

ANTIPREDATOR DEFENSE OF BIOLOGICAL CONTROL
AGENT *Oxyops vitiosa* IS MEDIATED BY PLANT VOLATILES
SEQUESTERED FROM THE HOST PLANT
Melaleuca quinquenervia

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Abstract—The weevil *Oxyops vitiosa* is an Australian species imported to Florida, USA, for the biological control of the invasive weed species *Melaleuca quinquenervia*. Larvae of this species feed on leaves of their host and produce a shiny orange secretion that covers the integument. When this secretion is applied at physiological concentrations to dog food bait, fire ant consumption and visitation are significantly reduced. Gas chromatographic analysis indicates that the larval secretion qualitatively and quantitatively resembles the terpenoid composition of the host foliage. When the combination of 10 major terpenoids from the *O. vitiosa* secretion was applied to dog food bait, fire ant consumption and visitation were reduced. When these 10 terpenoids were tested individually, the sesquiterpene viridiflorol was the most active component in decreasing fire ant consumption. Fire ant visitation was initially (15 min after initiation of the study) decreased for dog food bait treated with viridiflorol and the monoterpenes 1,8-cineole and α -terpineol. Fire ants continued to avoid the bait treated with viridiflorol at 18 $\mu\text{g}/\text{mg}$ dog food for up to 6 hr after the initiation of the experiment. Moreover, ants avoided bait treated with 1.8 $\mu\text{g}/\text{mg}$ for up to 3 hr. The concentrations of viridiflorol, 1,8-cineole, and α -terpineol in larval washes were about twice that of the host foliage, suggesting that the larvae sequester these plant-derived compounds for defense against generalist predators.

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INTRODUCTION

The Australian weevil, *Oxyops vitiosa*, Pascoe (Coleoptera: Curculionidae) was introduced in south Florida in 1997 for the biological control of the invasive weed *Melaleuca quinquenervia* (Cav.) S. T. Blake (Myrtaceae) (Center et al., 2000). This weevil species has since been established throughout the infested area of Florida. Larvae feed on young leaves and have a characteristic odor that resembles that of the foliage of their host tree. The bright orange larvae are diurnally active and feed exposed during all instars on the foliage, then drop to the ground, where they form a protective pupal cell in the soil (Purcell and Balciunas, 1994). The cuticle of the larvae is completely covered with a shiny, oily substance that may function in defense against generalist predators (Montgomery and Wheeler, 2000). However, the chemical nature of this defense has yet to be determined. Although few weevil species have been reported to produce defensive secretions (Pavis et al., 1992), similar integumental slime found on the sluglike sawfly larvae of *Caliroa cerasi* reduced the incidence of predation by generalist ants (Eisner, 1994).

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) constitutes one of the most invasive invertebrate predators in the southeastern United States, invading forests, pastures, croplands, and natural areas (Lofgren et al., 1975). This ant is among the most common invertebrate predators in the southeastern United States. (Elvin et al., 1983; Kharboutli and Mack, 1991). Generalist predators, including ants, have prevented the establishment or reduced the effectiveness of many weed biological control agents (Goeden and Louda, 1976), and this ant species, in particular, has been directly implicated in reducing the efficacy of *Tyta luctuosa*, a biological control agent of field bindweed (Ciomperlik et al., 1992).

Biological control efforts may benefit by recruiting agents that sequester plant defensive compounds that impart protection from generalist predators. Sequestration of plant defenses may be more common than appreciated by biological control practitioners, as this phenomenon is common among herbivores with narrow host ranges (Bowers, 1990). Several weed biological control agents are known to sequester plant defensive compounds, namely the pyrrolizidine alkaloids of the *Senecio jacobaea*–*Tyria jacobaeae* association (Aplin et al., 1968), the quinolizidine alkaloids of the Scotch broom–aphid association (Wink et al., 1982), and the bisanthraquinone hypericin obtained by the *Chrysolina* beetles from their host plant *Hypericum* (Rees, 1969). The purpose of this study was to determine the nature of the defensive secretions produced by the larvae of *O. vitiosa* toward the generalist predator *S. invicta*.

METHODS AND MATERIALS

Insects. Larvae of *O. vitiosa* were collected ($N = 20$) from a field site infested with *M. quinquenervia* in Ft. Lauderdale, Broward County, Florida. All larvae were weighed alive prior to analysis. Solvent washes were conducted on fourth instars by dipping each in 1 or 2 ml of chloroform (CHCl_3) for 15 sec. The CHCl_3 larval wash was filtered through glass wool and dried over sodium sulfate (Na_2SO_4) prior to analysis by gas chromatography.

Red imported fire ants were collected at the University of Florida, Ft. Lauderdale Research and Education Center, as described previously (Montgomery and Wheeler, 2000). Colonies, consisting of brood, eggs, and workers, were established in plastic boxes (20×12 cm) and provided with deionized water in a glass tube (45×4 mm) plugged with cotton. Ants were fed kibbled dog food (Pedigree Prime) freely for two to five days prior to each test. The food was removed 24–36 hr prior to each test. Newly collected ant colonies were used for each test.

Plants. Leaves were collected from the same location as the larvae described above. Tip leaves were clipped from young trees and brought to the laboratory where they were frozen (-10°C) for further processing. The leaf components were extracted by a modified microwave technique (Southwell et al., 1995; Degen and Stadler, 1998; Gomez et al., 1999). Fresh leaf samples (75–150 mg fresh weight) were immersed in 1 ml EtOH (95%) and irradiated with microwaves (750 W) for 60 sec. An aliquot (500 μl) of the EtOH extract was mixed with deionized water (500 μl), followed by 500 μl of CHCl_3 . The sample was vortexed for 1 min and then centrifuged for 10 min at 10,000 rpm. An aliquot (200 μl) of the CHCl_3 layer was removed and dried over Na_2SO_4 . Internal standards were added as 10 ng/ μl each of *n*-tridecane and *n*-eicosane in 10 μl CHCl_3 to the extract prior to analysis by gas chromatography.

