Repellency of Five Essential Oils to *Linepithema humile* (Hymenoptera: Formicidae)[1]

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Abstract Laboratory bioassays were performed to test the repellent properties of 5 plant-extracted essential oils against the Argentine ant, *Linepithema humile* (Mayr). Three concentrations (0.10%, 1%, and 10% in n-hexane) of peppermint, spearmint, wintergreen, cinnamon and clove oils, as well as a negative control [n-hexane] and positive control [Cinnamite™] were evaluated in choice tests to evaluate repellency against Argentine ant workers by counting the number of ants entering a preferred harborage that was treated and then aged for 2 h (fresh) or 7 d (one-wk-old). When deposits were fresh, all oils at all concentrations were repellent, with repellency defined as statistically fewer ants in harborages compared with harborages treated with only solvent (hexane). After the deposits were aged for 7 d, 4 of the 5 oils formulated at the 0.10% concentration were no longer repellent, whereas only spearmint had retained its repellent property. At 1% and 10% oil concentrations all 5 oils were repellent, whereas only 1% wintergreen was slightly less repellent.

Key Words *Linepithema humile*, Argentine ant, essential oil, repellency, deterrency

The Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), was introduced into the United States around 1891 via the port of New Orleans, LA (Foster 1908, Newell and Barber 1913, Suarez et al. 2001). In the United States, it is a major pest in urban, agricultural and natural environments due to its unicolonial structure, which allows the formation of supercolonies with multiple shared nests (Newell and Barber 1913, Suarez et al. 1999, Tsutsui et al. 2000, Vega and Rust 2001, Tsutsui and Suarez 2003). Because Argentine ants have lost genetic diversity, they lack intraspecific aggression, allowing populations to increase rapidly (Newell and Barber 1913, Holway et al. 1998, Holway 1999, Human and Gordon 1999, Tsutsui et al. 2001, Suarez et al. 2002). Lack of aggression provides a competitive advantage to *L. humile* when competing against native ants, other introduced ant species (Kabashima et al. 2007), and other insect and arthropod species (Holway et al. 1998, Holway 1999, Tsutsui and Suarez 2003).

According to Suarez et al. (2001), *L. humile* is distributed throughout the southeastern United States and in most of California. It is most successful in disturbed environments, including urban habitats (McGlynn 1999). Argentine ants move indoors in search of food and/or water, as well as relief from extreme temperatures (Vega and Rust 2001, Schilman et al. 2007). Control of Argentine ants around and in homes has

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typically relied on modern, synthetic insecticides, more specifically slow-acting baits and perimeter sprays (Vega and Rust 2001). Plant-derived essential oils can be effective at repelling, deterring, and/or killing a variety of arthropods, such as ants (Meissner and Silverman 2001, Appel et al. 2004, Wiltz et al. 2007), cockroaches (Appel et al. 2001, Peterson et al. 2002), termites (Peterson et al. 2004, Cheng et al. 2007), silverfish (Wang et al. 2006), beetles (Hori 2003), thrips (Koschier et al. 2007), ticks (Jaenson et al. 2006), whiteflies (Zhang et al. 2004), and mosquitoes (Rutledge et al. 1983, Omolo et al. 2004, Yang and Ma 2005, Amer and Mehlhorn 2006, Chang et al. 2006). The use of essential oils in structural pest control products provides an alternative to traditional pesticide chemistries whereas retaining the ideal characteristics of repellency, deterrency, and/or toxicity (Isman 2000, Cheng et al. 2007, Wiltz et al. 2007).

Nerio et al. (2010) reviewed studies of essential oils used as repellents against insects and other arthropods and concluded that they have potential to be used as repellents. However, the mode of repellency (through volatilization) also reduces the length of time these chemicals remain active. Even so, they recommended further experimentation aimed at prolonging the repellency of essential oils. The long-term repellent characteristics of essential oils has been challenged by some (Buescher et al. 1982, Rutledge et al. 1983, Nerio et al. 2010), who reported efficacy when freshly applied, but reduced effectiveness as the oils aged. The objective of this study was to evaluate the repellent effects of freshly-applied and aged essential oils to Argentine ants in a choice-based bioassay.

