# Argentine Worker Ant (Hymenoptera: Formicidae) Mortality Response to Sodium Salicylate and Sodium Cinnamate[1]

Megan E. Babb, ChulHee Kang[2], Mark A. Brinkman, and Cathy Lee[3]

Department of Biology, Gordon State College, Barnesville, Georgia 30204 USA
[2]Department of Chemistry, Washington State University, Pullman, Washington 99164.

J. Entomol. Sci. 49(4): 1-10 (October 2014)

**Abstract** The mortality response of Argentine worker ants, *Linepithema humile* (Mayr), following exposure to four phenolic compounds (sodium salicylate, sodium cinnamate, *p*-coumaric acid, caffeic acid) known to serve in plant defense mechanisms, boric acid, and sodium bicarbonate was assayed in laboratory testing. Argentine ant mortality after 3 days of exposure to sodium salicylate powder was significantly ($F$ = 129.69; df = 3,2; $P$ < 0.0001) higher than ant mortality in the untreated controls and in treatments with boric acid and sodium bicarbonate powders. Worker ant mortality after 3 days exposure to either sodium salicylate or cinnamic acid powder was significantly ($F$= 124.56, df =4,2, $P$ < 0.0001) higher than mortality of worker ants in control, *p*-coumaric acid, and caffeic acid treatments. Assays with a range of concentrations (0, 0.625, 1.25, 2.5, and 5.0%) of either sodium salicylate or sodium cinnamate dissolved in 10% sucrose solution showed significantly higher mortality with 5% sodium salicylate ($F$ = 15.03; df = 4,2; $P$ < 0.0001) and 5% sodium cinnamate ($F$ = 30.14; df = 4,2; $P$ < 0.0001) than the lower concentrations tested. In summary, salicylic acid and cinnamic acid, both in powder forms and liquid sucrose baits caused a higher Argentine worker ant mortality than either boric acid or sodium bicarbonate. Both appear to be promising candidates for further development as Argentine ant control products.

**Key Words** plant defensins, phenolic compounds, boric acid, Argentine ant

The Argentine ant, *Linepithema humile* (Mayr), is native to South America and was likely introduced to the U.S. around 1908 via the port of New Orleans, LA (Suarez et al. 2001). These large colony-producing ants quickly became common agricultural pests (Tsutsui et al. 2001) and are now in the top 100 of the U.S.'s worst animal invasive species (Simberloff and Gibbons 2004). Insecticides are available for use against Argentine ants, but repeated treatments are often needed adding to homeowner costs and safety concerns. Thus, there is a need to develop new control products that are effective, safe, and inexpensive.

Alternative candidates could be from widely distributed plant-origin compounds, some of which are effective at repelling, deterring, and/or are lethal to a variety of insect pests (Scocco et al. 2012). Numerous phenolic compounds have been found in the plant kingdom which function as defensive molecules against microbial pathogens, insects, and other herbivorous animals. When stimulated by external factors (e.g., microbial infections, UV light, mechanical wounding), most plants significantly

[1]Received 14 November 2013; accepted for publication 14 May 2014.
[3]Corresponding author (e-mail: clee@gordonstate.edu).

[AQ1] increase those phenolic compounds in their tissues (Benhamou 1996, Matsyki 1996, Kuc 1995). For example, methyl salicylate is a natural defense chemical that some plants use to fend off insect pests (Shulaev et al. 1997). Zinc salicylate is used in insect repellents and, thus, is one of the recommended methods for inhibiting the transmission of vector-borne diseases (Petrus1997).

Sodium salicylate is a water-soluble form of salicylic acid (Van Sumere 1989) that has a similar chemical structure to aspirin (acetylsalicylic acid). Aspirin is often used as an analgestic to minor aches and pains, as an antipyretic to reduce fever, and as an antiinflammatory medication. However, in addition to its multiple cellular and systemic effects, large doses of aspirin are known to be toxic and interfere with several cellular metabolic pathways (e.g., the Krebs cycle and oxidative phosphorylation) leading to metabolic acidosis (Hill 1973, Temple 1978, O'Malley 2007). In plants, salicylic acid is synthesized from *o*-coumarate through hydroxylation of cinnamate (Chadha and Brown 1974).

The objective of this research was to determine the response of Argentine worker ants to several plant phenolic metabolites of the salicylic acid biosynthesis pathway with specific focus on cinnamate, coumarate, and caffeic acid. Our ultimate purpose in these assays was to identify metabolites that may serve as efficacious and safe compounds for potential development for Argentine ant management.