Chemicals. Terpenoids were purchased from commercial sources, except where mentioned, and were of the highest purity available. The list included the primary compounds reported by Brophy et al. (1989) and Ramanoelina et al. (1994). These included (+)- α -pinene (Chemical Purity = 98%), (–)- β -pinene (99%), α -terpinene (98%), (–)-limonene (92%), 1,8-cineole (100%), γ -terpinene (98%), terpinen 4-ol (97%), α -terpineol (98%), and *trans*-nerolidol (95%). Viridiflorol (86%) was extracted from *M. quinquenervia* foliage by one of us (I.A.S.).

Gas Chromatography. Samples were analyzed with either a Hewlett-Packard model 6890 or 5890 gas chromatograph. Data collection, storage, and analysis were conducted with the ChromPerfect data system. Helium at a linear flow rate of 37 cm/sec was used as a carrier gas. All samples were analyzed on three fused silica capillary columns [HP-5 (Hewlett Packard Company, Wilmington, Delaware), DB-17MS and a DB-WAXetr (J & W Scientific, Folsom, California); all 30 m \times 0.32 mm ID, 0.25- μm -thick film]. Injector temperature was 200°C and FID temperature was 250°C . The oven temperature was held at 50°C for 2 min then increased

at 8°C/min to 250°C, where it was held for 5 min. Compounds were quantified relative to the peak areas of the internal standard and values were adjusted by calculation of their response factors relative to *n*-tridecane (Debbrecht, 1985).

Compound identities were confirmed by GC-MS using a Hewlett Packard 6890 instrument fitted with a HP-5MS (30.3 m × 0.25 mm, 0.25- μ m film thickness) FSOT column with helium (36 cm/sec) as a carrier gas, injector port (split 1:50) at 250°C, mass selective detector (HP 5973) at 250°C (source) and 150°C (quad) with transfer line 280°C and ion source filament voltage of 70 eV. Component identification was made on the basis of mass spectral fragmentation, retention index with *n*-paraffins, and comparison with authentic constituents and mass spectral and retention matching with commercial (NIST, Wiley, and Adams) libraries.

Bioassay of Defensive Secretions. The material washed from the *O. vitiosa* larvae or select commercial terpenoids was dissolved in 50 μ l of CHCl₃ and applied to kibbled dog food cut into 50-mg pieces. The solvent was allowed to dry for 0.5 hr in a fume hood under ambient conditions. The treated dog food bait was stored for 12–15 hr at 4°C. Each test consisted of offering the ants a choice between a solvent (CHCl₃)-treated control and either a crude larval wash or a select commercial terpenoid formulated individually or in a mixture. To determine the percentage loss of terpenoids during the experiment, treated dog food samples were CHCl₃-extracted before and after a 6-hr test and analyzed by GC. By comparing peak areas the concentration (mean \pm SE) of each terpenoid was found to decrease during the experiment from 53.9% \pm 2.5% for viridiflorol to 93.7% \pm 0.9% for α -pinene.

Four choice tests were conducted, all under ambient conditions between 9 AM and 3 PM. For all tests, ant consumption was determined by comparing the dog food weight before and after each 6-hr test. Additionally, the number of ants visiting each dog food bait was counted at regular intervals. In study 1, choice tests were conducted between a CHCl₃-treated control and the crude *O. vitiosa* larval wash [1-larval equivalent (LE)] applied to a piece of dog food ($N = 10$). In study 2, choice tests were conducted between a CHCl₃-treated control and a mixture of the 10 major terpenoids found from GC analysis of the larval washes ($N = 6$). These were formulated to include the range of concentrations of each compound found in the crude larval washes (Table 1). Additional treatments consisted of 10-fold and 100-fold dilutions of these mixtures. One major *M. quinquenervia* terpenoid, β -caryophyllene, was not identified during this phase of the research and, thus, its repellent activity was not determined. In study 3, choice tests were conducted between a CHCl₃-treated control and the same 10 terpenoids tested individually at the highest concentrations tested in study 2 ($N = 4$). In study 4, choice tests were conducted between a CHCl₃-treated control and three different viridiflorol concentrations using the same concentrations as in study 2 ($N = 6$).

Statistical Analysis. All data, terpenoid extraction yields, *S. invicta* consumption, and the number of fire ants visiting each dog food bait, were analyzed with

TABLE 1. CONCENTRATION OF 10 TERPENOID COMPONENTS APPLIED TO KIBBLED DOG FOOD AND FED TO RED IMPORTED FIRE ANT WORKERS^a

Compound	Concentration ($\mu\text{g}/\text{mg}$ dog food)
1,8-Cineole	22.00
Viridiflorol	18.00
<i>trans</i> -Nerolidol	5.02
α -Terpineol	5.47
α -Pinene	5.06
Limonene	3.09
β -Pinene	1.70
Terpinen 4-ol	1.80
γ -Terpinene	1.67
α -Terpinene	1.43

^aEach piece of dog food (50 mg) was treated with 1-fold (high), 10-fold (intermediate) or 100-fold (low) dilutions of one larval equivalent of these 10 components ($N = 6$).

SAS (SAS Institute, 1990). The terpenoid yields of the leaf extracts and the larval washes were compared with an ANOVA for each terpenoid analyzed. The consumption and the fire ant activity data were analyzed with a Kruskal-Wallis test.

