

MORTALITY OF ANT (HYMENOPTERA: FORMICIDAE) PEST SPECIES EXPOSED TO SODIUM HYDROGEN CARBONATE

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ABSTRACT

Laboratory bioassays enabled us to determine the mortality of Argentine ant (*Linepithema humile* [Mayr]) workers, and red imported fire ant (*Solenopsis invicta* Buren) workers exposed to sodium hydrogen carbonate (NaHCO_3 , sodium bicarbonate). The median lethal concentration (LC_{50}) of NaHCO_3 for Argentine ants was 5.64 mg per cm^2 after 5 d exposure and 3.96 mg per cm^2 after 6 d. Cumulative mortality for Argentine ants exposed to 28 mg NaHCO_3 per cm^2 was 89.5% on day 6. Workers of both species were exposed to concentrations of 9.92, 17.70, or 152.00 mg NaHCO_3 per cm^2 in separate tests. Mortality of Argentine ants was significantly higher than that of fire ants following exposure to 9.92 mg NaHCO_3 per cm^2 , while mortality for the two species did not differ following exposure to the two higher concentrations. Mortality of both species treated with the highest concentration exceeded 99% at 6 d. In tests with equivalent amounts of sodium in NaHCO_3 and NaCl treatments, mortality for fire ants exposed to NaHCO_3 was about 46% after 6 d. Mortality for fire ants exposed to NaCl was about 15% and was similar to that for untreated ants. Argentine ants were provided sugar water baits containing a range of NaHCO_3 concentrations. Argentine ant mortality after 6 d exposure to 5% NaHCO_3 -sugar water treatment was about 50%. Mortality was not higher for workers exposed to higher concentrations of NaHCO_3 in sugar water baits. Enzymatic dysfunction caused by unfavorable increases in internal pH is the most likely explanation for worker mortality following exposure to NaHCO_3 .

Key Words: Sodium bicarbonate, bicarbonate of soda, Argentine ant, red imported fire ant, pest ant, laboratory bioassays, Gut pH.

RESUMEN

Los bioensayos del laboratorio nos permiten determinar la mortalidad de las trabajadoras de la hormiga Argentina (*Linepithema humile* [Mayr]), y la hormiga de fuego roja importada, (*Solenopsis invicta* Buren) expuestas al hidróxido carbonato del sodio (NaHCO_3 , hidrogeno-carbonato del sodio). La concentración media letal (LC_{50}) del NaHCO_3 para la hormiga Argentina fue 5.64 mg por cm^2 después de exponerlas por 5 días y fue 3.96 mg por cm^2 después de 6 días. La mortalidad acumulativa de las hormigas Argentinas expuestas a 28 mg de NaHCO_3 por cm^2 fue 89.5% en el día 6. Las trabajadoras de ambas especies fueron expuestas a concentraciones de 9.92, 17.70, o 152.00 mg de NaHCO_3 por cm^2 en pruebas separadas. La mortalidad de las hormigas Argentinas fue significativamente más alta que la mortalidad de las hormigas de fuego roja importada después de exponerlas al 9.92 mg de NaHCO_3 por cm^2 , mientras que la mortalidad de las dos especies no fue diferente después de exponerlas a las dos concentraciones más altas. La mortalidad de ambas especies tratadas con la más alta concentración alcanzó el 99% al día 6. En pruebas con cantidades equivalentes de sodio en los tratamientos de NaHCO_3 y NaCl , la mortalidad de las hormigas de fuego roja importadas expuestas al NaCl fue aproximadamente 15% y fue similar a la mortalidad en las hormigas no tratadas. Las hormigas Argentinas fueron proveídas con cebos de agua azucarada que tenían varias concentraciones de NaHCO_3 . La mortalidad de la hormiga Argentina después de exponerlas por 6 días al tratamiento del agua azucarada con 5% de NaHCO_3 fue aproximadamente 50%. La mortalidad no fue mas alta para las trabajadoras expuestas al concentraciones mas altas de NaHCO_3 en cebos de agua azucarada. La disfunción enzimática causada por aumentos no favorables en el pH es la explicación mas probable para la mortalidad de las trabajadoras después de exponerlas al NaHCO_3 .

