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Chemotype variation of the weed *Melaleuca quinquenervia* influences the biomass and fecundity of the biological control agent *Oxyops vitiosa*

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Abstract

Host plant nutritional and non-nutritional variability can have a significant effect on herbivore populations by influencing survival, larval performance, and fecundity. The effect of chemical and physical variation of the leaves of two chemotypes of the weed *Melaleuca quinquenervia* was determined on the biomass and fecundity of the biological control agent *Oxyops vitiosa* (Coleoptera: Curculionidae). *M. quinquenervia* chemotypes were distinguished by the principal terpenoids *E*-nerolidol and viridiflorol using gas chromatography and mass spectroscopy. Not only were the terpenoid profiles of the two chemotypes different but the viridiflorol leaves had greater toughness (1.2-fold) and reduced nitrogen (0.7-fold). When the larvae and adults were fed leaves of the *E*-nerolidol chemotype increased adult biomass (1.1-fold) and fecundity were found (2.6- to 4.5-fold) compared with those fed leaves of the viridiflorol chemotype. Regardless of the larval diet, when adults were fed the *E*-nerolidol chemotype leaves they had greater egg production compared with those adults fed the viridiflorol leaves. Moreover, adult pre-oviposition period was extended (1.5-fold) when individuals were fed the viridiflorol leaves compared with those fed the *E*-nerolidol leaves. By rearing the *O. vitiosa* weevil on the more nutritious chemotype plants these results assisted in the mass production and establishment of the *M. quinquenervia* biological control agent.
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Keywords: Insect nutrition; *Melaleuca quinquenervia*; *Oxyops vitiosa*; Plant quality; Secondary metabolites; Terpenoids; Weed biological control; Toughness

1. Introduction

Host plant quality that varies in nutritional and non-nutritional components is an important factor that influences larval performance (e.g., consumption, growth, and development) and fecundity of herbivorous insects with potential for population- and ecosystem-level effects (Awmack and Leather, 2002; Schweitzer et al., 2004). Host quality can be influenced by both environmental (Krischik and Denno, 1983) and genetic factors (Berenbaum and Zangerl, 1992). Among the many plant quality components, secondary metabolites may be under genetic control resulting in quantitative and qualitative variability (e.g., Shelton et al., 2002). In many aromatic plant species, as in the families Myrtaceae (Brophy and Doran, 1996) and Lamiaceae (= Labiatae; e.g., Schmidt et al., 2004), different chemical variants are well-known and have been identified as distinct chemotypes. Examples of secondary compound variability in invasive weeds targeted for biological control include *Euphorbia esula* L. Euphorbiaceae (Holden and Mahlberg, 1992), *Hypericum perforatum* L. Clusiaceae (Sirvent et al., 2002), *Lantana camara* L. Verbenaceae (Randrianalijaona et al., 2005), and *Senecio jacobaea* L. Asteraceae (Macel et al., 2002). These variable levels of secondary metabolites can potentially have a significant impact on the performance and fecundity of adapted and non-adapted herbivore species, however, this has yet to be well documented.

Foliage quality of the invasive weed *Melaleuca quinquenervia* (Cavanilles) S. T. Blake (Myrtaceae) has been shown

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to reduce the survival, development rate, growth, and fecundity of the biological control agent *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae; Wheeler, 2001, 2003). The plant quality factors found to reduce larval performance included high leaf toughness, low leaf moisture, and low percent nitrogen (Wheeler, 2001, 2003). However, plants of the family Myrtaceae, including the *Eucalyptus* and *Melaleuca* spp., are also well known sources of volatile essential oils, many of which have medicinal value (Lassak and McCarthy, 1983). These same essential oils also protect plants from non-adapted herbivores or function in host location for adapted species (Gershenzon and Croteau, 1991; Langenheim, 1994). The essential oils in the *M. quinquenervia* foliage include some of the most bioactive compounds known (Aldrich et al., 1993; Clarke et al., 1999; Doskotch et al., 1980; Lawler et al., 1999; Muller and Hilker, 1999; Ndiege et al., 1996; Wheeler et al., 2003), however, their relevance to the biological control agent *O. vitiosa* growth and fecundity have yet to be determined.

