Journal of Economic Entomology, 112(2), 2019, 691–698
doi: 10.1093/jee/toy360
Advance Access Publication Date: 10 December 2018
Research

Insecticidal Activity of Methyl Benzoate Analogs Against Red Imported Fire Ants, *Solenopsis invicta* (Hymenoptera: Formicidae)

Jian Chen,[1,4] Tahir Rashid,[2] Guolei Feng,[2] Yan Feng,[3] Aijun Zhang,[3] and Michael J. Grodowitz[1]

[1]US Department of Agriculture, Agricultural Research Service, National Biological Control Laboratory, Stoneville, MS, [2]Alcorn State University, Extension/Research Demonstration Farm & Technology Transfer Center, 294 Grainger Dorsey Road, Mound Bayou, MS, [3]US Department of Agriculture, Agricultural Research Service, Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, MD, and [4]Corresponding author, e-mail: jian.chen@ars.usda.gov

Subject Editor: Michael Brewer

Received 31 July 2018; Editorial decision 28 October 2018

Abstract

Although insecticidal properties of certain benzoates have been investigated for pest insects and mites, toxicity of benzoates to the red imported fire ants, *Solenopsis invicta* Buren, has never been reported. In this study, 15 commercially available benzoates were assessed for their contact and fumigation toxicity to *S. invicta* workers and their chemical structure–activity relationships. Among tested benzoates, benzylbenzoate, *n*-pentybenzoate, and *n*-hexylbenzoate were three most potent contact toxins against *S. invicta* workers (mean LD_{50} value = 23.31, 35.26, 35.99 µg per ant, respectively) and methyl-3-methoxybenzoate, methyl-3-methylbenzoate, and methylbenzoate were the three most potent fumigants (mean LC_{50} value = 0.61, 0.62, 0.75 µg/ml, respectively). For nonsubstituted alkyl benzoates (esters of benzoic acid and C1–C6 linear alcohols), the contact toxicity was positively correlated to the alkyl chain length ($r = 0.89$), while the fumigation toxicity was negatively correlated ($r = 0.90$). Presence of a methoxyl group at either the ortho or meta position of methylbenzoate significantly increased its contact toxicity, so did a methyl group at meta position. However, presence of a methyl group at ortho position reduced the contact toxicity. Presence of methyl or methoxyl group at the meta position did not have significant effect on the fumigation toxicity; however, methyl, methoxyl, chloro, or nitro groups at the ortho position significantly reduced fumigation toxicity. Hexylbenzoate has neither known Occupational Safety and Health Administration hazards nor aquatic toxicity, and methyl 3-methoxybenzoate is not considered a hazardous substance, indicating a great potential for their application in fire ant management.

Key words: insecticide, benzoates, contact toxicity, fumigation toxicity, naturally occurring compound

The red imported fire ant, *Solenopsis invicta* Buren, is one of the most successful invasive ants in the world and is regarded as one of World's worst invasive alien species (Lowe et al. 2000). Native to South America, *S. invicta* has been introduced into many countries and regions, including the United States, Australia, China, Philippines, Thailand, Taiwan, Hong Kong, Macau, etc. (Ascunce et al. 2011). The ant has great potential to further expand its distribution (Morrison et al. 2004, 2005). Due to their aggressiveness and venomous sting, *S. invicta* are a significant threat to humans, wildlife, crops, and livestock (Vinson 2013). Like many other insect pests, management of *S. invicta* heavily depends on the application of synthetic insecticides (Williams et al. 2001, Drees et al. 2013). There is an increasing desire to use toxicologically and environmentally benign chemicals in insect pest management. Naturally occurring toxins often have more desirable properties than conventional synthetic pesticides for pest management programs because of their rapid environmental biodegradation, reduced nontarget effects, and generally lower toxicity to nontargets like natural enemies, humans, and other vertebrates (Copping and Duke 2007).

Various benzoates occur in plants (Pino et al. 2005, Wang et al. 2005, Adams 2007, Mojtaba et al. 2011, Huang et al. 2013, Yang et al. 2013). Certain benzoates also occur in insects, such as methylbenzoate in the scarab beetle, *Anomala albopilosa* (Hope) (Leal et al. 1996), harvester ant, *Messor barbarous* (L.) (Co et al. 2003), and orchid bee, *Euglossa cybelia* Moure (Schiestl and Roubik 2003); ethylbenzoate in the butterfly, *Bicyclus martius* (Fabricius) (Wang et al. 2014); and benzylbenzoate in the sawfly, *Nematus prasinus* Hartig (Boevé et al. 1992). Certain benzoates are well known for

their insecticidal (Yang et al. 2013, Feng and Zhang 2017) and acaricidal property (Harju et al. 2004, Suhaili and Ho 2008, Dressler et al. 2016). For example, benzylbenzoate is used to treat scabies, a contagious skin infestation caused by the mite *Sarcoptes scabiei* (Linnaeus) (Dressler et al. 2016). Benzylbenzoate is effective against the house dust mite, *Dermatophagoides pteronyssinus* (Trouessart) (Suhaili and Ho 2008) and the grain storage mite, *Tyrophagus putrescentiae* (Schrank) (Harju et al. 2004). Benzylbenzoate is ovicidal to the whitebacked planthopper, *Sogatella furcifera* (Horváth) (Yang et al. 2013). Methylbenzoate was recently reported to be insecticidal to a variety of insect pests, including the brown marmorated stinkbug, *Halyomorpha halys* Stål, diamondback moth, *Plutella xylostella* (L.), tobacco hornworm, *Manduca sexta* (L.), and spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Feng and Zhang 2017). These research results indicate that benzoate analogs may have broad-spectrum pesticidal properties.

Both contact insecticides and fumigants have been used for treating fire ant mounds (Thorvilson et al. 1989, Drees et al. 2013). A safe fumigant can be very useful for objects that cannot be treated directly with existing synthetic insecticides. Since insecticidal activity of benzoates has been discovered for many insect species, we hypothesized that certain benzoates may also be toxic to *S. invicta*. In this study, 15 commercially available benzoates were assessed for their contact and fumigation toxicity against *S. invicta* workers.

