Effects of Aromatic Cedar Mulch on the Argentine Ant and the Odorous House Ant (Hymenoptera: Formicidae)

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ABSTRACT In laboratory studies, the Argentine ant, *Linepithema humile* (Mayr), and the odorous house ant, *Tapinoma sessile* (Say), avoided aromatic cedar mulch as a nesting substrate. Both ant species were killed when confined with fresh aromatic cedar mulch in sealed containers. However, when confined with cedar mulch that had been aged outdoors for up to 140 d, mortality of *L. humile* was complete regardless of mulch age, whereas *T. sessile* mortality declined significantly over the mulch-aging period. Argentine ant susceptibility to aromatic cedar mulch was also greater than that of the odorous house ant when colonies were restricted to mulch in open trays. In addition, commercial aromatic cedar oil was lethal to both ant species. Our results suggest that aromatic cedar mulch may serve as an effective component of a comprehensive urban ant management program.

KEY WORDS *Linepithema humile*, *Tapinoma sessile*, aromatic cedar mulch, cultural control, landscaping

ANTS ARE IMPORTANT urban pests in the United States, with pest management professionals reporting a recent 30% increase in ant-related problems (Moreland and Dorsch 2000). The odorous house ant, *Tapinoma sessile* (Say), and the Argentine ant, *Linepithema humile* (Mayr), are among the species most frequently encountered in and around human dwellings (Smith 1965, Klotz et al. 1995, Rust and Knight 1990, Vega and Rust 2000). Both species predominate in urban areas in North Carolina (H.E.M. and J.S., unpublished data). Although *T. sessile* is strictly a household and industrial pest, *L. humile*, an introduced species from South America, has spread through parts of the southeastern United States and California (Newell and Barber 1913, Suarez et al. 2001), displacing native arthropods (Holway 1998, Kennedy 1998), and interfering with the biological control of certain pest homopterans (Frazer and van den Bosch 1973, Dreistadt et al. 1986).

Argentine ant and odorous house ant colonies contain multiple queens in numerous, interconnected nests, which they relocate frequently (Smallwood and Culver 1979, Smallwood 1982, Silverman and Nsimba 2000). The polydomous colonies may cover large areas. These life history traits make it difficult to achieve control with contact insecticides or baits (Knight and Rust 1990, Rust et al. 1996). Nevertheless, baits and perimeter insecticide treatments are commonly used to target ants (Haack and Granovsky 1990, Klotz et al. 1997).

Various chemicals are repellent to ants (Shorey et al. 1992, 1993, 1996; Sisk et al. 1996). Farnesol, for example, effectively disrupted foraging of *Formica aerata* Francouer on plum trees (Shorey et al. 1993) and prevented Argentine ants from tending homopterans on citrus trees (Shorey et al. 1992, 1996). Based on these successes in agriculture, it has been suggested that repellents, used in combination with traditional methods, may also lead to more successful ant control in residential areas (Klotz et al. 1997).

The wood of the eastern red cedar, *Juniperus virginiana* L., also known as aromatic cedar, has been long thought to possess insecticidal or insect-repellent properties (Back and Rabak 1922, Sweetman et al. 1953). However, very few investigations have been carried out to test the accuracy of this popular belief. Early studies demonstrated that cedar wood and its shavings killed various life stages of the clothes moth, *Tineola bisselliella* Hummel, and the black carpet beetle, *Attagenus unicolor* Brahm (Scott et al. 1918, Laudani and Clark 1954), and cedar oil volatiles were lethal to larval *T. bisselliella (*Huddle and Mills 1952). Milled aromatic cedar flake board repelled but did not kill the German cockroach, *Blattella germanica* L., while other cockroaches, *Periplaneta americana* L. and *P. fuliginosa* (Serville), were neither repelled nor killed (Appel and Mack 1989). To our knowledge, the effects of aromatic cedar on ants have never been reported.