## Materials and Methods

**Ants.** All Argentine ant workers used in these experiments were collected from nests in logs and leaf litter in different areas of a 14-ha wooded lot on the Gordon State College campus in Barnesville, GA (Lamar Co.). These ants were housed in a plastic Sterilite® container (0.079 $m^3$, Sterilite Corp., Townsend, MA) lined with Insect-a-Slip (BioQuip Products Inc., Rancho Dominguez, CA) to prevent escape. Pine litter was collected along with the live ants for nesting material, and the litter was sprayed with water occasionally to maintain moisture. The ants were provided 10% sucrose solution for food at the laboratory with the same temperature and humidity with the science building.

**Powdered compound tests (sodium salicylate, boric acid, sodium bicarbonate).** Sodium salicylate (modified from salicylic acid; Sigma Aldrich, St. Louis, MO), boric acid (Enoz Roach Away, Willert Home Products Inc., St. Louis, MO), and sodium bicarbonate (Thomas, 1997) were tested as powders at a concentration of $1.74 \times 10^{-3}$ g/$cm^2$. The respective powders were evenly distributed on the bottoms (104.5 $cm^2$ surface area) of plastic containers (8.5W x 12.3L x 7H cm; Glad® Products Co., Oakland, CA). These compounds were compared with a nontreated control. Eppendorf microcentrifuge tube (1.5 mL) caps containing 10% sucrose solution were placed in the plastic containers as food, and 20 Argentine ant workers were added to each container. Treatments were replicated 3 times (3 containers per treatment). Each container was checked daily for 3 days with dead workers being removed at each observation. The test was repeated 9 times between 5 March and 4 April 2013. Mortality data were analyzed by the PROC MIXED procedure with repeated measures in SAS (Littell et al. 1996), and means were separated with LSD ($P = 0.05$).

**Powdered compound tests.** Sodium salicylate, cinnamic acid, caffeic acid, and *p*-coumaric acid were tested in powder form in separate assays by methods previously described. All were compared with a nontreated control. This experiment was repeated 9 times between 22 August and 9 September 2011. Data were also analyzed by the PROC MIXED procedure with repeated measures in SAS, and means were separated with LSD ($P = 0.05$).

**Liquid bait tests.** Because salicylic acid and cinnamic acid were not readily soluble in water, sodium salicylate and sodium cinnamate were synthesized from salicylic acid and cinnamic acid (Sigma Aldrich, St. Louis, MO) by acid-base extraction (Harwood and Moody 2009). The acid-base extraction converted our compounds into salts which we then dissolved in a 10% (w/v) sucrose solution to create the test solutions.

Sodium salicylate and sodium cinnamate were individually serially diluted in 10% sucrose to create concentrations of 5, 2.5, 1.25, and 0.625% of each compound. Eppendorf microcentrifuge tube caps (1.5 mL) filled with 200 µL of assigned experimental solution were placed in each container along with 20 worker ants. Each sodium salicylate concentration plus a control of 10% sucrose without the sodium salicylate were replicated 3 times per experiment, and the experiments were repeated 5 times between 2 November 2010 and 10 February 2011. Mortality was checked every 24 h for 3 d. The sodium cinnamate concentrations plus a control were replicated 3 times per experiment and were repeated 4 times between 20 September and 24 October 2011.

As described above, ant mortality was checked daily for a period of 3 days for each experiment. Data were analyzed by the PROC MIXED procedure with repeated measures in SAS, and means were separated with LSD ($P = 0.05$).

## Results

**Powdered compound tests.** Cumulative mortality for Argentine worker ants exposed to sodium salicylate powder at a concentration of $1.74 \times 10^{-3}$ g/cm$^2$ was significantly ($F = 129.69$; df = 3,2; $P < 0.0001$) higher than mortality of nontreated workers and ants exposed to either boric acid or sodium bicarbonate. Following correction for control mortality (Abbott 1925), Argentine ant mortality after 3 d exposure to sodium salicylate, boric acid, and sodium bicarbonate was 100.0, 66.3, and 18.6% ($F = 129.69$; df = 3,2; $P < 0.0001$), respectively (Fig. 1). After correction for control mortality, Argentine ant mortality in containers treated with sodium salicylate, cinnamic acid, caffeic acid, and *p*-coumaric acid powders was 100.0, 80.4, 3.5, and 2.3% ($F = 124.56$; df = 4,2; $P < 0.0001$), respectively (Fig. 2). Argentine ant mortality after 3 d exposure to sodium salicylate and cinnamic acid powder was significantly higher than mortality for ants in nontreated control, *p*-coumaric acid, and caffeic acid ($F = 124.56$ df =4,2, $P < 0.0001$).