Materials and Methods

Insects

Red imported fire ant colonies were collected from Washington County, Mississippi. Colonies were separated from soil using water dripping method (Banks et al. 1981). Each colony was housed in a plastic tray (44.5 × 60.0 × 13.0 cm). A 14.0 × 2.0 cm petri dish with 10-cm-thick bottom layer of hardened dental plaster (Castone; Dentsply International Inc., York, PA) was used as an artificial nest. The lid of the petri dish was painted in black. The social form of *S. invicta* colonies was determined using Polymerase chain reaction on Gp-9 alleles (Valles and Porter 2003). All ants used in laboratory bioassays were from monogyne colonies. Ants fed ad lib on 10% sugar water and house crickets. Colonies were maintained in a rearing room at 25°C, 80% relative humidity (RH) with a photoperiod of 12:12 (L:D) h.

Toxicity Bioassays

Contact toxicity

Topical application was used for contact toxicity bioassays. Only large workers (body weight: 3.54 ± 0.37 mg [mean ± SD]) were used to provide relatively uniform body weight and facilitate test compound application onto the ant body. For all compounds, acetone (HPLC Plus, purity ≥99.9%, Sigma-Aldrich, Saint Louis, MO) was used as a solvent. The test solution or solvent control (0.779 µl) was applied using a capillary tube. Fifteen benzoates (Sigma-Aldrich) were tested, including methylbenzoate, ethylbenzoate, vinylbenzoate, methyl-2-methylbenzoate, methyl-3-methylbenzoate, methyl-2-chlorobenzoate, methyl-2-nitrobenzoate, *n*-propylbenzoate, *n*-butylbenzoate, *n*-pentylbenzoate, isobutylbenzoate, *n*-hexylbenzoate, benzylbenzoate, methyl-3-methylbenzoate, and methyl-3-methoxybenzoate (Fig. 1). Two experiments were conducted for assessing contact toxicity. In experiment 1, mortality was measured at a dose of 77.9 µg per ant for all 15 benzoates, to rank the toxicity of tested compounds. The 77.9 µg per ant concentration was used because a preliminary bioassay indicated that only benzylbenzoate achieved 100% mortality at 77.9 µg per ant and all other compounds had variable mortality levels. Three ant colonies were used and there were three replicates for each colony. Each replicate consisted of 11–18 ants. In experiment 2, LD_{50} values were established at the colony level for the nine most toxic benzoates selected from experiment 1. To investigate the chemical structure–toxicity relationship, LD_{50} values also were established for methylbenzoate and ethylbenzoate. For *n*-propylbenzoate the LD_{50} value was established for two colonies. For other 10 benzoates, LD_{50} values were determined for three colonies. For each LD_{50} value, five to six doses of benzoate were used except for *n*-butylbenzoate in which four doses were used for one colony. Each dose was replicated three times except for ethylbenzoate in which two replicates were used for each dose. A single replicate consisted of 13–17 ants. Treated ants were placed in a 30-ml loosely capped cup, and dead ants were counted after 24 h. For a positive control, the contact toxicity of malathion (Sigma-Aldrich) to fire ant workers also was determined for three colonies. For malathion, six doses were used for determining LD_{50} values. Each dose was replicated three times and each replicate consisted of 25 ants.

Fumigation toxicity

Fumigation toxicities also were assessed for all 15 benzoates. A glass flask (280 ml, Fisherbrand, Fisher Scientific, Pittsburgh, PA) was used to house worker ants in the fumigation toxicity bioassays. A 2-µl test solution containing one of each tested compound was applied onto a paraffin film (Bemis Company, Inc, Oshkosh, WI), which was used to seal the flask with ants. The treated spot of the paraffin film faced the inside of the flask. The upper part of the flask was coated with Fluon (Insect-A-Slip, BioQuip Products, Rancho Dominguez, CA) to prevent ants from contacting the test material. Two experiments were conducted. In experiment 1, 24 h mortalities were obtained for each benzoate compound at the dosage of 1.43 µg/ml, to rank the fumigation toxicity of tested compounds. The reason for selecting this concentration was that a preliminary bioassay indicated that only methylbenzoate achieved a 100% mortality at 1.43 µg/ml and only methyl-2-nitrobenzoate did not show any mortality. Three ant colonies were used for experiments. There were three replicates for each colony and each replicate consisted of 16–26 ants. In experiment 2, LC_{50} values were established for the five most toxic benzoates selected from experiment 1. For each benzoate, LC_{50} values were determined for three colonies. Each LC_{50} value was established using five dosages. Each dosage was replicated three times. A single replicate consisted of 15–26 ants. Ants that could not stand by themselves were considered dead. Dead ants were counted after 24 h. As a positive control, the 24-h mortality of dichlorvos (2,2-dichlorovinyl dimethyl phosphate, Sigma-Aldrich) was measured using five dosages (0.04, 0.09, 0.22, 0.44, and 0.66 ng/ml) for three colonies. There were three replicates for each dosage and 25 ants were used for each replicate.

Data Analysis

Polo Plus (Version 2.0, LeOra Software, Petaluma, CA) was used to estimate LD_{50} and LC_{50} values, with 95% CIs. The relative toxicity ratio with their upper and lower 95% confidence limits was used to evaluate the significance of difference between LD_{50} values and LC_{50} values. The significance was set at $P = 0.05$ probability level. If the 95% CI of the ratio between two LD_{50} or LC_{50} values include 1, they were not considered significantly different (Robertson et al. 2007). For comparison of mortality among different treatments, analysis of variance (PROC GLM, SAS Institute, Cary, NC) was performed and means were separated using Tukey's multiple comparison test ($P < 0.05$). Arcsine square root transformation was performed on the data before the statistical analysis. Correlations between toxicity