RESULTS

Chemical Analyses

Considerable quantitative and qualitative similarity was found in the chemical constituents from leaf extracts and larval washes (Table 2; Figure 1). However, some of the terpenoids analyzed (e.g., viridiflorol, 1,8-cineole, α -terpineol, limonene, terpinen 4-ol) were present in greater concentrations in the larval washes than in the leaf extracts. This suggests that as the *O. vitiosa* larvae consume *M. quinquenervia* foliage, they absorb and deposit the foliar terpenoids on their cuticle. The larval fresh weight averaged (\pm SE) 101.9 (\pm 3.0) mg and the amount of material removed by washing each larva averaged 7.9 (\pm 0.5) mg.

Bioassay of Larval Secretions

Study 1—Larval Washes. Fire ant consumption and visitation were significantly greater on the control dog food bait (treated with CHCl_3) compared with that treated with the *O. vitiosa* larval wash (Table 3). During the 6-hr test, the fire

TABLE 2. COMPARISON OF MEAN (\pm SE) CONCENTRATIONS OF 10 TERPENOID COMPONENTS FROM CHCl_3 -EXTRACTED *M. quinquenervia* LEAVES ($N = 4$) AND CHCl_3 -WASHED *O. vitiosa* LARVAE ($N = 10$)^a

Compound	Leaf extract		Larval wash		<i>F</i>	<i>df</i>	<i>P</i>
	$\mu\text{g}/\text{mg}$	SE	$\mu\text{g}/\text{mg}$	SE			
Viridiflorol	6.06	0.86	11.85	1.25	5.59	1,13	0.034
1-8 Cineole	3.15	0.41	6.73	0.76	7.67	1,13	0.016
<i>trans</i> -Nerolidol	nd		nd				
α -Terpineol	0.89	0.12	2.23	0.22	11.96	1,13	0.004
α -Pinene	1.47	0.35	1.44	0.18	0.01	1,13	>0.900
β -Pinene	0.44	0.08	0.45	0.04	0.01	1,13	>0.900
Limonene	1.13	0.16	0.66	0.08	5.86	1,13	0.031
γ -Terpinene	0.08	0.01	0.09	0.01	1.13	1,13	>0.300
Terpinen 4-ol	0.07	0.01	0.15	0.01	24.33	1,13	<0.001
α -Terpinene	0.01	0.00	0.03	0.01	4.02	1,8	0.080

^aResults of ANOVA are presented for comparisons between the leaf extract and larval washes for each compound. Values estimated by gas chromatography and expressed as $\mu\text{g}/\text{mg}$ of fresh leaf or larval weight.
nd: not detected.

TABLE 3. CONSUMPTION AND VISITATION BY RED IMPORTED FIRE ANTS OF DOG FOOD TREATED WITH CHCl_3 (CONTROL) OR *O. vitiosa* LARVAL WASHES (6 HR TEST)^a

Colony	Consumption (mg)		Number fire ants visiting bait			
			3 hr		6 hr	
	Control	Treated	Control	Treated	Control	Treated
1	4.4	1.5	10	0	9	0
2	2.8	0.7	7	0	11	0
3	11.2	1.8	10	0	12	0
4	4.3	2.3	8	0	7	0
5	40.0	0.7	30	0	30	0
6	3.7	0.7	15	0	10	0
7	9.0	0	10	0	6	0
8	3.0	0	17	0	11	0
9	4.7	0	12	0	7	0
10	3.9	0	13	0	9	0
Mean	8.7	0.8	13.2	0	11.2	0
SE	3.6	0.3	21	0	2.2	0
	$\chi^2_1 = 14.438; P < 0.001$		$\chi^2_1 = 16.365; P < 0.001$		$\chi^2_1 = 16.351; P < 0.001$	

^aEach piece of dog food (50 mg) was treated with one larval equivalent.