**Sucrose bait tests.** The corrected mortality of Argentine ants exposed to 5% sodium salicylate was 29.3% and was significantly ($F = 15.03$; df = 4.2; $P < 0.0001$) higher than that observed in the other treatments (Fig. 3). In like manner, the corrected mortality of worker ants exposed to 5% sodium cinnamate was 63.3% (Fig. 4) and was significantly higher ($F = 30.14$; df = 4,2; $P < 0.0001$) than for the control and other sodium cinnamate treatments.

## Discussion

The mode of action for salicylic acid and cinnamic acid in Argentine worker ant mortality is unclear, but the results from the tests in which workers were immersed in salicylic acid and cinnamic acid powder and solution containing salt forms of salicylic acid and cinnamic acid suggest that brief exposure to these agents is sufficient to

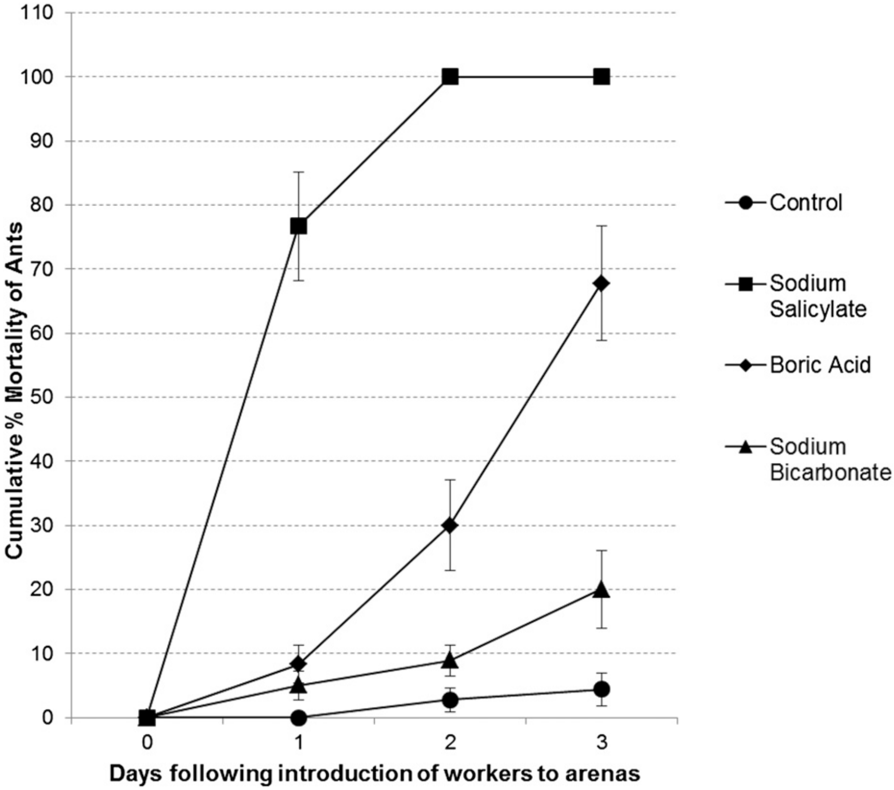


**Fig. 1. Cumulative mortality of Argentine ants exposed to powdered sodium salicylate (■), boric acid (◆), sodium bicarbonate (▲). Control (●) was a 10% sucrose liquid food bait only and experimental groups were exposed to the powdered compounds respectively in their arena.**

noticeably impact ant health. Furthermore, worker ant mortality increased following 2 - 3 days of exposure to salicylic acid and cinnamic acid on surfaces or in baits. Many of the ants surviving exposure to salicylic acid and cinnamic acid-treated containers were sluggish, but their foraging behavior did not completely cease. Some ants were motionless and appeared to be dead, but moved appendages after being touched with forceps. Although worker ants could avoid liquid baits containing sodium salicylate or sodium cinnamate in our study, the ants were observed foraging the treated baits. This suggests that sodium salicylate and sodium cinnamate are not repellent. However, in these experiments, we measured the mortality of ants, not the uptake of sucrose containing sodium salicylate or sodium cinnamate. There may have been some degree of preference for untreated dishes; however, the reduced number of live ants in treated dishes was more likely due to mortality caused by treatments rather than potential repellency of sodium salicylate and sodium cinnamate. When comparing with control group, dead ants in the treated group must have ingested food baits containing sodium salicylate and sodium cinnamate. It is interesting to note that