Tramp ants such as *L. humile* and *T. sessile* nest opportunistically in a large variety of substrates (Passera 1994), and we frequently have found nests in organic landscaping mulches such as pine straw, pine bark, cypress, and hardwoods. These mulches are frequently placed alongside buildings and may facilitate ant entry indoors. In this study we evaluated the toxic and repellent effects of aromatic cedar mulch on laboratory colonies of *L. humile* and *T. sessile.*

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Materials and Methods

Insects. Odorous house ant colonies were collected from the J. C. Raulston Arboretum of North Carolina State University in Raleigh, NC; Argentine ants were collected from the main campus of Forsyth Community College in Winston Salem, NC. The collections were made on several occasions during the summer of 2000. The colonies collected were taken to the laboratory and transferred to a plastic tray (40 by 52 by 8 cm, Panel Controls, Greenville, SC) coated with Fluon (AD-1, Northern Products, Woonsocket, RI) to prevent escapes. For shelter, each colony of ants was provided with several artificial nests, each of which consisted of a petri dish (15 mm diameter) filled with sculptured dental plaster to increase nest surface area, and glued on top of a water-filled glass jar, from which a cotton wick conducted moisture to the ants. The ants were provided a 25% sucrose solution, artificial diet (Nonacs and Dill 1988), and German cockroaches, *B. germanica* ad libitum.

Mulch Choice Test. This study assessed the attractiveness of aromatic cedar mulch as a nesting substrate in both the presence and absence of alternative mulches. Individual artificial nests containing Argentine ant or odorous house ant colonies were placed in the center of a fluon-coated plastic tray (40 by 52 by 8 cm). The size of the ant colonies varied, but was in all cases >1,000 workers, plus brood and numerous queens. Each tray contained two choices of garden mulch, placed in piles (≈20 by 30 by 10 cm) on opposite sides. The choices were fresh shredded aromatic cedar and one of the following: pine straw, pine bark, shredded cypress wood, or chipped hardwood (P&L Bark, Pageland, SC).

To determine whether ants would vacate their artificial nests when cedar was provided as the nesting substrate, we placed laboratory nests of either ant species in trays containing only fresh aromatic cedar. If no colony movement into the cedar mulch had occurred within 24 h, cedar mulch was subsequently replaced with cypress mulch and nest establishment in the cypress mulch was assessed.

All mulches were moistened slightly before the experiment to provide conditions suitable for ants. Water content (grams) per mulch dry weight (grams) was determined for each mulch type. Mean water content (±SEM) was aromatic cedar, 1.10 (0.20); cypress, 1.20 (0.35); hardwood, 1.20 (0.22); pine bark, 1.24 (0.23); and pine straw, 0.58 (0.26).

At the beginning of a trial, ants were induced to leave their artificial nest by removing an opaque shield above the nest to admit light and by tapping the nest with the index finger several times. The experiment was replicated 10 times for each mulch combination and ant species. Ten replicates were also performed when cedar was provided in a non-choice arrangement. After 24 h, the mulch piles were checked for the presence or absence of accumulated brood (signifying nesting behavior). Where a choice of two mulches was provided, only those replicates where the ants left the artificial nest were used in the data analyses.

Toxicity of Mulch. We assessed the toxicity of aromatic cedar mulch as a function of mulch age. Glass vials (20 ml) were filled with 1.5 g of aromatic cedar mulch or fresh cypress mulch (control). Piles of aromatic cedar mulch (≈50 cm high) were placed outdoors alongside a building wall and aged. They were partially shaded from the sun and exposed to minimal precipitation. Ten to 30 ants were anesthetized with CO_2, placed into each vial and allowed to recover from anesthesia before the vials were sealed. Mortality was assessed 24 h later. Ten to 20 replicates were performed for each ant species and for each of 18 mulch ages between 0 and 140 d.