The volatile essential oil constituents of many species of the Myrtaceae are highly variable and are controlled by genetic and/or environmental factors (Butcher et al., 1992, 1994; Doran and Bell, 1994; Doran and Matheson, 1994; Shelton et al., 2002). The variation in constituents studied from the leaves of *M. quinquenervia* in its native range (Ireland et al., 2002) and elsewhere (Moudachirou et al., 1996; Philippe et al., 2002; Ramanoelina et al., 1994) indicate that numerous chemotypes exist within the species. Preliminary studies indicate that similar chemical variation exists among different Florida populations of *M. quinquenervia* (Wheeler, unpublished data). Analysis of the essential oils of several Florida *M. quinquenervia* trees indicates that at least two distinct chemotypes exist. One is referred to here by its primary sesquiterpene *E*-nerolidol (chemotype I). Another chemotype is referred to here by a primary sesquiterpene viridiflorol (chemotype II).

Few studies have addressed the biological relevance of these chemotype differences toward biological control agents. Oviposition by another species introduced for *M. quinquenervia* biological control, the psyllid *Boreioglycaspis melaleucae* Moore showed a preference for the viridiflorol chemotype plants even though no difference in performance was detected (Wheeler and Ordung, 2005). When larvae of *O. vitiosa* were fed leaves of the viridiflorol chemotype survival decreased, as did larval biomass gain compared to those fed the *E*-nerolidol leaves (Dray et al., 2004). However, the effect of these chemotypes on adult fecundity has not been determined. Possibly, the success of biological control agent impact on the target weed will be influenced by the nutritional value of the chemical variant on which it feeds. To improve establishment of this species and future insects imported to control *M. quinquenervia* it may be critical to understand how this variability impacts biological control agent biology. The goals of these studies were to determine the terpenoid composition, percent nitrogen, and level of toughness of the leaves of each chemotype and to evaluate their nutritional suitability for larval biomass gain, adult preoviposition, and fecundity.

2. Methods and materials

2.1. Plants

Seedlings of *M. quinquenervia* were obtained by germinating seeds collected from trees in south Florida. Plants from each chemotype were obtained from vegetative cuttings from trees whose chemotype had previously been determined by gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS; see below). All plants were transplanted into larger pots (11.4 L) when about 25 cm tall. These plants were fertilized with 90 g/pot Osmocote Plus 15-9-12, N–P–K (Scotts-Sierra Horticultural Products, Marysville, OH) in a slow-release 'southern' formulation (Wheeler, 2003). Plants were grown in a screenhouse that received rainwater and daily irrigation from overhead sprinklers for approximately 6 months at which time the plants were about 1 m tall. Three times weekly, leaves were clipped from trees and brought back to the laboratory. As *O. vitiosa* is a known flush-feeder (Wheeler, 2001), only the silky terminal 15 cm tip leaves of each tree were collected and either used for plant quality analysis or fed to larvae.

2.2. Plant quality

Several leaf quality factors that are relevant to herbivore nutrition were investigated including leaf toughness, percent moisture (for determination of nutrient dilution), nitrogen content, and terpenoid constituents. Leaves were tested for toughness using a modified gram gauge (Wheeler, 2001) which estimates the pressure required to puncture leaf tissues. Leaf toughness was measured on leaves 1–10 counting from the tip leaves toward the branch base. Replicates consisted of 20 leaves of each position where four leaves were analyzed from five trees of each chemotype. Leaf percent moisture ($n = 50$) was determined gravimetrically by weighing 10 branch tips fresh and after drying (60 °C) for 48 h from five trees of each chemotype. Percent nitrogen ($n = 3$) was determined for pooled tip leaves from five trees of each chemotype with a modified Kjeldahl method on a dry mass basis as previously described (Wheeler, 2001). The tip leaves were pooled in order to have sufficient material (500 mg dry mass) for analysis.

2.3. Terpenoid analysis

Flush leaves were clipped from young trees of *M. quinquenervia* ($n = 10$) and brought to the laboratory where they were frozen (−10 °C) as described previously (Wheeler et al., 2003). The leaf components were extracted by a modified microwave technique (Wheeler et al., 2003).

2.4. Chemicals

Standards were purchased from commercial sources, or donated (viridiflorol and 2,4-dihydroxy-6-methoxytoluene)

by I. A. Southwell (NSW Agriculture, Wollongbar Agricultural Institute, NSW, Australia) and were of the highest purity available (details described in Wheeler et al., 2003). The standards included the primary compounds reported by Brophy et al. (1989) and Wheeler et al. (2003).