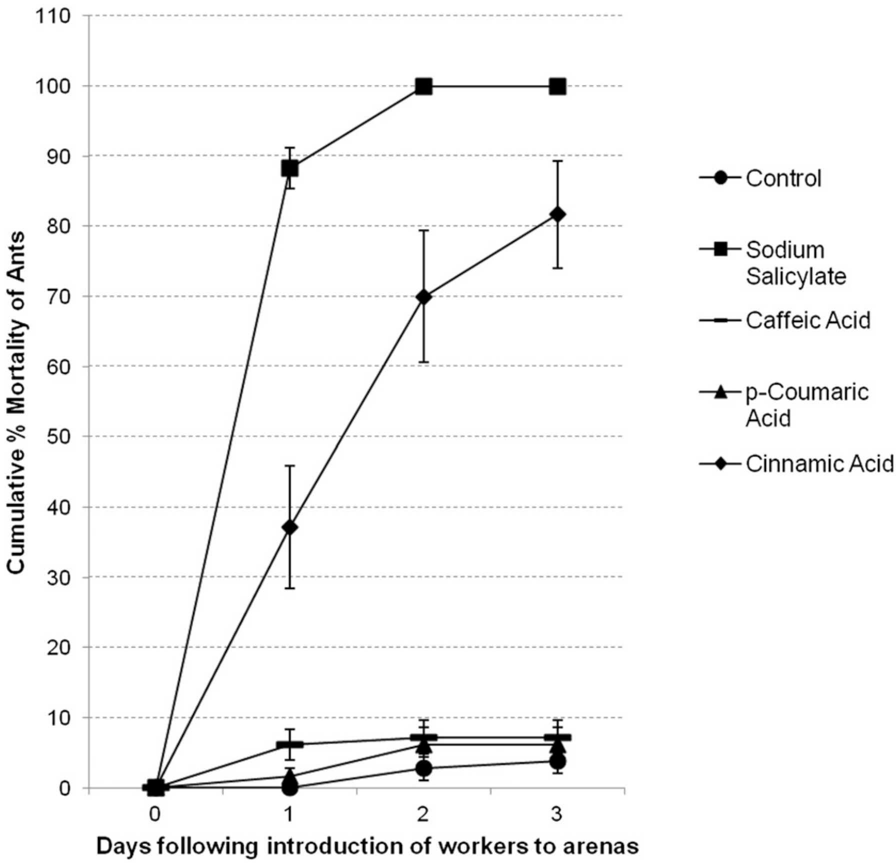


**Fig. 2. Cumulative mortality of Argentine ants exposed to powdered sodium salicylate (■), cinnamic acid (◆), caffeic acid (▬), and *p*-coumaric acid (▲). Control (●) was a 10% sucrose liquid food bait only and experimental groups were exposed to the powdered compounds respectively in their arena.**

Scocco et al. (2012) in the study of repellency of 5 essential oils to *L. humile,* showed that cinnamon essential oil in n-hexane solution exhibited repellent activity to Argentine ants. A major challenge of developing liquid bait insecticides for Argentine ants is to formulate an active ingredient that is highly preferred by Argentine ants (Baker et al. 1985). The concentration of active ingredient must be high enough to cause mortality. On the other hand, it should be low enough to kill ants in 1 - 4 days, allowing enough time for bait to spread throughout the exposed colony (Rust et al. 2004). Therefore, the level of mortality observed with sodium salicylate and sodium cinnamate is promising and deserves further investigation in both laboratory and field setting.