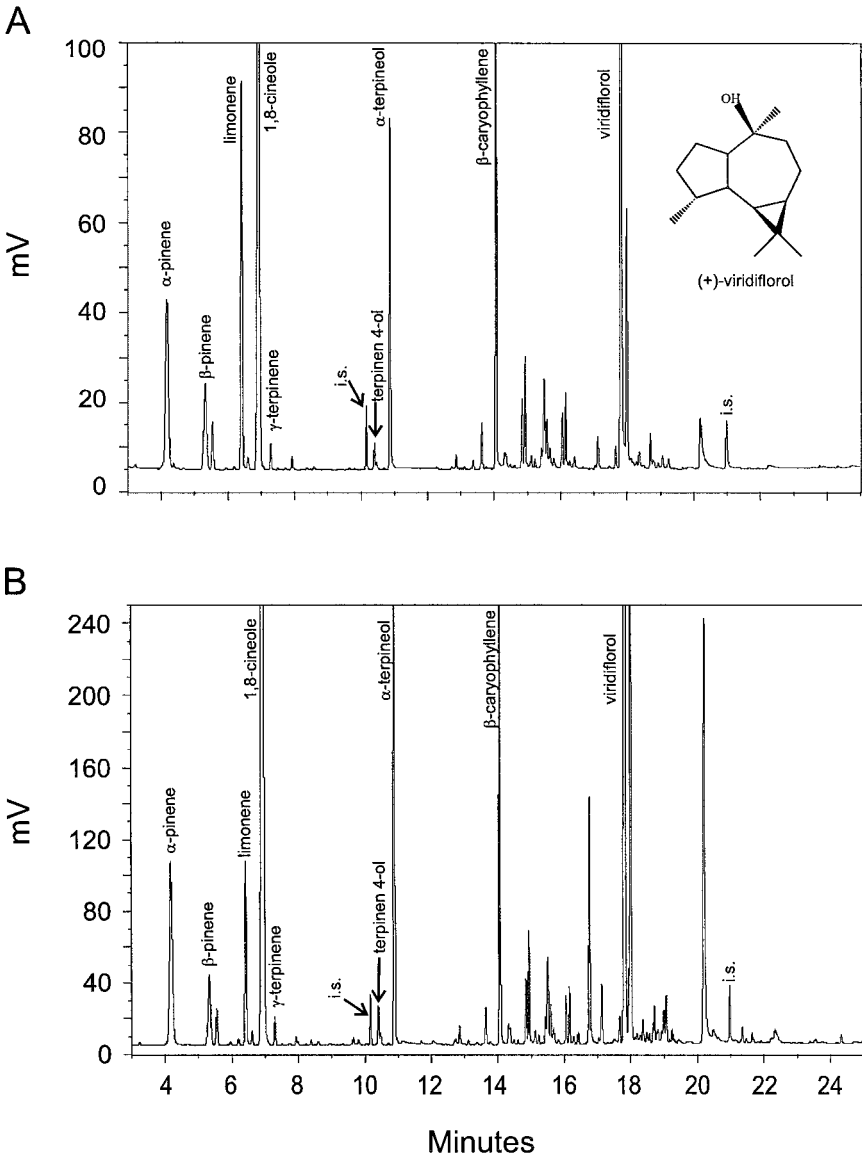


FIG. 1. Representative gas chromatograms of *M. quinquenervia* leaves (A) and *O. vitiosa* larval washes (B). Leaves were digested in 95% EtOH by 60-sec microwave irradiation. Larval washes consisted of dipping fourth-instar larvae in CHCl_3 for 15 sec. Internal standards *n*-tridecane and *n*-eicosane (10 ng/ μl of each) were added to each sample.

ants consumed an average of 8.7 (± 3.6) mg of dog food in the controls compared with 0.8 (± 0.3) mg of dog food in the larval-wash treatment. Additionally, fire ants frequently visited the control dog food at both 3 and 6 hr after initiation of the experiment, whereas they were never observed visiting the larval wash-treated dog food (Table 3).

Study 2—Mixture of Terpenoids. Fire ant consumption and visitation of dog food were significantly lower following treatment with the mixture of the 10 terpenoids (about 1 LE) than with the solvent-treated controls. In the high ($\chi_1^2 = 8.932$; $P = 0.003$) treatment of the terpenoid mixture, consumption was significantly reduced compared with that of the solvent-treated dog food (Figure 2). Furthermore, fire ant visitation was reduced (Figure 3) during the 15-min observation period in the high ($\chi_1^2 = 9.466$; $P = 0.002$), intermediate ($\chi_1^2 = 8.425$; $P = 0.004$), and low ($\chi_1^2 = 8.366$; $P = 0.004$) treatments of the terpenoid mixture

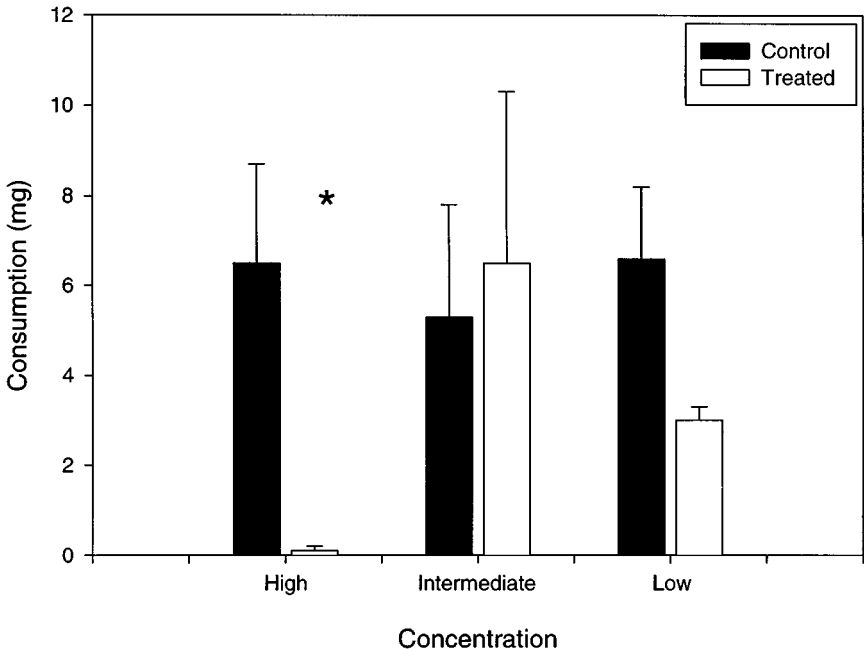


FIG. 2. Mean consumption (\pm SE) by *S. invicta* workers offered a choice between a dog food bait treated with CHCl_3 (control) and a mixture of the 10 major terpenoids occurring in CHCl_3 washes of *O. vittosa* larvae. The high concentration approximated the amount washed from one larva (Table 1), whereas the intermediate and the low levels were 10-fold and 100-fold dilutions, respectively. Asterisk indicates significant differences between the control and treated means.