2.5. Gas chromatography

Samples were analyzed with an Agilent (Hewlett-Packard) model 6890 GC. Data collection, storage, and analysis were conducted with the Agilent ChemStation (Wilmington, DE) data system. Helium at a linear flow rate of 37 cm/s was used as a carrier gas. All samples were analyzed on a fused silica capillary column (DB-17MS J&W Scientific; 30 m × 0.32 mm i.d., 0.25μm thick film). Injector temperature was 250 °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 10 min.

Compound identities were confirmed by GC/MS using an Agilent 6890 instrument fitted with either a HP-5MS (Agilent, 30 m × 0.25 mm, 0.25μm film thickness) or a DB-17MS (30 m × 0.32 mm, 0.25μm thick film) FSOT column with helium at 36 or 42 cm/s (HP-5MS and DB-17MS, respectively) as a carrier gas, injector port (split 1:20) 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, comparison with authentic constituents when available, and mass spectral and retention matching with commercial libraries (NIST, Wiley, and Adams).

2.6. Identifying the chemotype source of each larva

To determine the influence of larval and adult diets on preoviposition and fecundity, *O. vitiosa* individuals were collected on a single date as 3rd or 4th instars on *M. quinquenervia* at one of two sites: Davie, Broward or Estero, Lee, Co. FL. Plants from the Davie and the Estero sites were known to be predominantly (82–83%) viridiflorol and *E*-nerolidol chemotype plants, respectively (Wheeler, unpublished data).The chemotype of the plant from which each larva was collected was confirmed by GC and GC/MS. Larvae were discarded that were collected on plants that were not of the predominant chemotype at each site. Field collected 3rd to 4th instars were fed leaves from screenhouse-grown *M. quinquenervia* plants of the same chemotype from which they were collected until the prepupal stage. Larvae were reared in petri dishes (15 × 2 cm) lined with moistened filter paper and sealed with Parafilm to retain moisture. Larvae were fed every 3 days and mortality was 5% for those reared on each chemotype leaf. Prepupae were transferred to individual 30 ml plastic cups for pupation containing ground floral blocks (Smithers-Oasis, Kent, OH, USA) (Wheeler and Zahniser, 2001). All insects were reared at 28 °C, 90% RH, and L14:D10 h photoperiod.

2.7. Adult performance and fecundity

To determine if chemotype nutrition and adult gender influenced adult biomass, 1-day-old adult males and females were weighed individually (±0.1 mg) prior to feeding. Then adult male and female pairs ($n = 25$/treatment combination) were transferred to Plexiglas cylindrical cages (30 × 15 cm) and supplied with fresh *M. quinquenervia* tips. To distinguish between the effect of larval and adult nutrition on adult pre-oviposition periods and fecundity, adults were fed every 3 days either flush leaf tips (ca. 15 cm) of the same chemotype upon which they had fed as larvae or flush leaves of the alternate chemotype. Of the original 25 adult pairs, 20, 22, 23, and 22, survived, produced eggs, and were included in the analysis from the nerolidol–nerolidol, nerolidol–viridiflorol, viridiflorol–nerolidol, and viridiflorol–viridiflorol diets, respectively. In addition to the adult biomass data collection described above, data were collected on preoviposition period, and the total number of eggs produced over the course of the experiment. The eggs were removed and counted every 3 days for 140 days.

2.8. Data analysis

All analyses were conducted with SAS/PC (PROC GLM) unless otherwise noted (SAS Institute Inc., 1990). Leaf percent nitrogen and moisture were determined from leaves pooled from the branch tips and were analyzed by one-way ANOVA. The leaf toughness results were analyzed on different leaves and comparisons of regression coefficients of the different chemotypes were performed by ANCOVA where leaf position served as the covariate. To determine if larval diets and gender influenced adult biomass a two-way ANOVA with interaction was conducted. To determine the influence of larval and adult diets, the adult preoviposition, and fecundity data were analyzed with two-way ANCOVAs with interactions where adult biomass served as the covariate and larval and adult diets served as the main effects.

3. Results

3.1. Plant quality

Percent foliar water did not differ significantly between the two chemotypes ($P > 0.4$), where the average value was 76.6 ± 1.2% fresh mass. Percent nitrogen levels were greater for leaves from the *E*-nerolidol plants compared with those from viridiflorol plants (Fig. 1).