When we compared worker ant mortality following exposure to the powder forms of sodium salicylate and cinnamic acid with caffeic acid and *p*-coumaric acid, the greatest

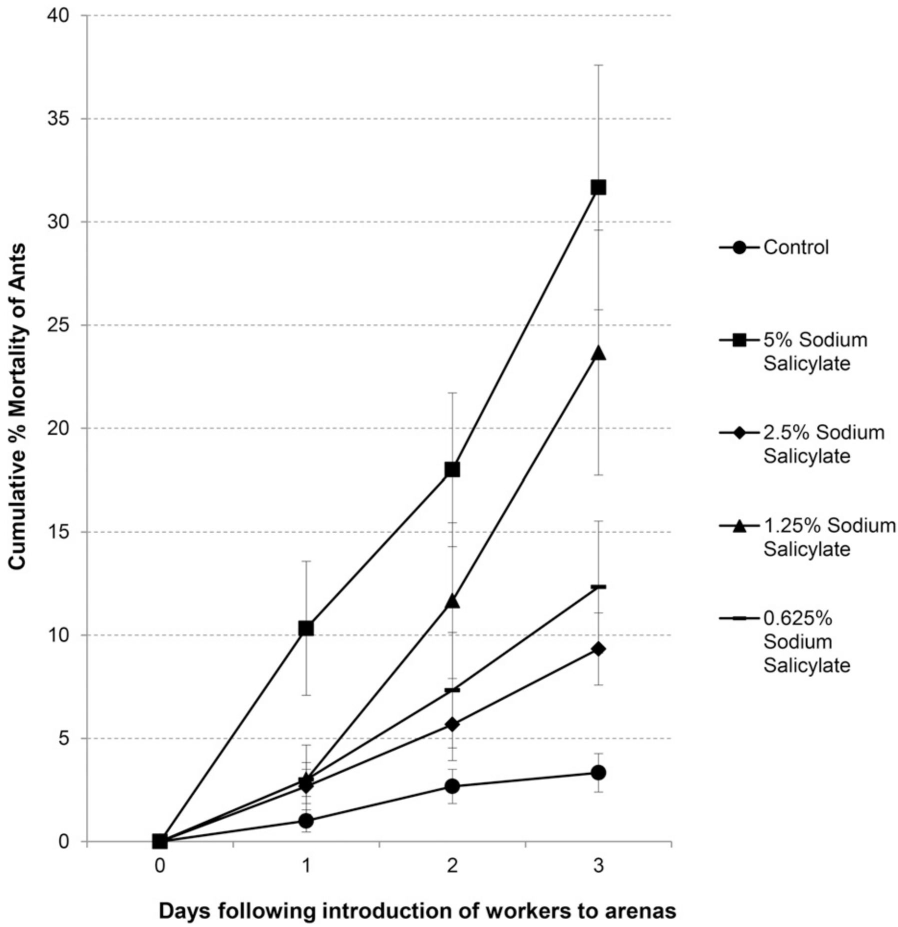


**Fig. 3. Cumulative mortality of Argentine ants fed various concentrations of sodium salicylate in a 10% sucrose liquid food bait with 5% (■), 2.5% (◆), 1.25% (▲), 0.625% (▬) respectively. Control (●) was a 10% sucrose liquid food bait only.**

mortality was observed in the sodium salicylate treatment with 100% mortality on day 2, 70% mortality by cinnamic acid on day 2, and less than 7% by caffeic acid and *p*-coumaric acid during the same time interval. These results show a striking difference on Argentine ant mortality by these structurally-similar phenolic compounds. One of salicylic acid biosynthetic pathways in plants is started from phenylalanine, which is deaminated to transcinnamic acid then, to *o*-coumarate to salicylic acid. Therefore, chorismate/prephenate/phenylalanine is a starting point of the synthetic pathway for those phenolic compounds such as salicylic acid (Raskin 1992). Phenylalanine ammonia lyase catalyzes the deamination of phenylalanine to cinnamate (Sewalt et al. 1997). Sodium cinnamate is a water-soluble form of cinnamic acid, which is found in cinnamon,