The Argentine ant, *Linepithema humile* (Mayr), and red imported fire ant, *Solenopsis invicta* Buren, are indigenous to South America. Both have become important pests in urban and agricultural areas in the southern United States (Callcott & Collins 1996; Suarez et al. 1999). Fire ants infest lawns and are nuisances as well as

dangerous pests because of their aggressive behavior and sting. In surveys of South Carolina residents conducted by Lemke & Kissam (1989), 87% of respondents felt that they had a severe fire ant problem on their property, and 89% reported having one or more members of their immediate family stung by fire ants. Although Argentine

ants do not sting humans and livestock, they are considered a nuisance pest because they invade homes in search of food and nesting sites. Argentine ants are opportunistic feeders and will forage in garbage receptacles and pet food dishes (Rust et al. 2003).

Ant control in urban environments usually is accomplished with chemical insecticides (Pereira & Stimac 1997). Argentine ant control strategies have focused on the use of baits and the application of contact and barrier sprays and granules (Rust et al. 2003); however, most toxic or repellent barriers fail to provide long-term control, and commercial baits are not always accepted by foraging Argentine ants (Rust et al. 2003). Retreatments are often necessary, adding to the expense of ant control. Many homeowners find extensive use of insecticides in and around the home undesirable. Therefore, additional control methods with low toxicity are needed for urban pest ant management (Klotz et al. 1997a).

Brinkman et al. (2004) previously determined that *S. invicta* workers were susceptible to sodium hydrogen carbonate (NaHCO_3 , also known as sodium bicarbonate) placed on surfaces and in liquid baits. Workers were not repelled by concentrations of NaHCO_3 , and mortality was over 78% in treated arenas with liquid bait. They further reported that the median lethal concentration (LC_{50}) decreased from 9.66 mg per cm^2 on day 5 to 8.16 mg per cm^2 on day 6. Vinson (1970) tested the preferences of fire ants (*S. richteri* Forel) for various electrolytes (including NaHCO_3) in solution, but did not report on potential mortality following ingestion of those electrolytes. If effective against *S. invicta* and Argentine ants, NaHCO_3 could prove to be a safe alternative to conventional insecticides. The objective of this research, therefore, was to compare the mortality of Argentine ants and red imported fire ants after exposure to NaHCO_3 in simultaneous laboratory tests.

MATERIALS AND METHODS

Fire ant workers used in this study were obtained from monogyne field populations in Griffin, GA (Spalding Co.), and were removed from soil by procedures described by Jouvenaz et al. (1977). Argentine ants were collected from nests in logs and leaf litter on the Georgia Experiment Station campus. Although these ants were collected from different areas on the campus, they likely belonged to the same unicolony (Giraud 2002). These laboratory colonies were maintained in plastic trays containing artificial nests constructed of plastic Petri dishes (150 × 10 mm) with dental plaster on the bottom to maintain moisture (Stimac et al. 1993). Fluon® (Northern Products Inc., Woonsocket, RI) was applied to the inside walls of trays to prevent ant escape. Ants were fed 10% sugar water (v/v) and tuna in oil.

The LC_{50} of NaHCO_3 against Argentine ants was established in laboratory bioassays. Test arenas were clear 35-ml plastic cups. Each cup had a 5-mm diam hole in the bottom and contained dental plaster to about 10% of total cup volume. Lids for the cups were plastic and had a 1.2-cm diam hole to allow for air exchange. Fluon was applied to the inside walls of cups and undersides of lids. Ten cups were randomly assigned to each of the treatments.

Treatment concentrations were 0, 0.85, 1.7, 3.5, 7.0, 14.0, and 28.0 mg NaHCO_3 per cm^2 . The NaHCO_3 was deposited as powder on the surface of dental plaster in the appropriate cups. Cups were lightly tapped to evenly distribute the material on the surface. Ten Argentine ant workers were placed in each container with a small quantity of sugar water for food. Cups were placed on a wet foam pad to maintain moisture within cups over the duration of the tests. Initially, the NaHCO_3 treatments were dry, but as water was drawn up through the dental plaster, they became slightly moistened. Mortality was checked daily for 6 d; dead workers were removed each day. Treatments were replicated 10 times in a randomized complete block design (RCBD). These tests were conducted four times between 03 October and 15 December 2003. Data were subjected to probit analysis (SAS Institute 1985) to obtain estimates of lethal concentrations and associated parameters. Concentration of NaHCO_3 was transformed by $\log(x + 1)$ prior to regression analysis and graphing of ant mortality data (SPSS Inc. 1998).