Leaf toughness was significantly greater in the viridiflorol chemotype compared with that of the *E*-nerolidol (Fig. 2). Leaf toughness ranged from 106 to 310 g/mm^2 and increased significantly from the tips toward the branch base of both chemotypes. The slope of the leaf toughness line across leaf positions for the viridiflorol chemotype leaves was significantly greater than that of the *E*-nerolidol chem-

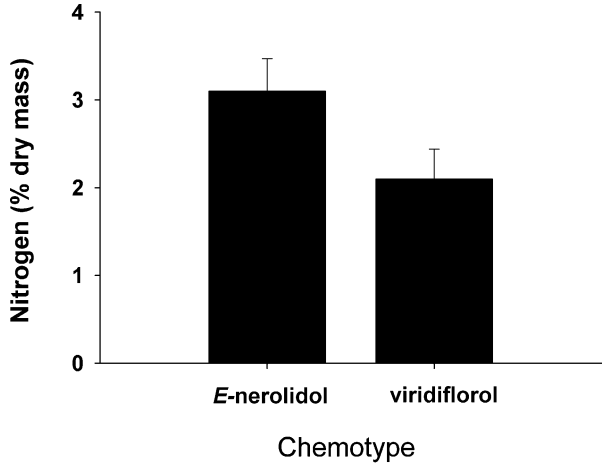

Fig. 1. Mean percent (±SE) nitrogen of *M. quinquenervia* leaves from two chemotypes, *E*-nerolidol and viridiflorol. The *E*-nerolidol leaves had significantly greater percent nitrogen content ($F_{1,8} = 3.95$; $P = 0.0821$) compared with the viridiflorol leaves.

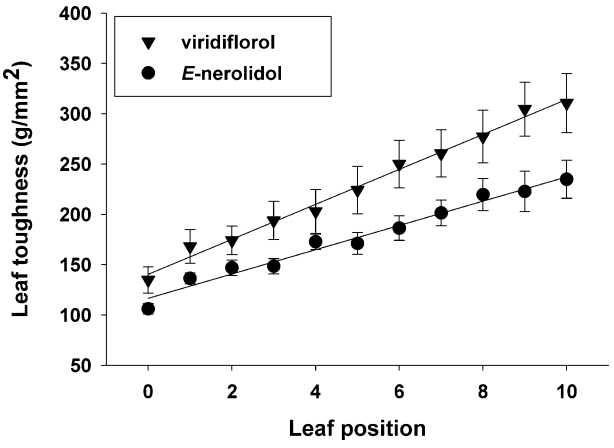

Fig. 2. Mean toughness (±SE) of *M. quinquenervia* leaves located on branches with emerging leaves. The slope of the line for the viridiflorol chemotype leaves (17.4 ± 0.6) was significantly greater ($F_{1,18} = 40.73$; $P < 0.0001$) than that of the *E*-nerolidol chemotype (12.1 ± 0.6). Moreover, the elevation of the line for the viridiflorol chemotype leaves (140.4 ± 3.3 g/mm^2) was significantly ($F_{1,18} = 23.12$; $P < 0.0001$) greater than that of the *E*-nerolidol chemotype (116.7 ± 3.7 g/mm^2).

otype. Moreover, the elevation of the line for the viridiflorol chemotype leaves was significantly greater than that of the *E*-nerolidol chemotype.

The two *M. quinquenervia* chemotypes could be readily distinguished by GC and GC/MS analysis of the foliar constituents (Fig. 3). The profile of the *E*-nerolidol chemotype contained relatively high concentrations of the sesquiterpene *E*-nerolidol, whereas this compound was not recovered from the viridiflorol chemotype leaves (Fig. 3; Table 1). Moreover, the profile of the *E*-nerolidol leaves either lacked or had much lower concentrations of nearly all the other constituents compared with the viridiflorol leaves. The only exception was relatively high concentrations of 2,4-dihydroxy-6-methoxytoluene in the *E*-nerolidol leaves (Table 1). The viridiflorol leaves also had relatively high concentrations of the sesquiterpenoids viridiflorol and β-caryophyllene and the monoterpenoids α-pinene, limonene, 1,8-cineole, and α-terpineol.

3.2. Adult performance and fecundity

Adult biomass was significantly influenced by both the larval diet and the adult gender (Fig. 4). However, the interaction between these two effects was not significant. Although male biomass did not differ, females collected from and fed the *E*-nerolidol plants had greater biomass compared with females from the viridiflorol chemotype (Fig. 4). Additionally, regardless of diet, females had greater biomass than males.

After the field-collected larvae were reared to the adult stage they were either maintained on leaves from plants of the same chemotype or switched to leaves of the alternate chemotype. The pre-oviposition period was only influenced by adult diet where those fed the viridiflorol chemotype required a significantly longer pre-oviposition period compared with the adults fed the *E*-nerolidol chemotype (Fig. 5A). Neither adult biomass ($P > 0.4$), larval diet ($P > 0.5$), nor the two-way interactions adult biomass × larval diet ($P > 0.6$), adult biomass × adult diet ($P > 0.2$) and larval diet × adult diet ($P > 0.3$) were significant.