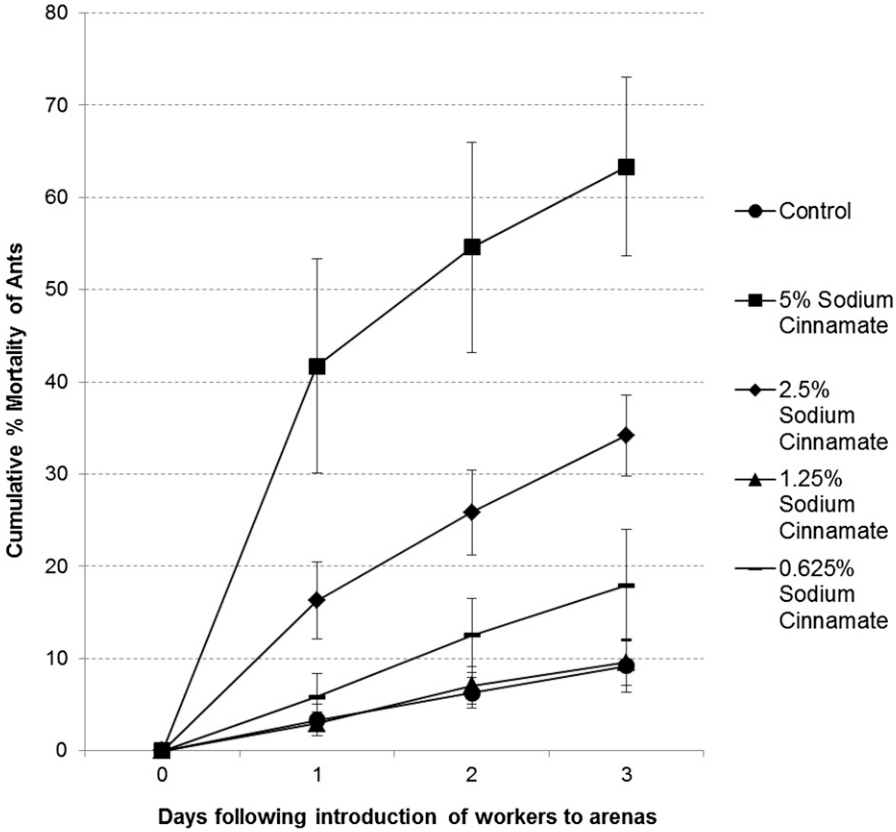


**Fig. 4. Cumulative mortality of Argentine ants fed various concentrations of sodium cinnamate in a 10% sucrose liquid food bait with 5% (■), 2.5% (◆), 1.25% (▲), 0.625% (▬) and control (●). Control was a 10% sucrose liquid food bait only.**

*Cinnamomum* spp., and is also a plant defensin in turnips (*Brassica rapa* [L.]) (Abdel-Farid et al. 2009). In this pathway, other phenolic intermediates such as *p*-coumaric acid and caffeic acid are produced through transferase and oxidoreductases. All of these phytochemicals are considered bioactive. However, caffeic acid is not a major intermediate of salicylic acid biosynthetic pathway even if its structure is similar to other compounds tested. It showed very low mortality, suggesting that more than phenolic moieties are involved in causing the worker ant mortality. The *p*-coumaric acid is one of the indirect metabolites of salicylic acid biosynthesis, and we observed low Argentine ant mortality, less than 7% caused by *p*-coumaric acid treatment. Among the intermediates we tested on worker ants, the powder forms of sodium salicylate and sodium cinnamate were most potent inducing the death of the worker ants.

In humans, salicylic acid is an active metabolite of aspirin, which is known as acethyl salicylic acid. Aspirin is used for antipyretic, antiinflammatory, and analgesic medication. It inhibits the cyclooxygenases in an irreversible manner, which affects more the COX-1 variant than the COX-2 variant enzymes (Burke et al. 2006).

Boric acid is widely used as common household insecticide. Boric acid has been reputed to be a low-toxicity pesticide for indoor-nesting ants (Klotz and Shorey 2000). Klotz et al. (2002) found extensive damage to cell and microvilli of the midguts of Argentine ants following consumption of boric acid. Our data showed that the mortality of Argentine ants by sodium salicylate and cinnamic acid is much higher than those observed with boric acid and sodium bicarbonate (Fig. 1). Furthermore, on the second day of exposure, sodium salicylate resulted in 100% mortality whereas boric acid caused 30% mortality and sodium bicarbonate caused less than10% mortality. On the third day of treatment, sodium salicylate caused 100% mortality, boric acid 68%, and sodium bicarbonate 20%. Mortality from exposure to salicylic acid and cinnamic acid powders was much higher than exposure to the same compounds in liquid bait form of 5% sodium salicylate in 10% sucrose solution. Slightly less mortality was shown in the powder forms of cinnamic acid. Our data show that lethality of ant worker by sodium salicylate powder is higher (100% on day 2) than that of boric acid (30% on day 2) and sodium bicarbonate (less than 10% on day 2).