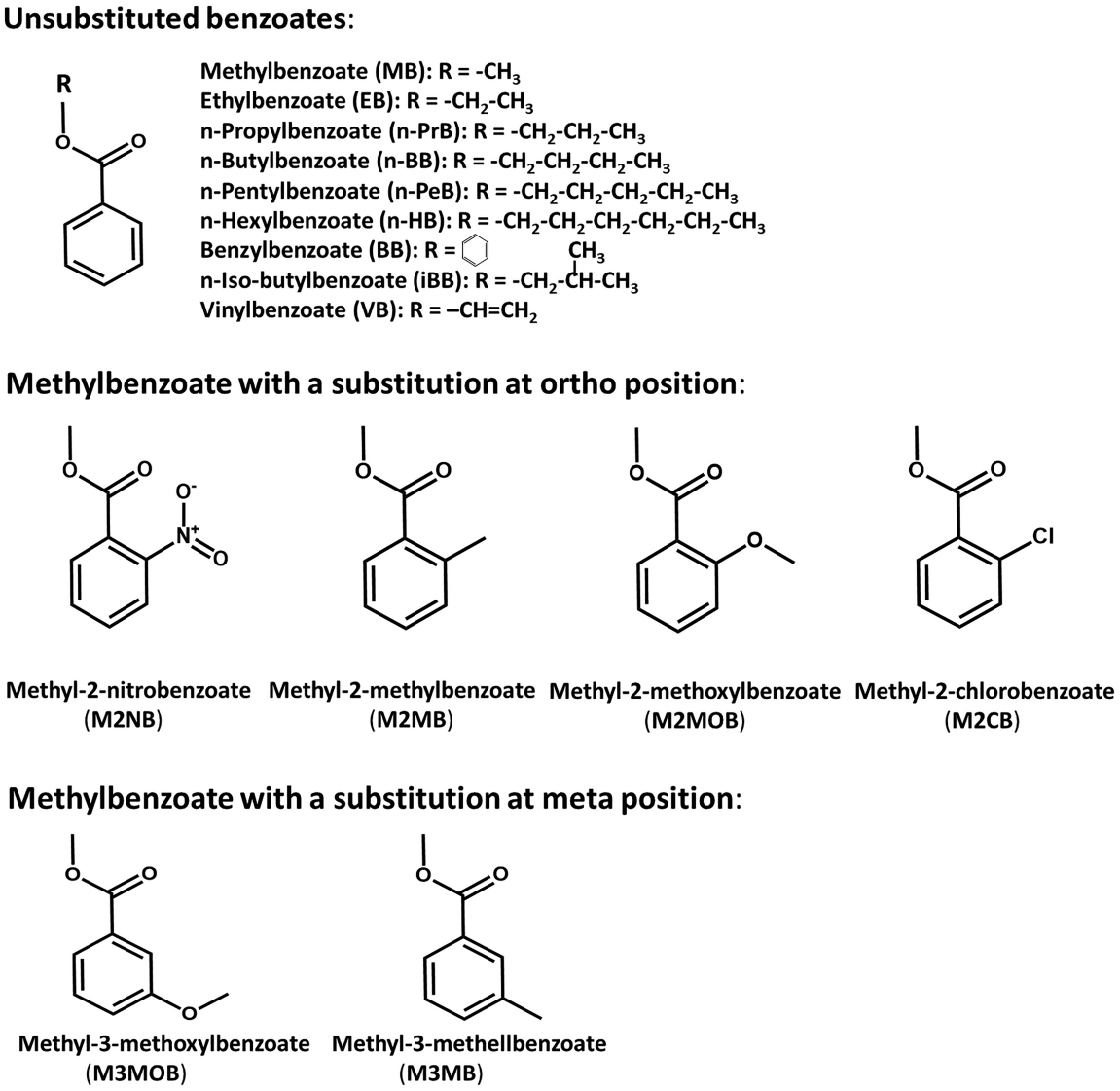

Fig. 1. Chemical structures of fifteen tested benzoates.

of alkyl benzoates (LD_{50} values for contact toxicity and mortality for fumigation toxicity) and their alkyl chain length were established (PROC Corr, SAS Institute).

Results

Contact Toxicity

In experiment 1, mortality was measured at a dose of 77.9 µg per ant for 15 benzoates. The adjusted mortality for each benzoate is shown in Fig. 2. There were significant differences in mortality among benzoate treatments (F =74.13; df =14, 90; $P < 0.0001$). The difference among colonies also was significant (F =5.60; df = 2, 90; $P = 0.005$). In experiment 2, LD_{50} values were established for the nine most toxic benzoates selected from experiment 1 (Table 1). To investigate the chemical structure–toxicity relationship, LD_{50} values also were established for methylbenzoate and ethylbenzoate (Table 1). At the dose of 77.9 µg per ant, benzylbenzoate, *n*-pentylbenzoate, *n*-hexylbenzoate, and *n*-butylbenzoate caused the greatest mortality (Fig. 2). In experiment 2, among the 11 benzoates, benzylbenzoate, pentylbenzoate, and hexylbenzoate had the lowest LD_{50} values. Malathion was more toxic than all tested benzoates. LD_{50} values for 11 benzoates ranged 18.83–164.32 µg per ant, whereas LD_{50} values for malathion ranged 0.013–0.014 µg per ant (Table 1). Based on the data of experiment 1, benzoate compounds with a methyl or nitro group at the ortho position had less contact toxicity (mortalities for methylbenzoate, methyl-2-methylbenzoate, and methyl-2-nitrobenzoate were 7.4 ± 4.52, 3.67 ± 1.56, and 2.84 ± 2.23%, respectively), but a chloro or methoxyl group at the same position increased toxicity (mortalities for methyl-2-chlorobenzoate and methyl-2-methoxylbenzoate were 20.56 ± 5.13 and 55.74 ± 6.42%, respectively). However, only the impact of the methoxyl group was statistically significant (Fig. 2). Methyl-2-methoxylbenzoate caused 6.50× greater mortality than methylbenzoate at the dose of 77.9 µg per ant (50.17 vs 7.40%) and *n*-butylbenzoate caused twice the mortality of isobutylbenzoate (84.12 vs 42.51%) (Fig. 2). Based on the data collected in experiment 2, correlation between acute toxicity (LD_{50} values) of alkyl benzoates and the alkyl chain length was established (Fig. 3). A negative correlation was found between LD_{50} values and alkyl chain length. The *r*-value (correlation coefficient) was 0. 89336, meaning that 79.81% variation was related.