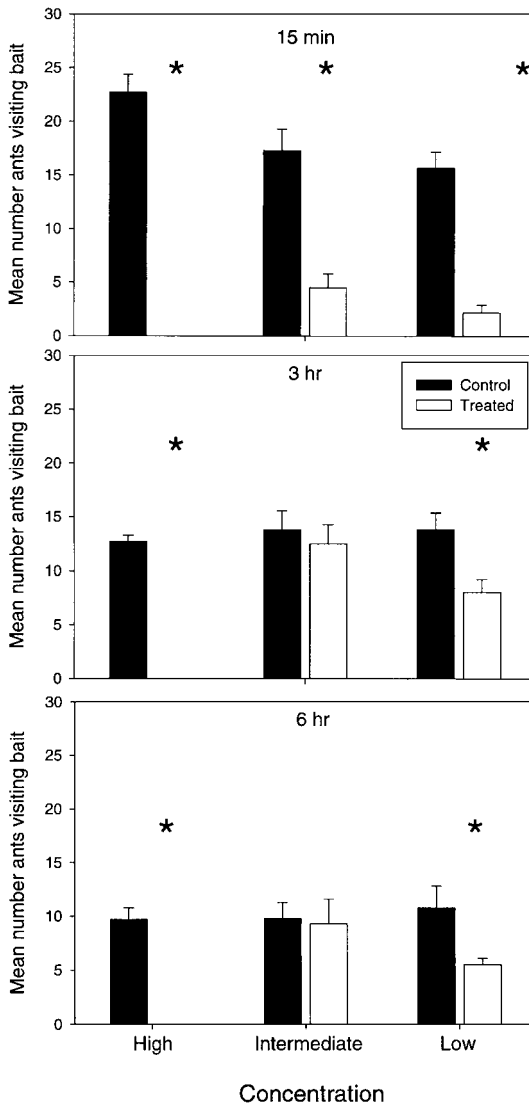


FIG. 3. Mean number (\pm SE) of *S. invicta* workers visiting dog food bait treated with CHCl_3 (control) and a mixture of the 10 major terpenoids occurring in CHCl_3 washes of *O. vitiosa* larvae. The high concentration approximated the amount washed from one larva (Table 1), whereas the intermediate and the low levels were 10-fold and 100-fold dilutions, respectively. The number of ants contacting each dog food bait was counted at 15 min, 3 hr, and 6 hr after the beginning of the experiment. Asterisks indicate significant differences between the control and treated means.

compared with the solvent-treated controls. Significant differences were also found 3 hr after the study began in the high ($\chi_1^2 = 9.542$; $P = 0.002$) and the low ($\chi_1^2 = 5.448$; $P = 0.020$) concentrations. Finally, fewer fire ants were observed visiting the terpenoid mixture-treated dog food at 6 hr after the study began on the high ($\chi_1^2 = 9.466$; $P = 0.002$) and the low ($\chi_1^2 = 6.226$; $P = 0.012$) concentrations.

Study 3—Individual Terpenoids. When each terpenoid was applied individually to the dog food, the only treatments that reduced fire ant consumption of bait were α -terpineol (Figure 4; $5.47 \mu\text{g}/\text{mg}$; $\chi_1^2 = 5.333$; $P = 0.021$) and viridiflorol ($18.0 \mu\text{g}/\text{mg}$; $\chi_1^2 = 5.398$; $P = 0.020$). Fire ant visitation was reduced after 15 min (Figure 5) in those treated with 1,8-cineole ($22.0 \mu\text{g}/\text{mg}$; $\chi_1^2 = 3.607$; $P = 0.058$), α -terpineol ($\chi_1^2 = 4.083$; $P = 0.043$) and viridiflorol ($\chi_1^2 = 5.463$; $P = 0.019$).

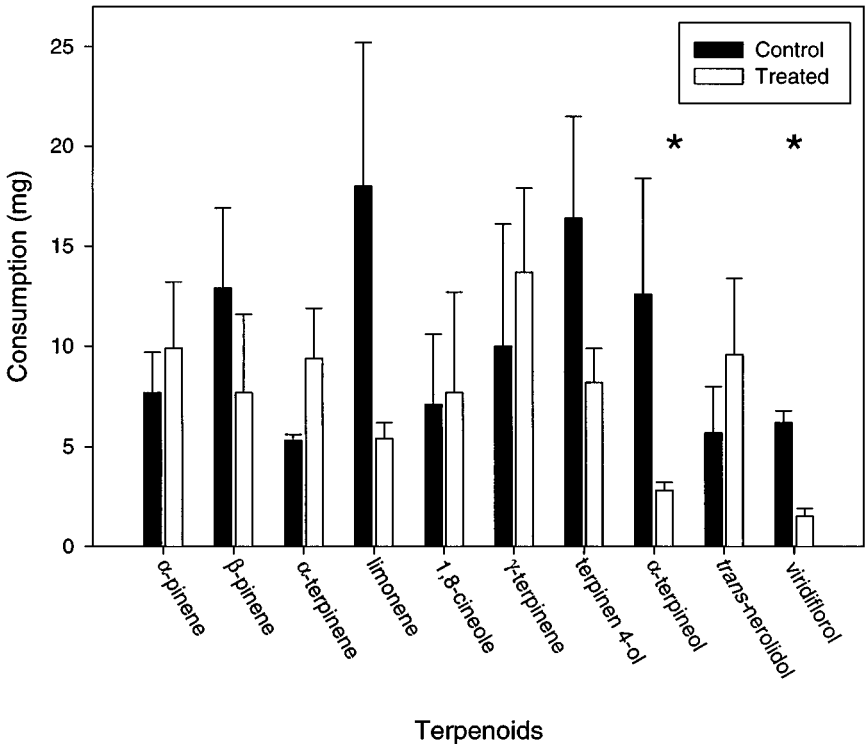


FIG. 4. Mean consumption (\pm SE) by *S. invicta* workers offered a choice between a dog food bait treated with CHCl_3 (control) and each of the 10 major terpenoids occurring in CHCl_3 washes of *O. vitiosa* larvae. Asterisks indicate significant difference between the control and treated means.