We also assessed the mortality of ants held in open containers filled with mulch. Plastic trays (40 by 52 by 8 cm) coated with Fluon were filled with fresh, dry aromatic cedar or cypress mulch (≈5 cm deep). Approximately 300–500 workers and 100–200 brood of either *L. humile* or *T. sessile* were placed on top of the mulch in these trays and provided with artificial diet and fresh water in 1.5-ml centrifuge tubes. No artificial nest was provided. Percentage worker mortality was recorded for each of 10 d by counting daily the number of live and dead workers in each tray.

Toxicity of Aromatic Cedar Oil. We determined whether mulch toxicity could be attributed to the oil in aromatic cedar wood. Pieces of cotton were placed at the bottom of plastic centrifuge tubes (50 ml) and moistened with 250 μl of water. Ten to 30 *L. humile* or *T. sessile* workers were then aspirated from a stock colony and added to the tubes. One hour after introduction of the ants, 50, 100, 200, 300, 400, or 500 μl of an extract of aromatic cedar oil (100% purity, Giles and Kendall, Huntsville, AL) was applied to pieces of cotton, which were positioned in the centrifuge tube lids. The side with the oil was facing up, so that ant contact with the oil was minimal. As a control, 500 μl of mineral oil was applied in the same manner. Mortality was checked after 24 h, and a dose–response curve was established for the aromatic cedar oil treatments based on ten replications for each ant species and oil quantity

Statistical Analysis. For the mulch choice experiment, 95% binomial confidence intervals were determined for the probability of aromatic cedar mulch not being colonized in the presence of an alternative mulch, or a colony dish, respectively, in a single trial (Mainland et al. 1956, Steel et al. 1997). Percentage mortality in the mulch and oil toxicity trials was plotted against mulch age and oil volume, respectively, and a logit analysis (PROC GENMOD, SAS Institute 1998) was performed on each data set. Overdispersion of data were accounted for using the SAS p-scale option. For mortality studies in mulch-filled trays, ant mortality curves were compared using the Wilcoxon method (Minitab 2000).

Results and Discussion

Mulch Choice Test. When offered a choice between fresh aromatic cedar and any of the other mulches, ants always colonized the noncedar mulch

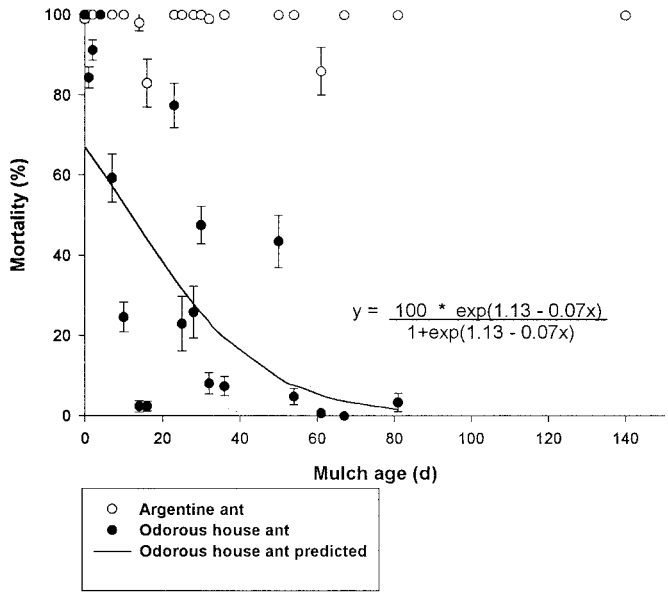

Fig. 1. Mortality of Argentine ants and odorous house ants confined within sealed vials containing aromatic cedar mulch aged for up to 140 d.