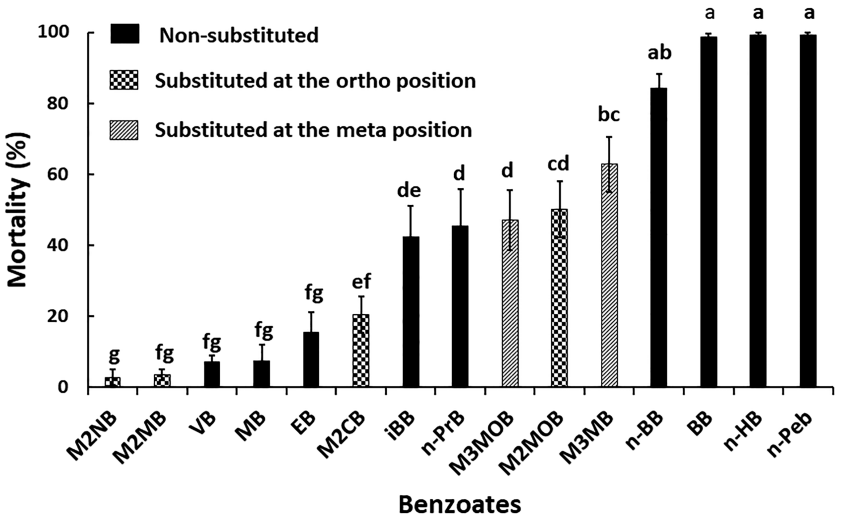

Fig. 2. Mortality (%, mean ± SE) of *S. invicta* workers 24 h after being topically treated with benzoates at a dose of 77.9 μg per ant. Mortalities labeled with different letters are significantly different ($P < 0.05$). Analysis of variance was performed and means were separated using Tukey's Multiple Comparison Test ($P < 0.05$). Arcsine square root transformation was performed on the data before the statistical analysis. For comparison of mortality among treatments, analysis of variance (PROC GLM; SAS Institute) was performed and means were separated using Tukey's Multiple Comparison Test ($P < 0.05$). M2NB: methyl-2-nitrobenzoate, M2MB: methyl-2-methylbenzoate, VB: vinylbenzoate, MB: methylbenzoate, EB: ethylbenzoate, M2CB: methyl-2-chlorobenzoate, iBB: isobutylbenzoate, *n*-PrB: *n*-propylbenzoate, M3MOB: methyl-3-methoxylbenzoate, M2MOB: methyl-2-methoxylbenzoate, M3MB: methyl-3-methylbenzoate, *n*-BB: *n*-butylbenzoate, BB: benzylbenzoate, *n*-HB: *n*-hexylbenzoate, *n*-PeB: *n*-pentylbenzoate.

Table 1. Contact toxicity (LD_{50} values) of 11 benzoates and malathion against *S. invicta* workers

Benzoate	Colony	LD_{50} (μg per ant)	95% CI	Slope (mean± SE)	χ^2 (df, *P*)
Methyl benzoate	1	149.39	130.20–168.11	7.01 ± 0.76	30.26 (13, 0.0043)
	2	93.65	88.85–98.814	11.72 ± 1.55	6.27(13, 0.94)
	3	128.45	121.23–136.88	9.49 ± 1.13	20.23(16, 0.44)
Ethyl benzoate	1	152.87	133.32–176.09	3.74 ± 0.65	9.91(8, 0.27)
	2	129.62	120.84–139.11	7.60 ± 0.85	4.96 (13, 0.98)
	3	125.76	116.11–135.69	7.13 ± 0.81	16.98 (13, 0.20)
Methyl 2-methoxybenzoate	1	67.73	65.03–70.22	24.94 ± 3.96	3.27(13, 0.99)
	2	96.43	90.63–103.99	8.43 ± 1.17	13.15 (13, 0.44)
	3	78.34	72.11–84.54	7.1 9 ± 0.88	16.05 (13, 0.25)
n–Propyl benzoate	1	109.89	101.48–124.46	7.104 ± 1.26	7.55 (13, 0.87)
	2	109.26	100.98–125.21	11.39 ± 2.47	21.23 (13, 0.07)
n-Butyl benzoate	1	77.13	72.80–82.23	10.31 ± 1.33	14.23 (13, 0.36)
	2	68.51	63.33–76.01	6.34 ± 1.00	5.61 (10, 0.85)
	3	50.04	40.54–57.83	4.73 ± 0.54	40.52 (16, 0.0007)
n-Penty benzoate	1	35.97	32.93–38.70	8.60 ± 1.17	7.44 (13, 0.88)
	2	41.16	37.80–44.34	11.66 ± 1.42	17.5 (13, 0.18)
	3	28.64	25.80–31.41	6.15 ± 0.68	10.77 (13, 0.63)
Isobutyl benzoate	1	80.07	74.30–86.15	7.06 ± 0.86	13.77 (13, 0.39)
	2	96.57	85.67–116.11	7.34 ± 1.00	39.34 (13, 0.0002)
	3	55.75	51.11–59.96	9.11 ± 1.01	15.09 (13, 0.30)
n-Hexyl benzoate	1	37.1	32.98–41.00	5.54 ± 0.62	13.15 (13, 0.44)
	2	42.23	39.30–45.01	9.14 ± 0.1.10	3.55 (13, 0.99)
	3	28.64	25.80–31.41	6.15 ± 0.68	10.77 (13, 0.63)
Benzyl benzoate	1	25.44	22.21–28.46	4.72 ± 0.52	10.48 (13, 0.65)
	2	25.68	21.48–29.46	4.15 ± 0.51	5.81 (13, 0.95)
	3	18.83	16.44–21.09	5.75 ± 0.79	5.26 (13, 0.97)
Methyl 3-methylbenzoate	1	70.32	62.00–77.63	8.40 ± 0.95	32.10 (13, 0.002)
	2	107.48	93.37–142.99	5.65 ± 0.87	28.72 (13, 0.007)
	3	67.73	65.03–70.22	24.93 ± 3.96	3.27 (13, 0.99)
Methyl 3-methoxybenzoate	1	112.72	99.69–143.06	5.69 ± 0.93	20.35 (13, 0.087)
	2	81.78	74.69–88.14	7.73 ± 1.22	13.38 (13, 0.42)
	3	47.52	41.99–52.18	7.33 ± 0.88	15.32 (13, 0.29)
Malathion (positive control)	1	0.013	0.012–0.014	9.09 ± 0.87	27.85 (13, 0.0095)
	2	0.014	0.013–0.015	9.71 ± 0.85	16.21 (13, 0.24)
	3	0.014	0.014–0.015	9.573 ± 0.88	17.01 (13, 0.20)

Fumigation Toxicity

In experiment 1, 24-h mortality was measured at a dosage of 1.43 µg/ml for 15 benzoates. The adjusted mortality for each benzoate is shown in Fig. 4. There were significant differences in mortality among benzoate treatments (F =149.42; df = 14, 90; P < 0.0001). The difference among colonies also was significant (F = 4.86, df = 2, 90; P = 0.01). Methylbenzoate, methyl-3-methybenzoate, methyl-3-methoxlbenzoate, vinylbenzoate, and ethylbenzoate caused the highest mortality. Correlation between mortalities and the alkyl chain length of alkyl benzoates was established (Fig. 5). A negative correlation was found between mortality and alkyl chain length. The r-value was 0.89941, meaning that 81.89% variation was related. In experiment 2, LC_{50} values were established for the five most toxic benzoates (Table 2). Ranked by the mean LC_{50} value, methyl-3-methoxybenzoate, methyl-3-methylbenzoate, and methylbenzoate were the three most potent fumigants among 15 tested benzoates (mean LC_{50} value = 0.61, 0.62, and 0.75 µg/ml, respectively), followed by vinylbenzoate and ethylbenzoate (LC_{50} value = 0.89 and 0.93 µg/ml, respectively). Due to a steep slope, LC_{50} values for dichlorvos could not be established for all three colonies; therefore, mortality data are presented (Fig. 6). There was a significant difference in mortality among different dichlorvos concentrations (F =120.69; df = 5, 36; P < 0.0001). The difference among colonies also was significant (F = 5.99; df = 2, 36; P = 0.0057). Complete mortality was achieved for all three colonies at concentration of 0.66 ng/ml. Dichlorvos was more toxic (LC_{50} value seemed within 0.088–0.22 ng/ml) than all of the benozates tested (LC_{50} values were within 0.54–1.14 µg/ml).