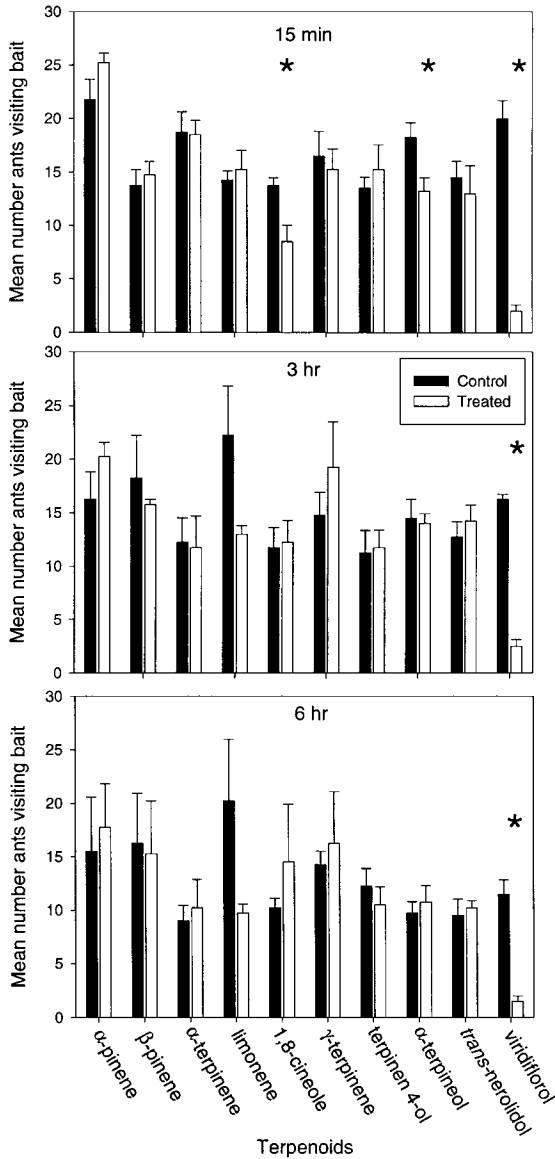


FIG. 5. Mean number (\pm SE) of *S. invicta* workers visiting dog food bait treated with CHCl_3 (control) and each of the 10 major terpenoids occurring in washes of *O. vitiosa* larvae. The number of ants contacting each dog food bait was counted at 15 min, 3 hr, and 6 hr after beginning the experiment. Asterisks indicate significant differences between the control and treated means.

After 3 hr ($\chi_1^2 = 5.398$; $P = 0.020$) and 6 hr ($\chi_1^2 = 5.600$; $P = 0.018$) fire ant visitations were reduced only on the viridiflorol-treated dog food bait.

Study 4—Viridiflorol. Viridiflorol tested alone was active over a range of concentrations at decreasing fire ant consumption and repelling ants. Compared with the solvent-treated controls, consumption was significantly reduced only by viridiflorol at the highest concentration ($\chi_1^2 = 4.395$; $P = 0.036$) tested (Figure 6). However, fire ant visitation to viridiflorol-treated bait was reduced at the highest ($\chi_1^2 = 9.466$; $P = 0.002$) and intermediate ($\chi_1^2 = 9.103$; $P = 0.003$) concentrations 15 min after the beginning of the study (Figure 7). This activity continued for 3 hr after the beginning of the study at the highest ($\chi_1^2 = 9.466$; $P = 0.002$) and intermediate ($\chi_1^2 = 3.480$; $P = 0.062$) viridiflorol concentrations. After 6 hr, a difference in activity was only found at the highest viridiflorol concentration ($\chi_1^2 = 7.174$; $P = 0.007$).

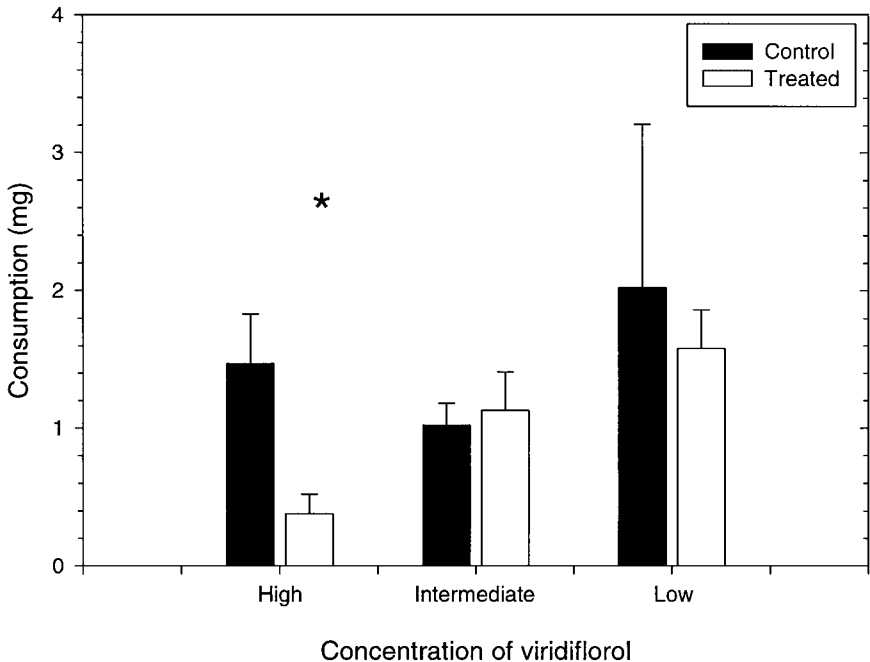


FIG. 6. Mean consumption (\pm SE) by *S. invicta* workers offered a choice between a dog food bait treated with CHCl_3 (control) and viridiflorol at high ($18 \mu\text{g}/\text{mg}$ dog food bait), intermediate ($1.8 \mu\text{g}/\text{mg}$ dog food bait), and low ($0.18 \mu\text{g}/\text{mg}$ dog food bait) concentrations. Asterisk indicates significant difference between the control and treated means.