The mean number of eggs produced after 140 days (Fig. 5B) was significantly influenced by the larval diet, the adult diet, and the interaction of these two. Adult biomass had no significant influence on egg production ($P > 0.7$) and was dropped from the analysis. Larvae fed the *E*-nerolidol plants and maintained as adults on leaves of the same chemotype increased egg production 2.6-fold over larvae fed viridiflorol leaves and maintained as adults on the viridiflorol leaves. Larvae fed the *E*-nerolidol plants and maintained as adults on leaves of the same chemotype increased egg production 3.1-fold over larvae fed *E*-nerolidol leaves then switched as adults to the viridiflorol leaves. Similarly, larvae fed the viridiflorol leaves and switched as adults to the *E*-nerolidol plants increased egg production 4.5-fold over those individuals fed as larvae and adults the viridiflorol leaves. These results indicate that regardless of the larval diet, when adults are fed the *E*-nerolidol chemotype leaves they had greater egg production compared with those adults fed the viridiflorol leaves. Moreover, insects fed the viridiflorol leaves as larvae and *E*-nerolidol as adults produced more eggs compared to insects fed *E*-nerolidol during larval and adult stages.

4. Discussion

Despite the diversity of secondary metabolites found in nearly all plant species, their impact on weed biological control programs has rarely been addressed (Jordon-Thaden and Louda, 2003). Secondary metabolite differences among plant species can influence the host selection and utilization by non-adapted (Rosenthal and Berenbaum, 1992) and adapted herbivores, including those developed

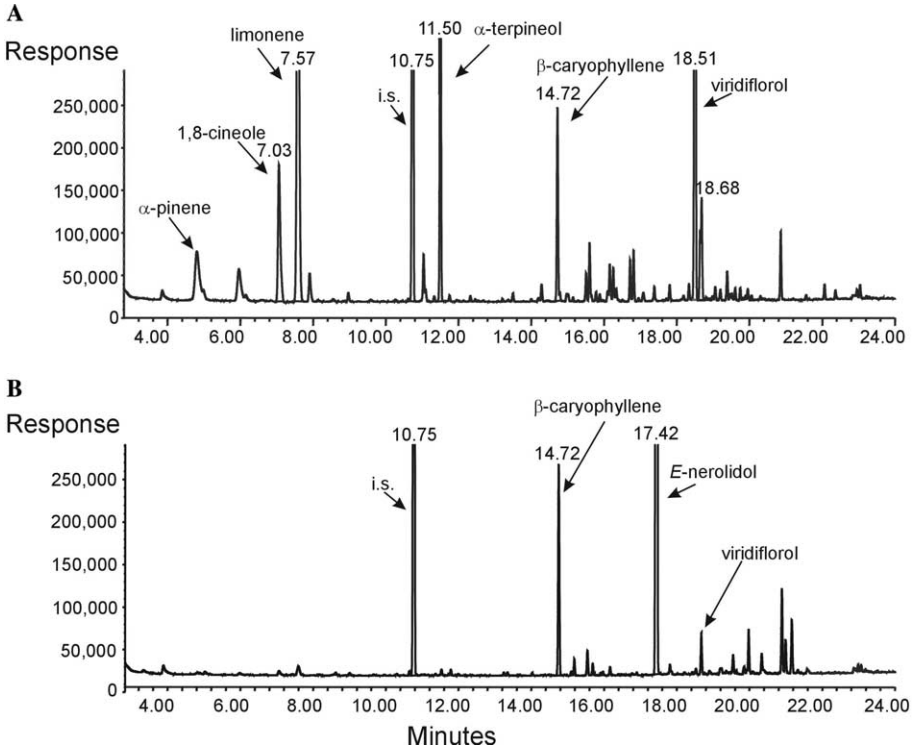

Fig. 3. Representative gas chromatographs (GC) of *M. quinquenervia* leaves from two chemotypes, viridiflorol (A) and *E*-nerolidol (B). The viridiflorol leaves had higher concentrations of several constituents, among them 1,8-cineole, limonene, α-terpineol, and viridiflorol compared with the *E*-nerolidol leaves. GC analysis was conducted on a DB-17MS, 30 m × 0.32 m, 0.25μm film thickness. Leaves were digested in 95% EtOH by 60 s microwave (750 watt) irradiation. The internal standard (i.s.) *n*-tridecane (50 ng/μl) was added to each sample.

for weed biological control (Wheeler, 2005). Diversity in secondary metabolite profiles and their concentrations can also vary within a species, as found here, creating distinct chemical variants that may be less susceptible to biological control.