Based on the data of experiment 1, methylbenzoate compounds with a methyl or methoxyl group at meta position of benzene have fumigation toxicity close to methylbenzoate; however, methylbenzoate compounds having methoxyl, chloro, or nitro groups at ortho position had significantly lower fumigation toxicity than methylbenzoate. The least potent fumigant is methyl-2-nitrobenzoate, which did not cause any mortality at a dosage of 1.43 µg/ml.

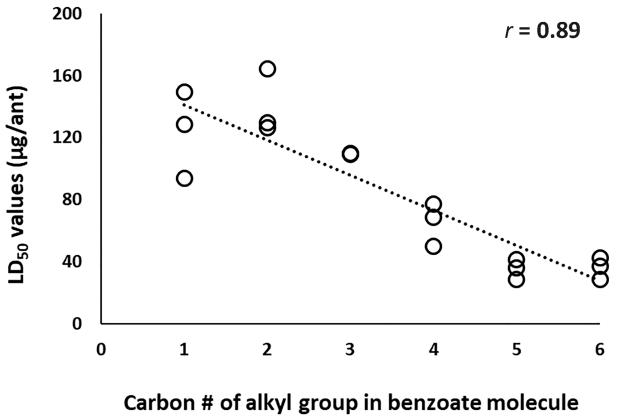

Fig. 3. Correlation between acute toxicity (LD_{50} values) of alkyl benzoates and their alkyl chain length.

Discussion

In this study, 15 commercially available benzoates were assessed for their contact and fumigation toxicity to *S. invicta* workers. Among tested benzoates, benzylbenzoate, *n*-pentylbenzoate, and *n*-hexylbenzoate were three most potent contact toxins (mean LD_{50} value = 23.31, 35.26, 35.99 µg per ant, respectively) and methyl-3-methoxybenzoate, methyl-3-methylbenzoate, and methylbenzoate were three most potent fumigants (mean LC_{50} value = 0.61, 0.62, 0.75 µg/ml, respectively). Some benzoates are used as flavor ingredients for human consumption (George 2010), indicating that they have low mammalian toxicity. For example, up to 98.91 mg/kg of benzylbenzoate has been used in alcohol beverages; up to 10.0 mg/kg of hexylbenzoate in baked goods; and

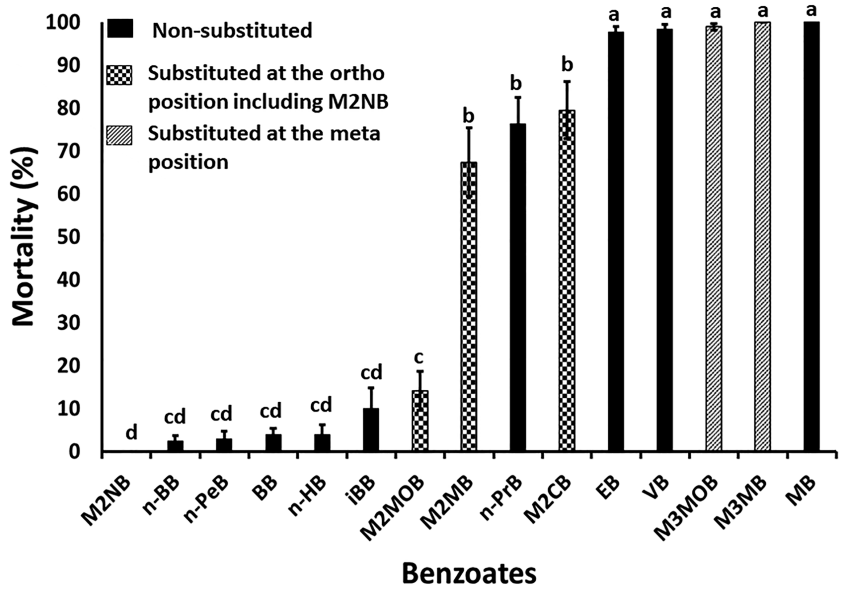

Fig. 4. Mortality (%, mean ± SE) of *S. invicta* workers 24 h after being fumigated with benzoates at a dosage of 1.43 µg/ml. Mortalities labeled with different letters are significantly different (P < 0.05). Analysis of variance was performed and means were separated using Tukey's multiple comparison test (P < 0.05). Arcsine square root transformation was performed on the data before the statistical analysis. For comparison of mortality among benzoates, analysis of variance was performed and means were separated using Tukey's Multiple Comparison Test (P < 0.05). M2NB: methyl-2-nitrobenzoate, *n*-BB: *n*-butylbenzoate, *n*-PeB: *n*-pentylbenzoate, BB: benzylbenzoate, *n*-HB: *n*-hexylbenzoate, iBB: isobutylbenzoate, M2MOB: methyl-2-methoxylbenzoate, M2MB: methyl-2-methylbenzoate, *n*-PrB: *n*-propylbenzoate, M2CB: methyl-2-chlorobenzoate, EB: ethylbenzoate, VB: vinylbenzoate, M3MOB: methyl-3-methoxylbenzoate, M3MB: methyl-3-methylbenzoate, MB: methylbenzoate.