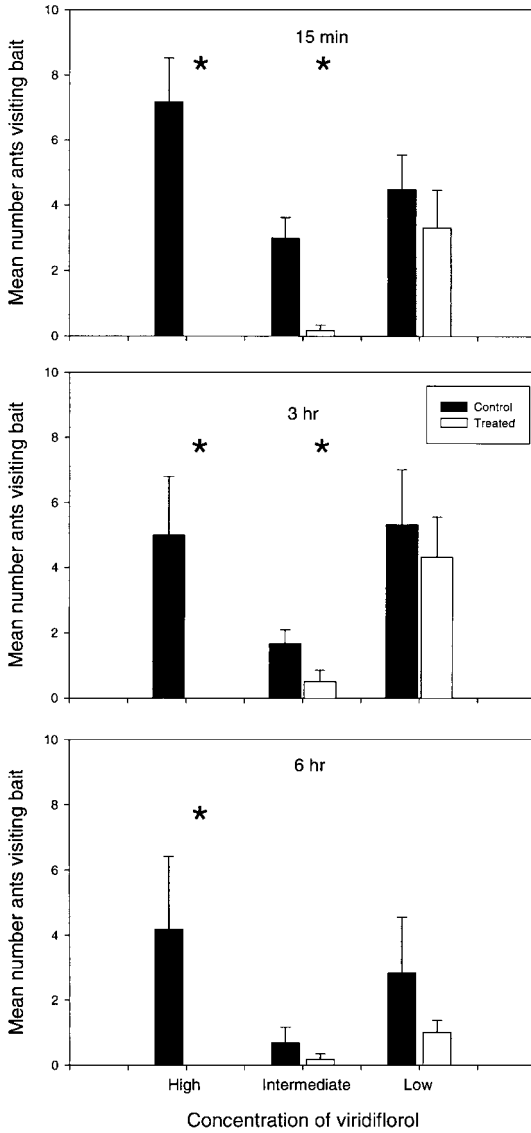


FIG. 7. Mean number (\pm SE) of *S. invicta* workers visiting dog food bait treated with CHCl_3 (control) and viridiflorol at high ($18 \mu\text{g}/\text{mg}$ dog food bait), intermediate ($1.8 \mu\text{g}/\text{mg}$ dog food bait) and low ($0.18 \mu\text{g}/\text{mg}$ dog food bait) concentrations. The number of ants contacting each dog food bait was counted at 15 min, 3 hr, and 6 hr after beginning the experiment. Asterisks indicate significant differences between the control and treated means.

DISCUSSION

These results demonstrate the repellent nature of the larval secretions of the *M. quinquenervia* biological control agent *O. vitiosa* toward the red imported fire ant *S. invicta*. They indicate repellent activity from the crude larval washes, mixtures of the 10 major terpenoids contained in the larval washes, and individual compounds from this mixture. In all experiments, fire ant consumption and visitation to the treated baits significantly decreased compared with the solvent-treated controls. The repellency of the most active component, viridiflorol, was demonstrated throughout the duration of the experimental observations (6 hr). Additionally, 1,8-cineole and α -terpineol were repellent during the initial observation (15 min). Possibly limonene was also repellent, although variable, and thus was not statistically significant in these analyses.

In the larval secretions, the three most active compounds, viridiflorol, 1,8-cineole, and α -terpineol, were recovered at about twice the concentration extracted from the host foliage, suggesting that the larvae sequester these plant-derived compounds. In fact, nearly all of the 10 compounds investigated were in greater concentration (on a fresh mass basis) washed from the larvae compared with those measured in the leaf extracts. The most active ant repellents increased the most from the plant to the larvae, namely viridiflorol (2.0-fold), 1,8-cineole (2.1-fold), and α -terpineol (2.5-fold). The proportion of ingested terpenoids retained by larvae on the cuticle can be calculated and expressed as the conversion efficiency for each terpenoid (ECI_t), analogous to calculation of the food conversion efficiency (ECI_f) (Slansky and Scriber, 1985). The ECI_t values were estimated by: [larval mass (mean = 101.9 mg) \times concentration of the terpenoid ($\mu\text{g}/\text{mg}$) washed from the larvae (from Table 2)]/[food consumed (mg) \times concentration of the terpenoid ($\mu\text{g}/\text{mg}$) in the leaves (from Table 2)]. We used consumption (400 mg fresh mass) and food conversion efficiency (ECI_f ; 7.3% fresh mass) results from a similar study of *O. vitiosa* larvae fed leaves of *M. quinquenervia* (Wheeler, unpublished data). To determine if select terpenoids were actively sequestered and deposited on the larvae, the conversion efficiency of each active terpenoid washed from the cuticle (ECI_t) can be compared with the conversion efficiency of the food (ECI_f). The results of these calculations generally suggest that more than half the quantity consumed of the most repellent terpenoids was retained and deposited on the larval cuticle. For example, the ECI_t values all exceeded 46% for viridiflorol (49.8%), 1,8-cineole (54.5%), and α -terpineol (63.9%). Moreover, with the exception of α -terpinene and terpinen 4-ol, which were present in barely detectable levels, the conversion efficiencies of the less-repellent terpenoids were considerably lower ($\leq 30\%$). These results are consistent with the interpretation that these terpenoids were actively sequestered, as these values were considerably greater than the conversion efficiency value for food ($ECI_f = 7.3\%$). A similar analysis was conducted, using the approximate digestibility (AD) of the