Potential differences in mortality of the two ant pest species from NaHCO_3 exposure also were determined in laboratory tests. Test arenas, application of treatments, and maintenance of ants and arenas were the same as previously described. In this test, fire ants and Argentine ants were exposed to 9.92 mg NaHCO_3 per cm^2 , a concentration that approximated the LC_{50} for fire ant workers following 5 d of exposure (Brinkman et al. 2004). Groups of 10 workers of each species were placed in the appropriate arenas and maintained as previously described. Mortality was checked daily for 7 d in the treatment and control arenas. Dead ants were removed each day. Treatments were replicated 5 to 10 times in a RCBD with each arena being a replicate. These experiments were repeated five times between 21 July and 5 August 2003.

Two higher concentrations were tested in separate assays by methods previously described. Both were compared to an untreated control. The concentration of 17.7 mg NaHCO_3 per cm^2 was evaluated three times between 18 December 2003 and 15 January 2004. The highest concentration of 152.0 mg NaHCO_3 per cm^2 was evaluated in five experiments between 16 January and 27 January 2004. Data resulting from these experiments were analyzed by the PROC MIXED procedure

with repeated measures in SAS (Littell et al. 1996); means were separated with LSD ($P = 0.05$).

A range of concentrations of NaHCO_3 was tested in sugar water baits on Argentine ants. Test arenas and maintenance of ants and arenas were the same as previously described. A stock solution of sugar water was prepared by mixing 8.37 g (10 ml) of granulated sugar with 90 ml sterile distilled water. Concentrations of 0, 1, 5, 7.5, and 10% NaHCO_3 —sugar water (v/v) were prepared (Table 2). Treatments were pipetted into 0.65-ml microcentrifuge tube lids, and these were individually placed on the dental plaster in cups. Treatments were replicated 10 times in a RCBD. Mortality was checked daily for 6 d. Dead ants were removed from cups each day. These tests were conducted three times between 20 February and 8 March 2004. Data were analyzed by the PROC MIXED procedure, and means were separated with LSD ($P = 0.05$).

Tests were conducted with equivalent amounts of sodium in the form of NaHCO_3 and sodium chloride (NaCl) to determine whether or not fire ant mortality would be similar for the two compounds. The total amount of either NaHCO_3 or NaCl placed in each test arena was 84.0 mg (11.898 mg per cm^2) of NaHCO_3 and 58.0 mg (8.215 mg per cm^2) of NaCl. Formula weight of NaHCO_3 is 84.00687 and is 58.44277 for NaCl (Whitten & Gailey 1981). Untreated arenas (control) were also included in these tests. Test arenas, treatment application, and maintenance of ants were the same as previously described. Treatments were replicated 10 times in a RCBD. Mortality was checked daily for 6 d, and dead ants were removed from cups each day. These experiments were repeated five times between 30 October 2003 and 18 January 2004 using fire ants from four different colonies. Data were analyzed by the PROC MIXED procedure with repeated measures in SAS (Littell et al. 1996), and means were separated with LSD.

RESULTS AND DISCUSSION

A positive linear relationship ($R^2 = 0.3665$; $F_{1,278} = 160.81$; $P < 0.0001$) occurred between NaHCO_3 concentration on surfaces and Argentine ant mortality (Fig. 1). Probit analysis of the concentration-mortality response of workers after 5 d

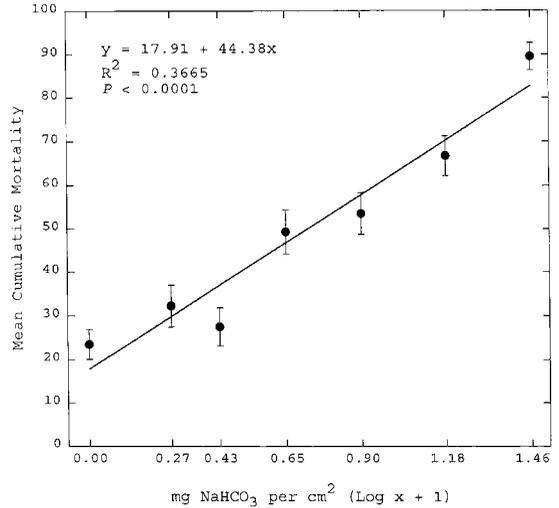


Fig. 1. Linear regression for NaHCO_3 concentration effects on Argentine ant mortality in test cups (Day 6). Vertical lines represent \pm SEM. Treatments were an untreated control, 0.85, 1.70, 3.50, 7.00, 14.00, and 28.00 mg NaHCO_3 per cm^2 . Concentration of NaHCO_3 was transformed by $\log(x + 1)$ prior to regression analysis and graphing of ant mortality data.