Table 2. Fumigation toxicity (LC_{50} values) of five benzoates against *S. invicta* workers

Benzoate	Colony	LC_{50} (μg/ml)	95% CI	Slope (mean± SE)	χ^2(df, P)
Methylbenzoate	1	0.77	0.73–0.81	9.14 ± 0.94	13.48 (13, 0.41)
	2	0.66	0.62–0.69	11.12 ± 1.35	11.82 (13, 0.54)
	3	0.82	0.69–0.88	12.11 ± 2.30	25.32 (13, 0.021)
Vinylbenzoate	1	0.95	0.89–1.03	9.46 ± 0.87	23.34 (13, 0.038)
	2	0.77	0.74–0.80	25.99 ± 2.39	3.08 (13, 0.99)
	3	0.96	0.92–0.10	16.07 ± 2.18	9.67 (13, 0.72)
Methyl-3-methylbenzoate	1	0.62	0.58–0.65	7.55 ± 0.73	10.17 (13, 0.68)
	2	0.7	0.62–0.78	10.52 ± 1.09	60.97 (13, <0.00001)
	3	0.63	0.55–0.69	9.72 ± 0.96	49.81 (13, <0.00001)
Methyl-3-methoxybenzoate	1	0.64	0.52–0.73	6.25 ± 0.84	28.57 (13, 0.0075)
	2	0.54	0.43–0.63	5.24 ± 0.56	29.51 (13, 0.0055)
	3	0.65	0.58–0.71	5.03 ± 0.51	14.77 (13, 0.32)
Ethylbenzoate	1	0.89	0.85–0.93	14.21 ± 1.39	18.08 (13, 0.16)
	2	0.76	0.70–0.81	9.81 ± 1.24	15.34 (13, 0.29)
	3	1.14	1.02–1.21	13.82 ± 2.01	30.90 (13, 0.0035)

Dichlorvos was used as a positive control. Due to a steep slope, LC_{50} values for dichlorvos could not be established for all three colonies. Mortality data were presented in Fig. 6. Complete mortality was achieved for all three colonies at concentration of 0.66 ng/ml.

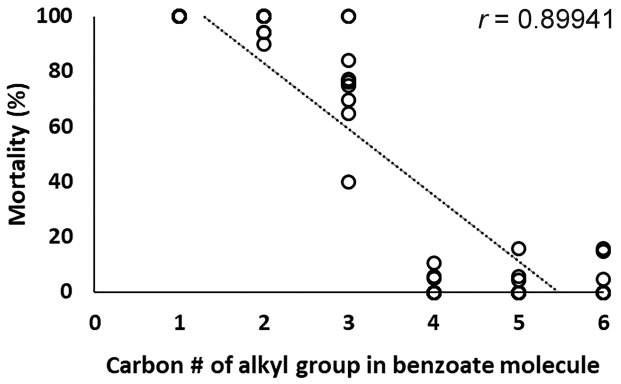

Fig. 5. Correlation between fumigation toxicity of alkyl benzoates (mortality at the dosage of 1.43 μg/ml) and their alkyl chain length.

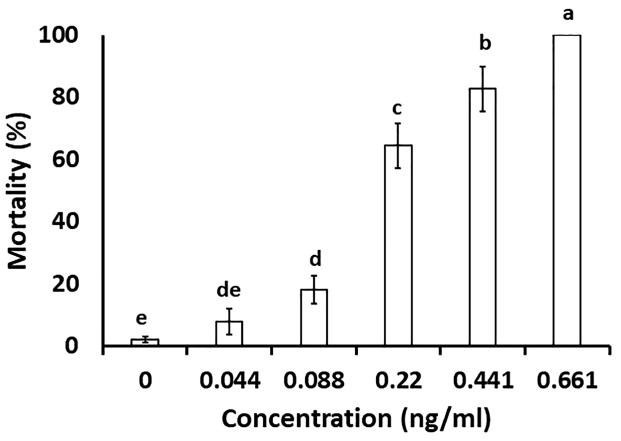

Fig. 6. Mortality (%, mean ± SE) of *S. invicta* workers 24 h after being fumigated with dichlorvos. Mortalities labeled with different letters are significantly different ($P < 0.05$). Arcsine square root transformation was performed on the data before the statistical analysis. For comparison of mortality among concentrations, analysis of variance was performed and means were separated using Tukey's multiple comparison test ($P < 0.05$).

up to 45.63 mg/kg of methybenzoate in chewing gum (George 2010). Benzylbenzoate, hexylbenzoate, and methybenzoate are all naturally occurring compounds (Brunke et al. 1993, Fombong et al. 2016, Chen 2017, Monteiro et al. 2017) that can be found in meadowsweet, *Filipendula ulmaria* (L.) Maxim (Brunke et al. 1993) and they have pleasant odors. Benzylbenzoate has a light, balsamic odor reminiscent of almond, methylbenzoate has an odor similar to cananga, and hexylbenzoate has a woody-green, piney, balsamic odor (George 2010).

Many naturally occurring compounds and materials have been reported to be toxic and/or repellant to *S. invicta*, such as citrus oil (Vogt et al. 2002), mint oil (Appel et al. 2004), essential oil from the leaf of *Cinnamomum osmophloeum* Kaneh (Cheng et al. 2008), sweet wormwood oil (Zhang et al. 2014), Nootka oil (Addesso et al. 2017), Sweet Orange essential oil (Hu et al. 2017), camphor essential oil (Fu et al. 2015), callicarpenal, and intermedeol isolated from leaves of American beautyberry (*Callicarpa americana* L.; Verbenaceae) and Japanese beautyberry (*Callicarpa japonica* Thunb.) (Chen et al. 2008), and a Chinese essential oil product (Chen 2009). Several nature-based products have been tested for fire ant control, such as Garden-Ville Soil Conditioner (30% citrus oil; Vogt et al. 2002), CitrexTM (78.2% d-limonene; Nester 2001), and Exxant (14.2% turpentine plus 0.2% ammonia; Barr and Best 2002). Toxicity and efficacy of two 2-tridecanone formulations were assessed against *S. invicta* (Chen 2016). The 2-tridecanone is a major constitute of the defensive secretion in tawny crazy ants, *Nylanderia fulva* Mayr that is reported to displace *S. invicta* in the field (Chen et al. 2013). Limonene is the major component of citrus oil and pinene is the major component of turpentine and pine oil. Both limonene and pinene are flammable and cause skin and eye irritation. Another obvious drawback for limonene, pinene, and 2-tridecanone based fire ant control products is their aquatic toxicity.

This study demonstrated the potent contact toxicity of benzylbenzoate, hexylbenzoate and pentylbenzoate; and fumigation toxicity of methyl-3-methoxybenzoate, methyl-3-methylbenzoate, methylbenzoate, vinylbenzoate, and ethylbenzoate against *S. invicta* workers. All these benzoates are nonflammable except methylbenzoate. With the exception of methyl-3-methylbenzoate, methyl-3-methoxybenzoate, and vinylbenzoate, all of the tested benzoates occur in the nature. In contrast to many benzoates, hexylbenzoate has neither known OSHA hazards nor aquatic toxicity. Methyl-3-methoxybenzoate is not considered a hazardous substance, indicating a great potential for their application in fire ant management.