Materials and Methods

Ants. Argentine ants used in this bioassay were collected from a field site on the University of Georgia Griffin Campus (N 33°15.973', W 84°17.336'). When collected, ants (including brood and queens) and accompanying soil, leaf litter, and other debris were placed in a plastic tub (model 400 - 5N, 57 x 45 x 13 cm, Del-Tec/Panel Controls Corporation, Greenville, SC). The tub was prepared in advance of ant collection by coating the inside walls with Fluon™ (Northern Products Inc., Woonsocket, RI) to prevent ant escape. In the laboratory, ants were provided several dead, adult crickets and 4 cotton balls soaked with 25% (w/v) sugar water. To prevent ants from desiccating, ≈250 ml of water was used to wet the leaf litter and debris daily. Ants were held at ambient humidity (55 - 60% as determined by a sling psychrometer) and temperature (20°C - 23°C) and allowed 24 h to acclimate to laboratory conditions before being used in a bioassay.

Worker ants used in the bioassay were collected from laboratory colonies by allowing several ants to climb onto a small paintbrush. The ants were then gently tapped into a clear, 30- ml plastic cup (Jet Plastica Industries Inc., Hatfield, PA) coated on the inner walls with Fluon to prevent escape, and then transferred to the floor of Fluon-coated boxes (discussed below).

Ant harborages. Harborages were prepared using polystyrene culture dishes (35 x 10 mm; Corning Inc., Corning, NY) half-filled with Castone™ (model #99044, Dentsply International Inc., York, PA) powder, a high strength, water-adsorbent dental molding material. Castone powder was mixed with water at a ratio of 30 g to 10 ml, after which approxi. 11 g of the mixture was placed into a culture dish so that each dish was approxi. half-filled. Before the Castone hardened, dishes were gently and repeatedly tapped on a horizontal surface to remove air bubbles and to evenly disperse the material to create a surface that filled the entire dish. Newly-prepared dishes were placed in an oven (40°C) and dried for at least 1 d before use. After drying, 2 holes (1.6 mm diam, 180° apart) were drilled through the side of the dish, just above the surface of the Castone, to provide entrance and exit holes for the ants. A third hole was drilled in the center of the accompanying lid of each dish. All dishes and lids were rinsed under running tap water to remove excess plastic and Castone dust prior to being placed in the drying oven.

Test treatments. Seventeen treatment combinations (5 oils at 3 concentrations each plus 2 controls) were evaluated for their repellency to Argentine ants. Spearmint, wintergreen, peppermint, cinnamon, and clove oils were acquired from Polarome International (Jersey City, NJ). With the exception of spearmint (60% purity), each oil was acquired as technical grade material. Oils were serially diluted in n-hexane to produce 5 ml each of 10%, 1% and 0.10% (v/v) solutions. Vials were sealed with Teflon tape, capped, and refrigerated (3 - 5°C) to prevent hexane evaporation. Two additional treatments (n-hexane alone [negative control] and 1% Cinnamite™ (cinnamaldehyde) suspension in water [positive control] [Mycotech Corporation, Butte, MT]) were prepared and used as controls.

Bioassay. For each of the 17 treatment combinations, 0.50 ml of test solution was applied to the dental stone surface in each of 2 dishes (34 dishes treated). One dish from each treatment (17 dishes) was designated for use 2 h after treatment (fresh deposit). The second dish was designated for an aged 7-d residual bioassay and remained on the laboratory workbench for 1 wk (aged deposit) without lids prior to the bioassy. The freshly-treated dishes remained on the laboratory workbench for 2 h to allow the hexane to evaporate. After oil treatment and just prior to the start of each bioassay, 250 µL of water was added to the dental stone because Argentine ants are highly susceptible to desiccation (Walters and Mackay 2003, Schilman et al. 2007). Immediately after the water had absorbed into the dental stone dishes, lids were placed on each dish and the dishes placed individually into clear, plastic boxes (19 x 14 x 9.5 cm; TriState Plastics, Dixon, KY). Fluon had been applied to the inside walls of each box to prevent ant escape. Twenty-five Argentine ant workers were then placed on the floor of each box. Ants then had the choice of either entering the covered dish or remaining outside of the dish. The number of ants that were inside the dish (alive + dead) was recorded after 2 h. For both residual ages (fresh and aged), each of the 17 treatment combinations was replicated 20 times ($n = 20$).