exposure to NaHCO_3 yielded a LC_{50} of 5.64 mg per cm^2 and 3.96 mg NaHCO_3 per cm^2 after 6 d (Table 1). This LC_{50} is lower than that obtained for red imported fire ants on day 5, as reported by Brinkman et al. (2004). Argentine ant LC_{50} followed a similar trend as that observed with fire ants by decreasing over time. Furthermore, fire ant mortality following exposure to 28 mg NaHCO_3 per cm^2 for 6 days was 66.0% (Brinkman et al. 2004), while Argentine ant mortality following 6 days of exposure to 28 mg NaHCO_3 per cm^2 was 89.5%. These results suggest that less NaHCO_3 is required to kill Argentine ant workers than fire ant workers.

Cumulative mortality for Argentine ants exposed to 9.92 mg NaHCO_3 per cm^2 was ($F = 9.85$; $df = 3, 6$; $P = 0.0001$) higher than mortality of fire ants exposed to the same concentration over the 7 d of exposure. On day seven, cumulative mortality among Argentine ants exposed to NaHCO_3 was 38.0% (± 5.5) and was 35.6% (± 4.9) for fire ants exposed to NaHCO_3 (Fig. 2A).

TABLE 1. CONCENTRATION—MORTALITY OF WORKER ARGENTINE ANTS AFTER EXPOSURE TO NaHCO_3 FOR 5 TO 6 D (N = 400 IN EACH TREATMENT).

Day	mg per cm^2		Slope \pm SE	χ^2	$P > \chi^2$
	LC_{50} (95% CL)	LC_{95} (95% CL)			
5	5.64 (2.74-13.10)	210.0 (50.0-26930.0)	1.05 \pm 0.21	24.6	0.0001
6	3.96 (1.84-7.60)	150.0 (40.0-7700.0)	1.04 \pm 0.20	28.2	0.0001

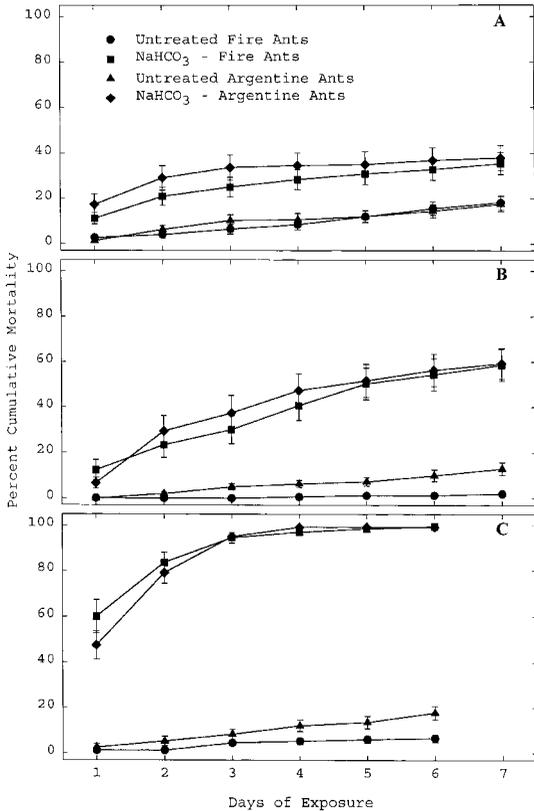


Fig. 2. Mortality of fire ants and Argentine ants exposed to a concentration of (A) 9.92, (B) 17.7, (C) 152.0 mg NaHCO₃ per cm² on dental plaster in test cups. Untreated workers of both species also were kept as controls.

Mortality of Argentine ants and fire ants exposed to 17.7 mg NaHCO₃ per cm² was ($F = 24.20$; $df = 3,6$; $P = 0.0001$) higher than mortality for their respective untreated controls over the 7 d of the test (Fig 2B). Argentine ant mortality did not differ ($P > 0.05$) from fire ant mortality following exposure to 17.7 mg NaHCO₃ per cm². Cumulative mortality at day 7 for Argentine ants was 59.3% (± 6.9) and 58.7% (± 7.1) for fire ants. This concentration was the predicted LC₇₅ for Argen-

tine ants after 6 d of exposure to NaHCO₃, but mortality for workers of both species was lower than 75%. The reason for the lower mortality is not known, but it was consistent for both ant species at this concentration.