Contact toxicity was positively correlated to alcohol chain length for nonsubstituted alkyl benzoates (esters of benzoic acid and C1-C6 linear alcohols). However, the longest alcohol chain tested in this

study was six carbons (hexylbenzoate). It may be worth further investigation of alkyl benzoates with longer alkyl chains.

Presence of a methoxyl group at either the ortho or the meta position of methylbenzoate significantly increased its contact toxicity, as did the presence of a methyl group at the meta addition. It may be feasible to improve the contact toxicity of other benzoates by modifying functional groups at those positions. For example, it may be worth further testing of the contact toxicity of hexyl-2 or 3-methoxylbenzoate and hexyl-3-methylbenzoate to *S. invicta*.

Fumigants have been used in fire ant mound treatment (Thorvilson et al. 1989). A safe fumigant would be very useful in some fire ant control scenarios in which existing synthetic insecticides cannot be directly used, such as fire ant control for baled hay. Based on the Safety Data Sheet from the producer (Sigma-Aldrich), methyl-3-methoxybenzoate is not considered a hazardous substance, meaning it has little potential to cause harm to humans, animals, or the environment, either by itself or through interaction with other factors. Considering its high fumigation toxicity to fire ants, further study on methyl-3-methoxybenzoate is warranted.

As naturally occurring compounds, these benzoates are not expected to have long-residual activities. How to achieve a long-term efficacy of these benzoates in the field will be a challenge and the solution may rely on the development of adequate delivery systems, such as slow-releasing formulations.

Acknowledgments

We thank Dr. Hamed Abbas and Dr. Mark Weaver, USDA-ARS, Stoneville, MS for critical reviews of an early version of the manuscript. We thank Mr. Leon Hicks for his technical assistance. The mention of trade names or commercial products in this publication is solely to provide specific information and does not imply recommendation or endorsement by the author institutions.

References Cited

Adams, R. P. 2007. Identification of essential oil components by gas chromatography/mass Spectrometry, 4th ed. Allured Publ. Corp, Carol Stream, IL.

Addesso, K. M., J. B. Oliver, P. A. O'Neal, and N. Youssef. 2017. Efficacy of nootka oil as a biopesticide for management of imported fire ants (Hymenoptera: Formicidae). J. Econ. Entomol. 110: 1547–1555.

Appel, A. G., M. J. Gehret, and M. J. Tanley. 2004. Repellency and toxicity of mint oil granules to red imported fire ants (Hymenoptera: Formicidae). J. Econ. Entomol. 97: 575–580.

Ascunce, M. S., C. C. Yang, J. Oakey, L. Calcaterra, W. J. Wu, C. J. Shih, J. Goudet, K. G. Ross, and D. Shoemaker. 2011. Global invasion history of the fire ant Solenopsis invicta. Science. 331: 1066–1068.

Banks, W. A., C. S. Lofgren, D. P. Jouvenaz, C. E. Stringer, P. M. Bishop, D. F. Williams, D. P. Wojcik, and B. M. Glancey.1981. Techniques for collecting, rearing, and handling imported fire ants. USDA, SEA, AATS-S-21, 9 p.

Barr, C. L., and R. L. Best. 2002. Product evaluation, field research and new products resulting from applied research. Southwest Entomol. Suppl. 25: 47–52.

Boevé, J. L., K. Dettner, W. Francke, H. Meyer, and J. M. Pasteels. 1992. The secretion of the ventral glands in *Nematus* sawfly larvae. Biochem. Syst. Ecol. 20: 107–111.

Brunke, E. J., F. J. Hammerschmidt, and G. Schmaus.1993. Flower scent of some traditional medical plants, pp. 282–296. *In* R. Teranishi, R. G. Buttery, and H. Sugisawa (eds.), Bioactive volatile compounds from plants. American Chemical Society Symposium Series. 525, Washington, DC.[AU: Please provide publisher details for ref. Brunke **et al**. 1993.]

Chen, J. 2009. Repellency of an over-the-counter essential oil product in China against workers of red imported fire ants. J. Agric. Food Chem. 57: 618–622.

Chen, J. 2016. Toxicity and efficacy of two emulsifiable concentrates of 2-tridecanone against red imported fire ants. Adv. Entomol. 4: 37–46.

Chen, J. 2017. Freeze-Thaw sample preparation method improves detection of volatile compounds in insects using headspace solid-phase microextraction. Anal. Chem. 89: 8366–8371.

Chen, J., C. L. Cantrell, S. O. Duke, and M. L. Allen. 2008. Repellency of callicarpenal and intermedeol against workers of imported fire ants (Hymenoptera: Formicidae). J. Econ. Entomol. 101: 265–271.

Chen, J., T. Rashid, G. Feng, L. Zhao, D. Oi, and B. B. Drees. 2013. Defensive chemicals of tawny crazy ants, *Nylanderia fulva* (Hymenoptera: Formicidae) and their toxicity to red imported fire ants, *Solenopsis invicta* (Hymenoptera: Formicidae). Toxicon. 76: 160–166.

Cheng, S. S., J. Y. Liu, C. Y. Lin, Y. R. Hsui, M. C. Lu, W. J. Wu, and S. T. Chang. 2008. Terminating red imported fire ants using *Cinnamomum osmophloeum* leaf essential oil. Bioresour. Technol. 99: 889–893.

Co, J. E., T. H. Jones, A. Hefetz, A. Tinaut, and R. R. Snelling. 2003. The comparative exocrine chemistry of nine old world species of *Messor* (Formicidae: Myrmicinae). Biochem. Syst. Ecol. 31: 367–373.

Copping, L. G., and S. O. Duke. 2007. Natural products that have been used commercially as crop protection agents. Pest Manag. Sci. 63: 524–554.

Drees, B. M., A. A. Calixto, and P. R. Nester. 2013. Integrated pest management concepts for red imported fire ants *Solenopsis invicta* (Hymenoptera: Formicidae). Insect Sci. 20: 429–438.

Dressler, C., S. Rosumeck, C. Sunderkötter, R. N. Werner, and A. Nast. 2016. The treatment of scabies. Dtsch. Arztebl. Int. 113: 757–762.

Feng, Y., and A. Zhang. 2017. A floral fragrance, methyl benzoate, is an efficient green pesticide. Sci. Rep. 7: 42168.

Fombong, A. T., J. M. Mutunga, P. E. Teal, and B. Torto. 2016. Behavioral evidence for olfactory-based location of honeybee colonies by the scarab *oplostomus haroldi*. J. Chem. Ecol. 42: 1063–1069.

