 d after treatment (Fig. 3). In comparison, there was no abandonment of any mounds in the other treatments and untreated controls. Application of mint oil granules to opened mounds also induced significant ($P < 0.05$) relocation and formation of satellite mounds. In these, ants abandoned 83% of treated mounds 2 d after treatment and 100% of treated mounds between days 2 and 5 (Fig. 4). Mint oil granules topically applied to mounds did not cause relocation and formation of satellite mounds 1 d after treatment, but ants in 67% of treated mounds relocated and formed satellite mounds at day 2 and 83% at day 4 (Fig. 4). Control mounds had 17% relocation or formation of satellite mounds throughout the test.

**Discussion**

Granules containing 2% mint oil were toxic and repellent to worker red imported fire ants in a variety of laboratory and field tests. Mint oil has relatively low mammalian toxicity (Reynolds 1982, Dreisbach 1983) and is derived from natural plant extracts. It is used commonly in candies, cough drops, flavorings, liqueurs, perfumes, and as topical ointments. Mint oil also volatilizes rapidly and leaves little or no residue when exposed to the air (our unpublished data). These qualities make mint oil an excellent candidate insecticide for the rapid control of insect pests in areas where conventional insecticides are not appropriate or preferred.

Mint oil granules were relatively toxic to worker red imported fire ants in both continuous and limited exposure tests. The cups that held the ants and mint oil granules were exposed to the air to avoid fumigation effects (Appel et al. 2001), and similar LT$_{50}$ results were obtained when cups containing ants and granules were held under a laboratory hood (our unpublished data). Even at the lowest rate of 1.65 mg/cm$^2$, with fewer than 12 1-mm-diameter granules that could be easily avoided by the ants, there was significant mortality. Most ants avoided contact with the granules at all granule densities, indicating contact repellency.

Limited exposure resulted in KD after 30 min and >90% KD after 2 h (Fig. 1). There were similar percentages of KD and 24-h mortality for exposures ≥30 min, but even exposure periods of <30 min that did not result in KD did result in significant 24-h mortality. Therefore, relatively short exposures to mint oil granules can produce delayed mortality without KD dur-
ing the exposure period. Because of the lipophillic nature of \( l \)-menthol and menthone, it is likely that these compounds were absorbed into the cuticular lipids of the fire ants and slowly moved into the hemocoel and nervous system. It is also possible that these compounds were absorbed into the tracheal system.

The mint oil granules were significantly repellent to worker red imported fire ants, with level of repellency increasing with rate of granules and exposure period. Mint oil is also repellent to American and German cockroaches (Inazuaka 1982, Appel et al. 2001) in laboratory experiments with relatively little air movement. However, in outdoor field trials, Held et al. (2003) found that crushed spearmint, Mentha spicata L., did not protect roses from Japanese beetle, Popillia japonica Newman, attack. Similarly, 0.001-1% menthol solutions were not repellent to starved Anopheles gambiae Giles in screen cage feeding trials with human volunteers (Barasa et al. 2002). It is likely that the highly volatile nature of mint oil compounds so reduces the concentration of these compounds in open air that repellent effects could not be detected. It is also possible that insufficient concentrations of mint oil were tested. Appel et al. (2001) found that concentrations of \( \geq 3 \)% mint oil were necessary for toxicity and repellency of cockroaches. Because the worker red imported fire ants were in direct contact or \( \leq 1 \text{cm} \) away from the granules, there was probably a greater concentration of volatilized mint oil compounds than in the air above the ants.

Mint oil vapor has fumigant effects against both American and German cockroaches (Appel et al. 2001) and house flies, Musca domestica L. (Rice and Coats 1994). Even though fumigation and direct mound injection is a rarely used tactic for red imported fire ant control (Drees et al. 2000), the presence of mint oil granules on mounds or sealed into mounds with soil could produce fumigation-induced mortality. We did not observe mound mortality after topical or internal application of mint oil granules, however. Mounds opened, treated with mint oil granules, and resealed, were abandoned rapidly (67\% at day 1 and 100\% at day 5), and 100\% of these mound had relocated or formed satellite mounds 2 d after treatment. Clearly, direct contact with the mint oil granules or vapor induced a repellent effect, namely, mound relocation or satellite mound formation. Topical application of mint oil granules resulted in 83\% of the mounds forming satellite mounds by day 4. Although not statistically different than untreated control mounds, more than twice as many topically treated mounds formed satellites as control mounds. These results indicate that repellent mint oil vapor may penetrate into mounds and provide residual repellency.

Repellents are not generally used for red imported fire ant management. Rather, slow-acting nonrepellent baits or highly toxic conventional insecticides are usually applied as broadcast or individual mound treatments. Repellent effects of insecticide treatments such as mound relocations or formation of satellite mounds are often considered as treatment failures. Use of repellents in potting soil mixtures and in protected areas around homes and other sensitive areas such as nursing homes and playgrounds may provide an additional tactic for fire ant quarantine and management.

The repellency and toxicity of mint oil granules to red imported fire ants make it a potential formulation for use in a comprehensive integrated pest management program. Mint oil has low mammalian toxicity, is a natural insecticide, and has been formulated as both a 2\% granule and a 4\% aerosol (Victor Poisen-Free Ant and Roach Killer, Woodstream Corp., Lititz, PA). A slow release granular formulation could be developed for use as a long-lasting repellent around homes, in quarantined plant material, and in voids or other harborage areas.

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