(*L. humile*, 40/40; *T. sessile*, 38/38). The probability of aromatic cedar not being colonized in the presence of an alternative mulch was 0.9–1.0 for both species (95% binomial confidence limit). When aromatic cedar mulch was provided as the only choice, it was not colonized by *L. humile* or by *T. sessile;* the ants subsequently colonized replacement cypress mulch in all cases. Thus, the probability of aromatic cedar mulch not being colonized even in the absence of other mulches lies between 0.7 and 1.0 (95% binomial confidence limit, 10 replicates per species). Since the water content of aromatic cedar was similar to those of cypress and pine bark, but was higher than that of pine straw (see *Materials and Methods*), mulch water content can be excluded as a reason for this avoidance.

Toxicity of Mulch. Argentine ants suffered total or very high mortality at all aromatic cedar mulch periods of outdoor exposure, whereas odorous house ant mortality decreased significantly with mulch age (Fig. 1; Table 1), indicating that the Argentine ant is considerably more susceptible than the odorous house ant to the effects of aromatic cedar. Control mortality (±SEM) in fresh cypress mulch was 1.8 (0.5) % for *L. humile* and 1.3 (0.6) % for *T. sessile.*

Our results suggest that aromatic cedar may be active against Argentine ants for at least 4 mo. However, it should be noted that the mulch we used was deposited in a somewhat protected outdoor location, being exposed to little rain or irrigation. We expect that ambient climatic conditions and irrigation frequency will affect the duration of aromatic cedar mulch activity against the Argentine ant.

Argentine ants and odorous house ants were affected differently when confined in open trays with aromatic cedar mulch, with all *L. humile* dying within 4 d, and 50% of the odorous house ants dead within 8 d (Wilcoxon, ChiSquare = 1,151.07, df = 1, $P = 0.0001$; Fig. 2). Neither ant species was killed by cypress mulch. We also observed that workers of both species formed large inactive aggregations on the aromatic cedar at locations where contact with the mulch was reduced. In addition *L. humile* and *T. sessile* workers neglected brood that was deposited on the aromatic cedar mulch surface.

Toxicity of Aromatic Cedar Oil. Aromatic cedar oil had a stronger effect on Argentine ants than on odorous house ants (Fig. 3), which is consistent with the results of the mulch toxicity study. Mortality of Argentine ants significantly increased with oil volume, whereas the dose-mortality slope for odorous house ants was not significant (Table 2). Ant mortality was presumably due to volatile constituents of the oil, as the treated cotton was positioned to minimize direct contact. However, contact toxicity cannot be com-

Table 1. Regression statistics for the effect of aged aromatic cedar mulch on ant mortality (logit analysis, PROC GENMOD)

	n	Slope (SE)	95% CL	χ^2	P
Argentine ant	260	−0.002 (0.009)	−0.02; 0.02	0.1	0.81
Odorous house ant	249	−0.07 (0.007)	−0.09; −0.06	108.9	<0.0001

Table 2. Regression statistics for the effect of aromatic cedar oil volume on ant mortality (logit analysis, PROC GENMOD)

	n	Slope (SE)	95% CL	χ^2	P
Argentine ant	60	0.007 (0.001)	0.005; 0.009	57.7664	0.0001
Odorous house ant	60	0.002 (0.001)	−0.0002; 0.004	3.272	0.07