Fu, J. T., L. Tang, W. S. Li, K. Wang, D. M. Cheng, and Z. X. Zhang. 2015. Fumigant toxicity and repellence activity of camphor essential oil from *cinnamonum camphora* siebold against *solenopsis invicta* workers (Hymenoptera:Formicidae). J. Insect Sci. 15: 129.

George, A. B. 2010. Fenaroli's handbook of flavor ingredients, 6th ed., CRC Press, Boca Raton, FL.

Harju, A. T., S. M. Pennanen, and J. Liesivuori. 2004. The efficacy of benzyl benzoate sprays in killing the storage mite *Tyrophagus putrescentiae* (Acari: Acaridae). Ann. Agric. Environ. Med. 11: 115–119.

Hu, W., N. Zhang, H. Chen, B. Zhong, A. Yang, F. Kuang, Z. Ouyang, and J. Chun. 2017. Fumigant activity of sweet orange essential oil fractions against red imported fire ants (Hymenoptera: Formicidae). J. Econ. Entomol. 110: 1556–1562.

Huang, J., H. Y. Han, G. Y. Li, H. Y. Wang, C. Zhang, K. Zhang, Y. Tan, P. Y. Li, and J. H. Wang. 2013. Two new terpenoid benzoates with antitumor activity from the roots of *Ferula dissecta*. J. Asian Nat. Prod. Res. 15: 1100–1106.

Leal, W. S., M. Hasegawa, M. Sawada, M. Ono, and S. Tada.1996. Scarab beetle *Anomala albopilosa albopilosa* utilizes a more complex sex pheromone system than a similar species *A. cuprea*. J. Chem. Ecol. 22: 2001–2010.

Lowe, S., Browne, M., Boudjelas, S., and M. De Poorter. 2000. 100 of the world's worst invasive alien species. A selection from the global invasive species database. Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), 12 pp. First published as special lift-out in Aliens 12, December 2000. Updated and reprinted version November 2004.

Mojtaba, T., G. H. Reza, S. Borzo, N. Shiva, and S. Esmaeil. 2011. In vitro antibacterial and antifungal activity of *Salvia multicaulis*. J. Essent. Oil. Bear Pl. 14: 255–259.

Monteiro, I. N., O. D. S. Monteiro, L. M. Costa-Junior, A. da Silva Lima, E. H. A. Andrade, J. G. S. Maia, and V. E. Mouchrek Filho. 2017. Chemical composition and acaricide activity of an essential oil from a rare chemotype of *Cinnamomum verum* Presl on *Rhipicephalus microplus* (Acari: Ixodidae). Vet. Parasitol. 238: 54–57.

Morrison, L. W., S. D. Porter, E. Daniels, and M. D. Korzukhin. 2004. Potential global range expansion of the invasive fire ant, *Solenopsis invicta*. Biol. Invasions. 6: 183–191.

Morrison, L. W., M. D. Korzukhin, and S. D. Porter. 2005. Predicted range expansion of the invasive fire ant, *Solenopsis invicta*, in the eastern United States based on the VEMAP global warming scenario. Divers. Distrib. 11: 99–204.

Nester, P. R. 2001. Evaluation of Citrex fire ant killer as a drench treatment for red imported fire ant mound. http://global.oup.com/us/companion.websites/fdscontent/ uscompanion/us/pdf/houp/15_4.pdf. Accessed 27 June 2018.

Pino, J. A., J. Mesa, Y. Muñoz, M. P. Martí, and R. Marbot. 2005. Volatile components from mango (*Mangifera indica* L.) cultivars. J. Agric. Food Chem. 53: 2213–2223.

Robertson, J. L., N. E. Savin, H. K. Preisler, and R. M. Russell. 2007. Bioassays with arthropods. CRC Press, Baco Raton, FL.

Schiestl, F. P., and D. W. Roubik. 2003. Odor compound detection in male euglossine bees. J. Chem. Ecol. 29: 253–257.

Suhaili, Z. A., and T. M. Ho. 2008. Residual activity of benzyl benzoate against *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae). Southeast Asian J. Trop. Med. Public Health. 39: 507–510.

Thorvilson, H. G., S. A. Phillips Jr., and A. A. Sorensen. 1989. An innovative thermo-fumigation technique for control of red imported fire ants (Hymenoptera: Formicidae). J. Agric. Entomol. 6: 31–36.

Valles, S. M., and S. D. Porter. 2003. Identification of polygyne and monogyne fire ant colonies (*Solenopsis invicta*) by multiplex PCR of Gp-9 alleles. Insectes Soc. 50: 199–200.

Vinson, B. S. 2013. Impact of the invasion of the imported fire ant. Insect Sci. 20: 439–455.

Vogt, J., T. G. Shelton, M. E. Merchant, S. A. Russell, M. J. Tanley, and A. Appel. 2002. Efficacy of three citrus oil formulations against *Solenopsis invicta* Buren (Hymenoptera: Formicidae), the red imported fire ant. J. Agric. Urban Entomol. 19: 159–171.

Wang, Y., C. Finn, and M. C. Qian. 2005. Impact of growing environment on Chickasaw blackberry (Rubus L.) aroma evaluated by gas chromatography olfactometry dilution analysis. J. Agric. Food Chem. 53: 3563–3571.

Wang, H. L., O. Brattström, P. M. Brakefield, W. Francke, and C. Löfstedt. 2014. Identification and biosynthesis of novel male specific esters in the wings of the tropical butterfly, *Bicyclus martius sanaos*. J. Chem. Ecol. 40: 549–559.

Williams, D. F., H. L. Collins, and D. H. Oi. 2001. The red imported fire ant (Hymenoptera: Formicidae): an historical perspective of treatment programs and the development of chemical baits for control. Am. Entomol. 47: 146–159.

Yang, J. O., N. Nakayama, K. Toda, S. Tebayashi, and C. S. Kim. 2013. Elicitor(s) in *Sogatella furcifera* (Horváth) causing the Japanese rice plant (*Oryza sativa* L.) to induce the ovicidal substance, benzyl benzoate. Biosci. Biotechnol. Biochem. 77: 1258–1261.

Zhang, N., L. Tang, W. Hu, K. Wang, Y. Zhou, H. Li, C. Huang, J. Chun, and Z. Zhang. 2014. Insecticidal, fumigant, and repellent activities of sweet wormwood oil and its individual components against red imported fire ant workers (Hymenoptera: Formicidae). J. Insect Sci. 14. pii: 241.