ingested terpenoid 1,8-cineole by a chrysomelid eucalypt-feeder, *Paropsis atomaria* (Olivier) (Ohmart and Larsson, 1989). Our calculations also suggest that sequestration was selective as the ECI_t values were greatest for the most repellent compounds. Similar selectivity was found with the larvae of other species where the most emetic iridoid glycoside, catalpol, was selectively sequestered compared with the less-emetic aucubin (Bowers and Collinge, 1992; Bowers et al., 1993). However, our calculations need to be confirmed with a more direct determination of the terpenoid budgets. Finally, our intention was to test the terpenoids at physiological concentrations. Thus, as the most repellent compounds were the most abundant terpenoids washed from the larvae, they were also tested at higher concentrations. Possibly, α -terpinene and γ -terpinene would be equally active if tested at higher concentrations.

Ant foraging activity was similar in studies 1–3, where typically more than 10 ants was reported on the control bait during each observation and 5 mg of the control bait was consumed. However, a decrease in the number of foraging ants was observed in study 4, resulting in less than 10 foragers reported during all observation periods and less than 2 mg of bait consumed by foragers. Similar variability in foraging activity occurred in a subsequent study (Wheeler et al., unpublished data). Several factors may influence ant activity including degree of satiation. However, the food was always removed from the ant colonies 24–36 hr prior to each test. Possibly seasonal or colony phenology changes influenced foraging activity. However, these factors were minimized as all the studies were conducted during July and August 2000. Moreover, studies were always conducted from 9 AM to 3 PM at ambient temperatures that varied only slightly on the days of the tests, with mean (range) temperatures between 27.3°C (22.8–31.7°C; study 3) and 28.6°C (23.3–33.9°C; study 4). Thus, it is unclear why consumption and foraging activity were lower during study 4.

Terpenoids are well known ant repellents (Shorey et al., 1992) that, when sequestered (e.g., cardenolides, cucurbitacins), may be repellent against many predatory types (Bowers, 1990). Several terpenoids have shown activity against both herbivorous and predaceous ant species. For example, plant foliar terpenoids may either repel (Hubbell et al., 1984; Howard et al., 1989) or be toxic to leaf-cutting ants (Howard et al., 1988). Additionally, insect-produced terpenoids from chrysomelid larvae also repel *S. invicta* (Blum et al., 1978). The plant sesquiterpene farnesol may be applied as a band around citrus tree trunks to exclude Argentine ants *Linepithema humile* (Mayr) for several months (Shorey et al., 1996). Numerous compounds with a range of volatilities have been reported to have ant repellent activity (Shorey et al., 1992); however, to our knowledge, this is the first report of repellent activity at physiological concentrations of the terpenoids viridiflorol, 1,8-cineole, and α -terpineol.

The ecological host range of this biological control agent *O. vitiosa* may be influenced by the availability of foliar compounds that serve as effective repellents

against generalist predators. Larvae may escape from predators on plant species that have the compounds that can be sequestered for antipredator defense (Jeffries and Lawton, 1984). Even though oviposition and larval development may occur on test plants in the laboratory, in field conditions, where natural enemies like fire ants are abundant, host use may be restricted to plant species that provide compounds that can be sequestered and impart defense against generalist predators. Although field observations indicated that this species was only collected on *M. quinquenervia* (Balciunas et al., 1994), quarantine host testing indicated that neonates complete development on the Australian myrtaceous species *Callistemon rigidus* and *C. viminalis* (Balciunas and Buckingham, 1996). Additionally, third instars previously fed *M. quinquenervia* will feed on the North American *Myrica cerifera* (Balciunas and Buckingham, 1996; Wheeler, unpublished data). Additional chemical variation in the *O. vitiosa* host range exists in the different *M. quinquenervia* chemotypes, both of which occur in Australia (Ireland, 1999) and Florida (Dray and Wheeler, unpublished data). The two *M. quinquenervia* chemotypes have distinct terpenoid profiles; one has primarily *trans*-nerolidol and only small concentrations of the primary components found in the present study. Different larval diets influenced the cuticular waxes of *Manduca sexta* when fed different plant species or an artificial diet (Espelie and Bernays, 1989) and these differences influenced the susceptibility of the larvae to ant predation (Cornelius and Bernays, 1995). The protection *O. vitiosa* larvae derive from these plant species and chemotypes and its ecological significance are the subject of an additional series of studies (Wheeler et al., unpublished data).

Predators are well-known antagonists of arthropods used for biological control of weeds (Crawley, 1989; Julien and Griffiths, 1998), and predation is so common that it may be assumed that it likely influences the establishment of all agents (Goeden and Louda, 1976). In general, natural enemies, including predators, parasitoids, and diseases interfere with approximately half of biological control projects (Goeden and Louda, 1976). *Oxyops vitiosa* is apparently well protected from one of the most common invertebrate generalist predators in the southeastern United States (Montgomery and Wheeler, 2000). However, recent observations indicate that another predator, the pentatomid, *Podisus mucronatus* Uhler, with relatively long mouthparts may penetrate the integumental defenses of *O. vitiosa* larvae (Pratt, unpublished data). Different mouthpart sizes have allowed heteropteran predators with long mandibles [e.g., *Nabis americanoferus* (Carayon) (Heteroptera: Nabidae) or *Podisus maculiventris* (Say)] (Heteroptera: Pentatomidae) to utilize otherwise well-defended prey compared with predators with shorter mandibles (e.g., coccinelids) (Olmstead and Denno, 1993). Nonetheless, *O. vitiosa* has become well established in Florida (Center et al., 2000). Several factors may contribute to its success, among them is their integumental terpenoid defense.

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