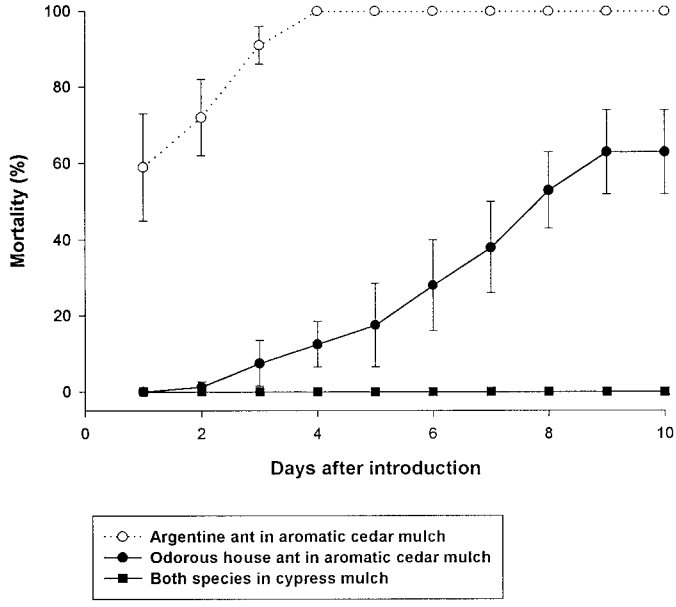

Fig. 2. Ant mortality in open trays of aromatic cedar or cypress mulch.

pletely ruled out as a contributing factor. In control treatments with 500 μl mineral oil, mean mortality (±SEM) was 3.0 (1.5) % and 1.0 (0.6) % for *L. humile* and *T. sessile*, respectively, after 24 h.

Mulch is widely used in residential landscaping for maintenance of soil humidity and temperature, and for cosmetic purposes (Porter 1998). Argentine ants and odorous house ants prefer to nest within the top 20 cm of well-drained, loose substrates, moving the nest higher or deeper as conditions change (Smith 1965, Markin 1968); consequently, mulch would appear to be an excellent substrate for these ants. Due to their relatively small size, Argentine ants may be more vulnerable to desiccation than many native species (Tremper 1976), and may thus benefit more from the constant moisture conditions provided by mulch.

Our laboratory studies demonstrated that *L. humile* and *T. sessile* avoided aromatic cedar mulch as a nesting substrate and died when confined to aromatic cedar mulch, presumably due to volatile chemical

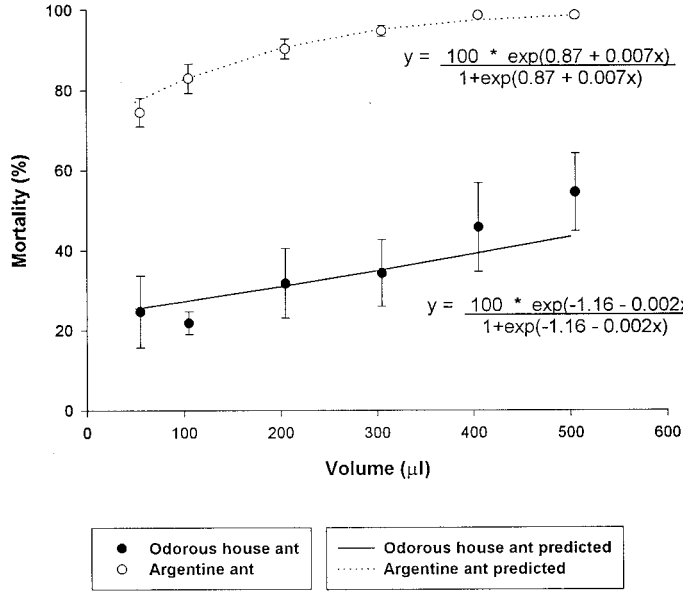

Fig. 3. Percentage mortality of Argentine ants and odorous house ants exposed to different volumes of aromatic cedar oil.

constituents in the wood. Therefore, aromatic cedar mulch may be effective in the field by limiting the availability of ant nesting sites and possibly reducing population size. If proven efficacious in field trials, it may be easily incorporated into a residential ant management program. Residential ant problems are increasing and many people prefer control methods with minimal pesticide input in the vicinity of their homes (Vega and Rust 2000). However, mulches such as pine straw and pine bark that support ant and other urban arthropod pest populations may require a greater insecticide load to effect control (Bello 1997). Klotz et al. (1997) have suggested that repellent materials could complement conventional control methods in the management of urban ants. However, to date, no repellent substances have been identified that could easily and effectively be used in residential situations. We suggest that aromatic cedar mulch may be a promising candidate for this management approach.

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