Salicylic acid and cinnamic acid at neutral pH are nonpolar. Therefore, we could not dissolve significant amounts of these compounds in sugar water baits. Instead we synthesized salts form of these compounds. When a range of concentrations of sodium salicylate and sodium cinnamate, mixed with sugar was tested, the greatest mortality was observed in the 5% sodium salicylate-sugar water treatment. Our results show concentration–dependent response, ranging from 0.625%, 1.25%, 2.5% and 5%. In those experiments, we normalized our concentrations following the $LC_{50}$ reported by Brinkman et al. (2004) for sodium bicarbonate. However, we could not conduct the test above 5% because sodium salicylate and sodium cinnamate were precipitated out of solution and, thus, were not available for ant consumption. Whereas direct observations of ant feeding were not made during our experiments, ants were provided a choice between sugar water with and without sodium salicylate and sodium cinnamate. Thus, these results suggest that ants were not deterred by sodium salicylate and sodium cinnamate. The similar approach and interpretation were reported in the previous experiments with sodium bicarbonate (Dethier et al. 1960).

A key challenge in the development of new pesticides is the balance among effectiveness, safety, and biodegradability. Despite cytotoxicity, the exact molecular mechanism of worker ant mortality by sodium salicylate and sodium cinnamate is unclear. Indepth understanding of how salicylic acid and cinnamate function from multidimensional investigation will provide additional tools to tailor the safer compounds for Argentine ant control.

## Acknowledgments

This work was supported in part by the Division of Mathematics and Natural Sciences of Gordon State College and the Faculty Development Fund of Gordon State College. The authors thank M.C. Fermin-Ennis and A. Osborne for their technical assistance, A. Duffus for reviewing earlier drafts of this manuscript, and J. Parker, B. Mcelroy, J. Marks, S. Connell, N. Baker, S. Kelly, J. Stephens, E. Antakikam, Jr., and S. Rountree in the Special Research Topics and Undergraduate Research Topics in Biology Courses.

## References Cited


**Abbott, W. S. 1925.** A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

**Abdel-Farid, I. B., M. Jahangi, C. A. von den Hondel, H. K. Kim and R. Verpoorte. 2009.** Fungal infection-induced metabolites in *Brassica rapa.* Plant Sci. 176: 608-615.

**Baker, T. C., S. E. Van Vorhis Key and L. K. Gaston. 1985.** Bait–preference tests for the Argentine ant (Hymenoptera: Formicidae). J. Econ. Entomol. 78: 1083-1088.

**Benhamou, N. 1996.** Elicitor-induced plant defense pathways. Trends Plant Sci. 1: 233-240.

**Brinkman, M. A., W. A. Gardner and R. M. Ipser. 2004.** Red imported fire ant (Hymenoptera: Formicidae) mortality following exposure to sodium hydrogen carbonate (NaHCO3). J. Entomol. Sci. 39: 188-201.

**Burke, A., E. Smyth and G. A. FitzGerald. 2006.** Analgesic antipyretic and antiinflammatory agents.Goodman and Gilman's the pharmacological basis of therapeutics (11 ed.). New York: McGraw-Hill.

**Chadha, K. C. and S. A. Brown. 1974.** Biosynthesis of phenolic acids in tomato plants infected with *Agrobacterium tumefaciens.* Can. J. Bot. 52: 2041-2047.

**Dethier, V. G., L. B. Broan and C. N. Smith. 1960.** The designation of chemicals in terms of the responses they elicit from insects. J. Econ. Entomol. 53: 134-136.

**Harwood, L. M. and C. J. Moody. 2009.** Experimental organic chemistry: principles and practice. Wiley-Blackwell Scientific Publications, Hoboken, NJ.

**Hill, J. B. 1973.** Salicylate intoxication. N. Engl. J. Med. 288: 1110.

**Klotz, J. H. and H. Shorey, 2000.** Low-toxic control of Argentine ants using pheromone-enhanced liquid baits. California Depart. Consumer Affairs; CDCA 84SA8020-07.

**Klotz, J. H., C. Amrhein, S. McDaniel, M. K. Rust and D. A. Reierson. 2002.** Assimilation and toxicity of boron in the Argentine ant (Hymenoptera: Formicidae). J. Entomol. Sci. 37: 193-199.

**Kuc, J. 1995.** Phytoalexins, stress metabolism, and disease resistance in plants. Annu. Rev. Phytopathol. 33: 275-297.