Statistical analysis. The number of ants in each dish served as the response variable. Data were analyzed using a two-way analysis of variance (ANOVA) (PROC GLM in SAS; Schlotzhauer and Littell 1997, SAS Institute 2008) with treatment and oil age as the main effects. Because the interaction between these variables was significant ($F = 6.0$; d.f. = 16, 19; $P < 0.0001$), the difference in the number of ants between the 2 residual ages was analyzed, by one-way ANOVA, for each of the 17 treatment combinations; means were separated with Tukey's Studentized Range Test (SAS Institute 2008) (Table 1). The number of ants in dishes was further analyzed, by one-way ANOVA, at each of the 6 combinations of residual age (fresh or 1-wk-old) and oil concentration (0.10%, 1%, and 10%); these means also were separated with Tukey's Studentized Range Test (SAS Institute 2008) (Table 2; Fig. 1).

Results

Individual oil age comparisons. In comparing the effect of residual age (fresh versus 1-wk-old) for each of the 17 treatments, statistically significant ($P < 0.05$)

Table 1. Number of ants (mean ± SEM) entering freshly-treated and one-wk-old harborages treated with various essential oils, hexane, and 1% Cinnamite.

	Number of ants (mean ± SE) inside dish at given residual age		Results of One-Way Analysis of Variance*	
Treatment	**Fresh**	**1-wk-old**	***F*-value**	***P* value**
Hexane	20.6 ± 1.0a	20.7 ± 0.9a	0.01	0.9252
1% Cinnamite	1.0 ± 0.3a	10.2 ± 0.6b	34.31	<0.0001
0.10% Spearmint	6.3 ± 1.4a	11.9 ± 1.6b	6.13	0.0229
0.10% Peppermint	14.5 ± 1.4a	18.5 ± 1.3b	8.79	0.0080
0.10% Wintergreen	8.7 ± 1.5a	15.8 ± 1.9b	20.13	0.0003
0.10% Cinnamon	9.7 ± 1.4a	14.5 ± 1.8b	6.01	0.0241
0.10% Clove	8.8 ± 0.9a	14.5 ± 1.5b	15.99	0.0008
1% Spearmint	3.3 ± 0.8a	5.3 ± 1.1a	2.00	0.1736
1% Peppermint	3.0 ± 0.7a	5.4 ± 1.1a	3.73	0.0685
1% Wintergreen	2.2 ± 0.5a	10.3 ± 1.7b	20.85	0.0002
1% Cinnamon	3.2 ± 0.6a	3.1 ± 0.5a	0.02	0.8907
1% Clove	3.6 ± 0.6a	4.6 ± 0.8a	0.80	0.3835
10% Spearmint	5.0 ± 0.9a	1.7 ± 0.6b	12.69	0.0021
10% Peppermint	4.5 ± 0.8a	4.9 ± 0.7a	0.09	0.7707
10% Wintergreen	3.4 ± 0.7a	2.7 ± 0.9a	0.37	0.5477
10% Cinnamon	2.5 ± 0.5a	3.8 ± 0.7a	2.33	0.1438
10% Clove	2.3 ± 0.4a	4.0 ± 1.0a	2.55	0.1266

Means within a row followed by the same letter are not significantly different (one-way ANOVA; means were separated with Tukey's Studentized Range Test [SAS Institute 2008]).
*d.f. = 1, 19 for each ANOVA.

increases in the number of ants entering harborages (i.e., loss of repellency) were observed for all 5 essential oils at 0.10% and for 1% Cinnamite and 1% wintergreen (Table 1). The number of worker ants entering 10% spearmint-treated harborages was significantly lower in 1-wk-old dishes in comparison with fresh deposits (Table 1).