The results presented here indicate that at least two distinct chemical variants or chemotypes of the environmental weed *M. quinquenervia* exist in Florida and that significant differences exist in the *O. vitiosa* larval and adult performance when fed the different chemotypes. These results indicate that adults reared as larvae on the viridiflorol chemotype had reduced adult biomass. Additionally, adults fed this chemotype as adults had reduced fecundity compared with those fed the *E*-nerolidol chemotype. This supports previous results that indicated reduced larval survival and biomass gain when fed the viridiflorol chemotype (Dray et al., 2004). In contrast, an oviposition preference for the viridiflorol chemotype was found with another *M. quinquenervia* biological control agent, the psyllid *Boreioglycaspis melaleucae* (Wheeler and Ordung, 2005). Although *O. vitiosa* is specialized to feed on *M. quinquenervia* and is apparently well equipped to detoxify the secondary metabolites in this species, viridiflorol alone or in combination with other terpenoids occur(s) in concentrations that reduce the larval performance and fecundity in individuals of this species.

Our results indicate that regardless of larval diet, the adult diet could determine the fecundity of the individual. Adults fed the *E*-nerolidol chemotype leaves had greater egg production compared with adults fed the viridiflorol leaves, regardless of the larval diet. Other species, like these weevils, that have long-lived adult stages are apparently able to ameliorate the influence of a poor larval diet during the adult stage resulting in relatively high levels of fecundity (Hopkins and Ekbom, 1999). By comparison, in insect species with short-lived adult stages like Lepidoptera, fecundity may be determined primarily by larval diet (Boggs and Ross, 1993).

The large differences in secondary compound profiles (qualitative and quantitative) between leaves of these two chemotypes can explain the reduction in larval performance and adult fecundity. However, the plant quality factors, nitrogen, and leaf toughness, probably contributed little to the relatively poor performance of the individuals fed the viridiflorol chemotype. Although the viridiflorol chemotype leaves had 0.7-fold lower levels of nitrogen (3.1 vs 2.1%), it is doubtful that this difference can completely explain the decrease in biomass and fecundity reported here. Previous work (Wheeler, 2003) indicated that when *O. vitiosa* larvae were fed *M. quinquenervia* leaves in a similar nitrogen range (2.0–2.5%) no difference in larval performance (survival, consumption, and biomass gain) or fecundity was found. Additionally, although previous results from field samples (Wheeler, 2001) indicated that *M. quinquenervia* leaf toughness values of about 800 g/mm^2 were associated with a significant decrease in *O. vitiosa* neonate

Table 1
Mean (±SE) concentration of constituents identified by GC and GC/MS from flush leaves from two chemotypes of *M. quinquenervia* ($n = 10$)

	M. quinquenervi[a]					
	Viridiflorol			*E*-nerolidol		
Constituents	*n*[b]	μg/mg	SE	*n*[b]	μg/mg	SE
α-Thujene[c]	10	0.0302	0.0050	0	nd[a]	
α-Pinene	10	3.3929	0.2609	10	0.4647	0.0765
β-Pinene	10	1.7659	0.1845	10	0.0787	0.0052
Myrcene	10	0.7954	0.1527	10	0.0165	0.0064
Limonene	10	3.3074	0.3522	10	0.1050	0.0229
1,8-Cineole	10	26.7769	5.6446	10	0.0713	0.0132
p-Cymene[c]	10	0.0204	0.0043	0	nd[a]	
γ-Terpinene	10	0.3337	0.0538	2	0.0090	0.0025
Terpinolene	10	0.1167	0.0100	10	0.0372	0.0057
Linalool	10	0.1119	0.0197	10	0.0874	0.0148
Terpinen 4-ol	10	0.3482	0.0460	1	0.0087	
α-Terpineol	10	8.2491	1.6666	9	0.0242	0.0045
α-Copaene	10	0.1188	0.0143	9	0.0264	0.0067
α-Gurjunene	10	0.4560	0.0522	10	0.0240	0.0045
β-Caryophyllene	10	3.1393	0.3249	10	1.6810	0.1634
(+)-Aromadendrene	10	0.1179	0.0166	10	0.0759	0.0220
α-Humulene	10	0.5154	0.0520	10	0.2428	0.0243
(−)-Alloaromadendrene	10	0.7535	0.0967	10	0.0284	0.0057
β-Selinene	10	0.5813	0.0690	10	0.1310	0.0169
α-Selinene	10	0.2773	0.0366	9	0.0122	0.0016
γ-Cadinene	10	0.3241	0.0459	10	0.0286	0.0024
δ-Cadinene	10	0.4586	0.0648	10	0.0221	0.0040
E-Nerolidol	0	nd[a]		10	60.3586	11.9482
Globulol	10	0.1498	0.0162	8	0.0120	0.0041
Viridiflorol	10	24.6252	0.2519	10	0.7914	0.1439
Caryophyllene oxide[c]	10	1.3205	0.1353	10	0.0400	0.0075
Epi-α-cadinol[d]	10	0.2583	0.0227	9	0.0183	0.0028
α-Cadinol[d]	10	0.1502	0.0157	8	0.0142	0.0019
β-Eudesmol	10	0.1435	0.0242	10	0.0422	0.0052
2,4-Dihydroxy-6-methoxytoluene	10	0.7119	0.0945	10	1.5562	0.2610