Mortality for Argentine ants and fire ants exposed to 152.0 mg NaHCO₃ per cm² was ($F = 592.02$; $df = 3,5$; $P = 0.0001$) higher than mortality for their respective untreated controls over the 6 d of the test. Following correction for control mortality (Abbott 1925), Argentine ant mortality was 99.09% and fire ant mortality was 99.47% after 6 days of continuous exposure to 152.0 mg NaHCO₃ per cm² (Fig. 2C, non-corrected mortality). This concentration was the predicted LC₉₅ for Argentine ants following 6 d exposure to NaHCO₃. However, mortality for both species was higher than 95% in less than 6 d. In fact, almost all workers of both species were killed by this concentration after only 4 d of exposure. These results suggest that, at lower concentrations, there may be small differences in mortality of workers of these two species; however, as concentration of NaHCO₃ is increased, the mortality of the two species is similar.

Cumulative mortality for fire ants provided untreated sugar water was ($F = 14.46$; $df = 4,5$; $P = 0.0001$) lower than for the three highest concentrations of NaHCO₃ in sugar water at 6 d. The highest corrected mortality was 49.56% in the 5.0% NaHCO₃-sugar water treatment (Table 2, non-corrected mortality). However, this was not ($P > 0.05$) different from ant mortality for the 7.5% NaHCO₃-sugar water treatment. Corrected Argentine ant mortality in the highest concentration of NaHCO₃ in sugar water was 29.20% after 6 d. A range of concentrations of NaHCO₃ mixed in sugar water was tested, yet the greatest mortality was observed in the 5% NaHCO₃-sugar water treatments. A concentration-dependent relationship did not occur beyond this level. A similar trend was observed with fire ants provided sugar water treatments containing NaHCO₃ (Brinkman et al. 2004). Brinkman et al. (2004) concluded that excess NaHCO₃ had settled out of solution in the higher concentrations and was not available for ant consumption. In this study, some precipitate was observed in the bottoms of lids

TABLE 2. CUMULATIVE MORTALITY FOR ARGENTINE ANTS (N = 100 PER TREATMENT PER TEST) PROVIDED SUGAR WATER AND NaHCO₃.

Food Treatment	Mean # Dead (day 6)
Untreated sugar water	24.67 \pm 3.31a
0.113 g NaHCO ₃ in 9.9 ml sugar water (1%)	30.33 \pm 3.47a
0.563 g NaHCO ₃ in 9.5 ml sugar water (5%)	62.00 \pm 4.11c
0.844 g NaHCO ₃ in 9.25 ml sugar water (7.5%)	55.67 \pm 4.86c
1.125 g NaHCO ₃ in 9.00 ml sugar water (10%)	46.67 \pm 4.53b

Means (\pm SEM) followed by same letter are not different (LSD, $P > 0.05$).

containing 10% NaHCO_3 -sugar water treatment, yet mortality for Argentine ants exposed to this concentration was significantly higher than mortality for Argentine ants provided untreated sugar water. Therefore, we concluded that workers were not repelled by the higher concentrations of NaHCO_3 in sugar water. Vinson (1970) found that fire ants workers preferred NaHCO_3 in solution over NaCl in solution. Preferential response to sodium was variable in comparison with other cations (Vinson 1970). Brinkman et al. (2004) conducted tests in arenas in which fire ants could feed on sugar water or sugar water containing NaHCO_3 . In those tests, fire ant mortality was much higher in arenas with both sugar water and sugar water containing NaHCO_3 , than it was in arenas with sugar water only. Brinkman et al. (2004) concluded that fire ants were not repelled by NaHCO_3 in food.

Fire ant mortality following exposure to NaCl was ($F = 22.76$; $df = 2,5$; $P = 0.0001$) lower than mortality occurring among those workers exposed to NaHCO_3 (Fig. 3). Worker mortality in response to NaCl was 15.4% at 6 d and did not differ ($P > 0.05$) from mortality of untreated ants. Fire ant mortality following 6 d exposure to NaHCO_3 was 46.2%. Brinkman et al. (2004) found that whole-body pH of fire ant workers exposed to NaHCO_3 increased with increasing concentration of NaHCO_3 . They theorized that fire ants ingested NaHCO_3 while cleaning appendages and that the resultant changes in internal pH were unfavorable to normal enzymatic functions. According to Tortora & Grabowski (1996), sodium hydrogen