Fresh deposits. In fresh deposits, 20.6 ± 1.0 (mean ± SE) Argentine ant workers entered harborages treated with hexane alone. This number was significantly greater than the number of ants entering harborages treated with 1% Cinnamite or any of the 3 concentrations of each essential oil (Table 2; Fig. 1). The mean number of ants recovered from harborages treated with 1% Cinnamite was 1.0 ± 0.3, which was significantly lower than the number of ants recovered from harborages treated with a 0.10% concentration of all 5 essential oils and the harborages treated with a 10% concentration of either spearmint or peppermint (Table 2; Fig. 1). The number of ants recovered from Cinnamite-treated harborages did not differ significantly from the

Table 2. Results of individual one-way analyses of variance for each combination of oil concentration and residual age.

			Results of One-Way Analysis of Variance*	
Figure	Oil Concentration	Age of Treatment	*F*-Value	*P* value
1A	0.10%	Fresh	27.6	< 0.0001
		1-wk-old	5.5	< 0.0001
1B	1%	Fresh	108.4	< 0.0001
		1-wk-old	27.2	< 0.0001
1C	10%	Fresh	94.6	< 0.0001
		1-wk-old	48.3	< 0.0001

* d. f. = 1, 6 for each one-way ANOVA.

number of ants recovered from dishes treated with a 1% concentration of all 5 essential oils or with a 10% concentration of wintergreen, cinnamon, or clove oils.

One-wk-old deposits. For those treatments that were aged for 7 d, the mean (± S.E.) number of ants entering harborages treated with hexane alone was 20.7 ± 0.9. This number was significantly greater than the number of ants entering harborages treated with 1% Cinnamite, all 3 concentrations of spearmint oil, and the 1% and 10% concentrations of peppermint, wintergreen, cinnamon and clove oils (Table 2; Fig. 1). The number of ants entering harborages treated with 0.10% of either peppermint, wintergreen, cinnamon or clove were not statistically different from the number entering the hexane-treated harborage.

The mean (± S.E.) number of ants entering harborages treated with Cinnamite 1-wk earlier was 10.2 ± 0.6, which was significantly lower than only the hexane treatment and the 0.10% peppermint treatment (Table 2; Fig. 1). The number of ants entering Cinnamite-treated harborages was significantly higher than the numbers of ants recovered from harborages treated 1-wk earlier with either 1% cinnamon, 1% clove and all 5 oils at the 10% concentration.

Discussion

In this study, repellency was indicated by the number of Argentine ants entering treated harborages, with lower numbers indicating avoidance of the harborage by the ants and, thus, relative repellency of the active ingredient. The largest number of ants recovered (≈82%) was from the hexane-treated harborages, which served as a negative control in these bioassays. There also was no difference in the number of ants recovered from fresh deposits of hexane versus dishes treated with hexane and aged for 1-wk (Table 1), thus, confirming complete hexane evaporation from the Castone surface within the initial 2 h period in freshly-treated harborages.

Cinnamite was intended to be a positive control in these bioassays and was highly repellent to Argentine ants when the workers were placed with harborages treated only 2-h-earlier (fresh deposits). However, significantly greater numbers of ants entered