[a] Compound was not detected.
[b] Number of plants where the constituent was detected.
[c] Constituents quantified on the HP-5MS column, otherwise on the DB-17MS column.
[d] Tentative assignment as identification was based on matching spectra and retention index but no standard was available for comparison.

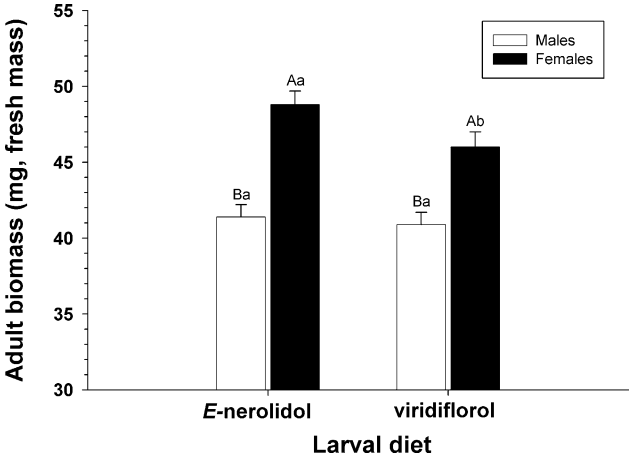

Fig. 4. Mean biomass (±SE) of *O. vitiosa* adult males and females collected from and fed as larvae *M. quinquenervia* leaves from either the *E*-nerolidol or the viridiflorol chemotype. Adult biomass was significantly influenced by both the larval diet ($F_{1,168} = 4.12$; $P = 0.0441$) and the gender of the adults ($F_{1,168} = 48.90$; $P < 0.0001$) but not by their interaction ($P > 0.2$). Females fed *E*-nerolidol leaves had significantly greater biomass than females fed viridiflorol leaves (lowercase letter comparisons within each gender). Male biomass was not influenced by larval diet. Additionally, females regardless of diet had significantly greater biomass than males (uppercase letter comparisons within each larval diet).

survival (7.5% survival), the leaf toughness values presented here were much lower, ranging from 106–310 g/mm^2. The impact of these high toughness values on *O. vitiosa* fecundity, however, have yet to be determined. Studies are planned that incorporate natural concentrations of these secondary compounds into an insect diet. These studies should assist in determining the impact of these chemotype compounds on larval performance and fecundity without the interference of other factors.

These results indicated that larvae collected on viridiflorol and switched to *E*-nerolidol plants had increased fecundity compared with those collected on *E*-nerolidol leaves and fed as adults on the same chemotype. Insect herbivores are able to detoxify dietary secondary plant compounds by various means and several detoxification systems are inducible as they have greater activity following previous exposure to these or similar compounds (Lindroth, 1991; Yu, 1986). Inducible detoxifying enzymes could be responsible for these results. The larvae fed the viridiflorol chemotype may have been exposed to an inducing concentration of terpenoids that predisposed the adults to continued challenges by these and other terpenoids. These induced individuals were better able as adults to metabolize subsequent dietary challenges of *E*-nerolidol compared with adults that had been fed as larvae leaves of the *E*-nerolidol chemotype. Similar terpenoids such as α-pinene, β-pinene, and limonene induced (1.2- to 2.4-fold) the aldrin epoxidase and α-pinene induced (1.4-fold) the general esterase detoxifying enzyme systems in fall armyworm larvae (Yu, 1982; Yu and Hsu, 1985). Whether the induced enzyme activity can be carried over from the larval into the adult stage has yet to be determined.