**Littell, R. C., G. S. Milliken, W. W. Stroup and R. D. Wolfinger. 1996.** SAS system for mixed models. SAS Institute, Inc., Cary, NC, pp 633.

**Matsuki, M. 1996.** Regulation of plant phenolic synthesis: from biochemistry to ecology and evolution. Aust. J. Bot. 44: 613-634.

**Oksana, S., B. Marian, R. Mahendra and S. H. Bo. 2012.** Plant phenolic compounds for food,pharmaceuticaland cosmetics production. J. Med. Plants Res. 6: 2526-2539.

**O'Malley, G. F. 2007.** Emergency department management of the salicylate-poisoned patient. Emerg. Med. Clin. North Am. 25: 333.

**Petrus, E. J. 1997.** Insect repellent composition and method of inhibiting the transmission and treatment of symptoms of vector-borne diseases. United States Patent 5965137.

**Raskin, I. 1992.** Role of salicylic acid in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43: 439-463.

**Rust, M. K., D. A. Reierson and J. H. Klotz. 2004.** Delayed toxicity as a critical factor in the efficacy of aqueous baits for controlling Argentine ants (Hymenoptera: Formicidae). J. Econ. Entomol. 97: 24.

**Scocco, C. M., D. R. Suiter and W. A. Gardner. 2012.** Repellency of five essential oils to *Linepithema humile* (Hymenoptera: Formicidae). J. Entomol. Sci. 47: 150-159.

**Sewalt, V. J. H., W. Ni, J. W. Blount, H. G. Jung, S. A. Masoud, P. A. Howles, C. Lamb and R. A. Dixon. 1997.** Reduced lignin content and altered lignin composition in transgenictobacco down-regulated in expression of L-phenylalanine ammonia-lyase or cinnamate 4-hydroxylase. Plant Physiol. 115: 41-50.

**Shulaev, V., P. Silverman and I. Raskin. 1997.** Airborne signaling by methyl salicylate in plant pathogen resistance. Nature 385: 718-721.

**Simberloff, D. and L. Gibbons. 2004.** Now you see them, now you don't – population crashes of introduced species. Biol. Invasions 6: 161-172.

**Stark, L. A., K. Reid, O. J. Sansom, F. V. Din, S. Guichard, I. Mayer, D. I. Jodrell, A. R. Clarkeand and M. G. Dunlop. 2007.** Aspirin activates the NF-κB signalling pathway and induces apoptosis in intestinal neoplasia in two *in vivo* models of human colorectal cancer. Carcinogenesis 28: 968-976.

**Suarez, A. V., D. A. Holway and T. J. Case. 2001.** Patterns of spread in biological invasions dominated by long-distance jump dispersal: insights from Argentine ant. Biol. Invasions 1: 43-53.

**Temple, A. R. 1978.** Pathophysiology of aspirin overdosage toxicity, with implications for management. Pediatrics 62: 873.

**Tsutsui, N. D., A. V. Suarez, D. A. Holway and T. J. Case. 2001.** Relationship among native and introduced populations of the Argentine ant (*Linepithema humile*) and the source of introduced populations. Mol. Ecol. 10: 2151-2161.

**Thomas, M. H. 1997.** Dry powder consisting of sodium bicarbonate, ant attractant, red chili powder. United States Patent 5618565.

**Van Sumere, C. F. 1989**. Methods in plant biochemistry, Pages 88-118. *In* Dey, P.M. and Harborne, J.B. (eds.), Plant Phenolics, Academic Press, London.

**AUTHOR QUERIES**

DATE 06/10/2014
JOB NAME ENTO
ARTICLE 1339
QUERIES FOR AUTHORS Babb et al.

This query form must be returned with all proofs for Corrections

PLEASE ANSWER THE AUTHOR QUERIES WHERE THEY APPEAR IN THE TEXT.

AU1) The in-text citation “Matsyki 1996” is not in the reference list. Please correct the citation, add the reference to the list, or delete the citation.
AU2) Reference “Matsuki, 1996” is not cited in the text. Please add an in-text citation or delete the reference.
AU3) Reference “Oksana, Marian, Mahendra, Bo, 2012” is not cited in the text. Please add an in-text citation or delete the reference.
AU4) Reference “Stark, Reid, Sansom, Din, Guichard, Mayer, Jodrell, Clarkeand, Dunlop, 2007” is not cited in the text. Please add an in-text citation or delete the reference.