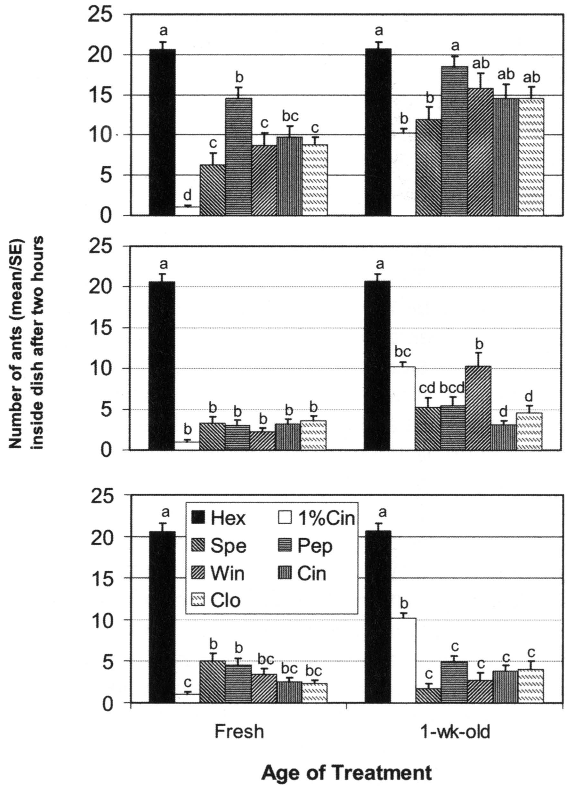

Fig. 1. Response of Argentine ant workers to harborages treated with 1% Cinnamite, hexane, or one of five essential oils at (A) 0.10%, (B) 1%, or (C) 10% and aged for 0 d (Fresh) or 7 d (1-wk-old). Within a concentration X residual age comparison, means followed by the same letter are not significantly different. For results of each one-way ANOVA, see Table 2. Means were separated with Tukey's Studentized Range Test (Schlotzhauer and Littell 1997, SAS Institute 2008). Hex, Hexane; 1% Cin, 1% Cinnamite; Spe, Spearmint; Pep, Peppermint; Win, Wintergreen; Cin, Cinnamon; Clo, Clove.

the Cinnamite-treated harborages after 1 wk (≈41%) in comparison with only 4% entering freshly-treated harborages (Table 1). Cinnamite clearly lost repellency within 7 d of application. Wiltz et al. (2007) reported that Cinnamite (1.5% concentration) kept starved Argentine ant workers from crossing treated areas to access food within 24 h of application, but they did not assess residual activity.

Repellency and residual activity of the 5 essential oils was concentration dependent (Fig. 1; Tables 1 and 2). For example, at 0.10% the number of ants entering harborages treated with the oils 2 h earlier was significantly less than the number of ants entering the hexane-treated harborages, but these numbers were also greater than the numbers of ants recovered from the Cinnamite-treated harborages (Fig. 1A, Fresh).

The percentage of ants recovered from freshly-treated harborages was 82.4% for hexane (negative control), 58% for peppermint, 38.8% for cinnamon, 35.2% for clove, 34.8% for wintergreen, 25.2% for spearmint, and 4% for Cinnamite (positive control). In comparison, at the higher concentrations the percentage of ants recovered from harborages freshly treated with the essential oils ranged from 8.8% to 14.4% at the 1% concentration (Fig. 1B, Fresh) and from 9.2 - 20% at the 10% concentration (Fig. 1C, Fresh), whereas the percentage recovered from the hexane-treated harborages was 82.4%.

At 0.10%, 7 d after application to harborages all 5 essential oils tested were less repellent than fresh deposits (Table 1). In addition, at 7 d after application, only spearmint oil could be statistically-defined as repellent as significantly fewer ants entered the treated harborages than entered hexane-treated harborages (Fig. 1A, 1-wk-old). At 1%, only wintergreen was significantly less repellent after 7 d (Table 1). Similar to the 0.10% oil concentrations, though, the number of ants entering freshly-treated harborages with any 1 of the 5 oils at 1% was significantly less than the number of ants entering the hexane-treated harborages (Fig. 1B, Fresh). There were no reductions in repellency over the 7 d period of any of the 5 essential oils when applied at a 10% concentration (Table 1; Fig. 1C).

These results, although only laboratory-based at this time, indicate the potential of using plant-derived essential oils to exclude Argentine ants from potential harborage sites. Essential oils are repellent and/or toxic to insects of medical (Buescher et al. 1982, Rutledge et al. 1983, Isman 2000, Omolo et al. 2004, Yang and Ma 2005, Amer and Mehlhorn 2006, Jaenson et al. 2006), agricultural (Curtis et al. 1990, Zhang et al. 2004, Wang et al. 2006, Koschier et al. 2007), stored product (Hori 2003), and structural or urban importance (Appel et al. 2001, Meissner and Silverman 2001, 2003; Peterson et al. 2002, Cheng et al. 2007, Wiltz et al. 2007). The use of essential oils are increasingly more popular among consumers as substitutes to traditional pest control chemistries (Isman 2000).