Work is currently underway to characterize the chemotypes at different potential release sites in Florida to confirm these laboratory results. Redistributions of agents and establishment of field nursery sites benefited from matching the chemotype that best suited agent larval performance and fecundity. By matching agent biotype to weed chemotype we will dramatically improve the control exerted by biological control agents. In Australia the native range of this weevil overlaps the distribution of both *M. quinquenervia* chemotypes (Ireland et al., 2002; Purcell and Balciunas, 1994). Biotypes of *O. vitiosa* may exist in Australia that are matched with each of the weed chemotypes. The *O. vitiosa* biotype already released in Florida is well adapted to the *E*-nerolidol chemotype of *M. quinquenervia*. Possibly a biotype adapted to the viridiflorol chemotype could be identified in its native range and after completing risk assessment analysis could complement the control already exerted on *M. quinquenervia* by the *E*-nerolidol-adapted *O. vitiosa*

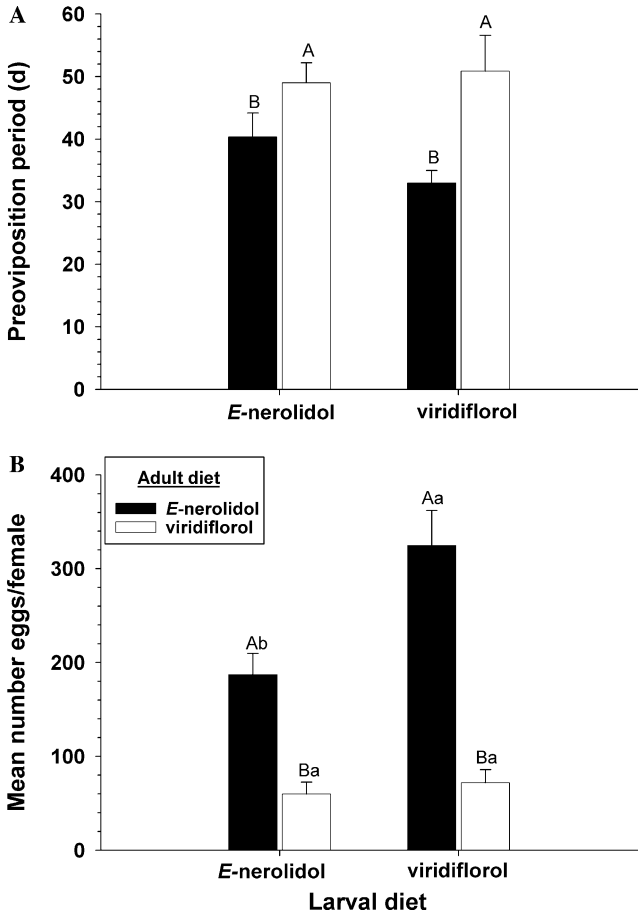

Fig. 5. Mean (±SE) *O. vitiosa* pre-oviposition period (A) and number of eggs produced per female (B) when fed as larvae or as adults leaves from each *M. quinquenervia* chemotype. The pre-oviposition period was significantly influenced only by adult diet ($F_{1,79} = 11.66$; $P = 0.0010$), where adults fed the viridiflorol leaves had a longer pre-oviposition period compared with adults fed the *E*-nerolidol leaves (A). Within larval diet, bars with the same letter are not significantly different. Additionally, the mean number of eggs produced after 140 days (B) was significantly influenced by both larval diet ($F_{1,83} = 9.36$; $P = 0.003$), adult diet ($F_{1,83} = 61.05$; $P < 0.0001$), and the interaction between these two effects ($F_{1,83} = 6.61$; $P = 0.0120$). Regardless of larval diet, adults fed *E*-nerolidol leaves had greater egg production than adults fed viridiflorol leaves. Bars in panel B with the same uppercase letters within each larval diet were not significantly different. Moreover, more eggs were produced by individuals fed as larvae the viridiflorol leaves and switched to the *E*-nerolidol leaves as adults compared with individuals fed as larvae and adults the *E*-nerolidol leaves. Bars with the same lowercase letters within the same adult diet were not significantly different.

biotype. Possibly, this compatible agent biotype/weed chemotype match may be found in other biological control agent/weed systems and could be exploited to improve control.

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