carbonate contributes hydroxide ions (OH^-) to solutions causing increases in pH. However, Bigner et al. (1997) attributed the alkalinizing action of NaHCO_3 to Na^+ and they based this on the strong ion difference theory. In the theory of strong ion difference in acid-base physiology (Stewart 1983), addition of non-metabolizable, positively charged cations to a body fluid compartment raises the pH of that compartment. Bigner et al. (1997) tested three Na compounds to determine which was best for treating metabolic acidosis in dairy cattle. Blood pH and blood HCO_3^- increased in the NaHCO_3 treatment, and was much higher than that observed for NaCl . They assumed, based on the theory, that the NaCl treatment did not alter blood pH because both the positively charged cation Na^+ and the negatively charged anion Cl^- were absorbed equally well and added no net charge to the blood fluid compartment. This suggests that Na^+ may have played a role in killing fire ants by raising pH, but only when delivered in the form of NaHCO_3 , and not as NaCl . According to Audesirk et al. (2002), salts dissociate into ions in solution, and may then form bonds with enzymes and interfere with the enzymes' normal three-dimensional structure. Also, changes in pH may modify the structure of enzymes and strongly alkaline solutions can denature enzymes (Conn & Stumpf 1976).

Hertel (1997) tested NaCl and KCl as protectants for pinewood against attack of a long-horn beetle species [*Hylotrupes bajulus* (L.)]. Both compounds were effective, but NaCl provided better protection than KCl . Dehydration of beetle larvae after feeding on salt treated wood was offered as a possible explanation for mortality. In our study, the role of dehydration in deaths of fire ants exposed to NaCl and NaHCO_3 on surfaces was minimal because workers had unrestricted access to untreated sugar water.

Optimal procedures for use of NaHCO_3 as an ant control treatment in the home have yet to be determined. According to Klotz et al. (1997a), dusts are an excellent formulation for insecticides because ants readily pick up dusts that are applied to their trails. This may be an acceptable application strategy for NaHCO_3 in that Brinkman et al. (2004) determined that fire ants were not repelled by NaHCO_3 and would readily forage over treated areas. Crust will develop on NaHCO_3 powder if it is exposed to moisture and then dries. Therefore, retreatment may be necessary on unprotected ant trails. Klotz et al. (1997a) suggest that dusts could be applied during home construction when there is easy access to wall voids. Knight & Rust (1990) reported that repellency often determines how much contact an insect will have with a toxicant and that very low repellency treatments may produce high kill, even with only intermediate toxicity, because of increased contact with the treatment.

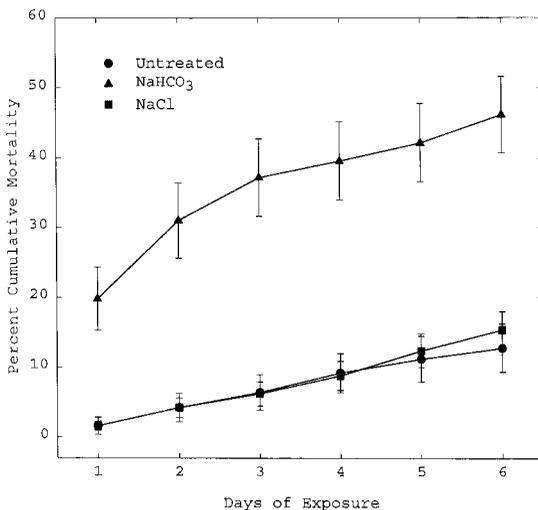


Fig. 3. Mortality of fire ants exposed to NaHCO_3 and NaCl treatments. Untreated workers of both species also were kept as controls. NaHCO_3 and NaCl treatments that were placed in respective arenas contained equal amounts of sodium.

Sugar water has been used as a bait carrier for boric acid against fire ants (Klotz et al. 1997b) and Argentine ants (Klotz et al. 2002). Sucrose solutions are attractive to Argentine ants and are a means of transporting toxicants into the colony (Hooper-Bui & Rust 2000). Sodium hydrogen carbonate is inexpensive, easy to handle, and generally recognized as safe (GRAS) for use in foods (Montville & Goldstein 1987). Thus, sugar water baits containing NaHCO_3 could be used safely in the homes with children or pets. Toxicants that are effective in baits exhibit delayed action, are readily transferred between ants and kill the recipient, and are not repellent (Stringer et al. 1964). Perhaps an ant trail treatment with NaHCO_3 powder, sucrose baits containing NaHCO_3 , or a combination of both, may provide safe methods of Argentine ant control in and around homes.

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