A number of examples are available for the use of essential oils for control of occasional invaders. Meissner and Silverman (2001) showed that aromatic cedar, *Juniperus virginiana* L., mulch was more efficient at repelling Argentine ants than cypress mulch or pine straw. When given a choice between cedar or noncedar mulch types, worker ants chose the noncedar varieties to construct or move a nest. In a later study, Meissner and Silverman (2003) reported that eliminating other possible landscape media, namely noncedar mulch varieties, may negatively impact ant nesting establishment. Their study also demonstrated the ability of cedar mulch to prevent Argentine ant foraging activity when compared with other mulches or media. Not only does aromatic cedar mulch hinder Argentine ant foraging activity and nest establishment, but it was also toxic to Argentine ants in laboratory bioassays (Meissner and Silverman 2001, 2003).

Appel et al. (2001, 2004) investigated the toxic and repellent effects of corn mint oil against German cockroaches, *Blattella germanica*, American cockroaches, *Periplaneta americana*, and red imported fire ants, *Solenopsis invicta* Buren. In continuous-exposure tests, both cockroach species were exposed to mint-oil impregnated filter paper for up to 24 h. They found toxicity to be concentration-dependent. At 3%, lethal time was slower (LT_{50} = 3,318 min for *B. germanica*, LT_{50} = 469.9 min for *P. americana*) than at concentrations of 100% (LT_{50} = 1 min for *B. germanica* and *P. americana*) (Appel et al. 2001). When red imported fire ants were treated in limited- and continuous-exposure tests, mint oil granules were toxic to the ants (Appel et al. 2004). Mint oil granules were toxic at low rates in the continuous-exposure test, and toxicity was positively correlated with granule quantity. The limited exposure test also showed that as little as a 15-min exposure resulted in 2X mortality in comparison with the untreated control after 24 h (Appel et al. 2004).

In repellency tests, Appel et al. (2001) concluded that mint oil granules were repellent to cockroaches, with 92.3 - 100% repellency to German cockroaches and 100% repellency to American cockroaches in a choice-box test. In contrast, in the untreated (control) choice-box, only 2% of American cockroaches and 13% of German cockroaches were repelled. Mint oil has the ability to repel and kill cockroaches, whereas leaving little-to-no residue (Appel, unpubl. data), but is also highly susceptible to volatilization in open environments.

Mint oil granules were also repellent to fire ants (Appel et al. 2004). Using an arrangement similar to the choice-box bioassay in 2001, Appel et al. (2004) placed 10 fire ant workers in a glass Petri dish with one-half of the dish bottom covered with mint oil granules and the other half left uncovered. They suggested that mint oil may have residual effects on fire ant mounds long after the ants initially leave the treated mounds. Although mound drenching is a labor-intensive and uncommonly used method of fire ant control, the oil-impregnated granules may be useful as a long-lasting residual for treating individual fire ant mounds.

More recently, Wiltz et al. (2007) tested basil, citronella, eucalyptus, lemon, peppermint and tea tree essential oils against *S. invicta* and *L. humile* for their repellent and toxic effects. Similar to the foraging activity study by Meissner and Silverman (2003), the repellency tests were designed to determine whether either ant species would cross oil-impregnated cardboard barriers to acquire a food source. All essential oils tested, except eucalyptus, were repellent to both ant species.

Individual essential oils may vary in their ability to repel, or kill by fumigation, specific pest species (Rutledge et al. 1983, Amer and Mehlhorn 2006). Rutledge et al. (1983) tested 31 repellents, including permethrin, against 5 species of *Aedes, Anopheles,* and *Culex* mosquitoes. They concluded that the activity of these various active ingredients was species specific. Even cogeneric species, such as using *B. germanica* in place of *B. asahinai*, did not provide consistent results (Rutledge et al. 1983). They also noted that to test a repellent for broad-spectrum properties, it was recommended to generate data on a variety of insect species.

In our study, fresh deposits of all the oils and all concentrations tested were repellent to Argentine ants, whereas concentrations $\geq 1\%$ were repellent even after being aged for 7 d. The positive control, Cinnamite, was more repellent when freshly applied, but lost its repellent nature when aged for 7 d. To use essential oils as repellents, further investigation is needed to slow the volatilization process and develop and target specific oils for specific insects. To facilitate an efficacious and economically-consistent repellent for Argentine ants, and potentially other occasional invader pests, the characteristics that make essential oils repellent need to be retained, whereas controlling the rate of volatilization.

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