Response of Two Chemotypes of Melaleuca quinquenervia (Myrtales: Myrtaceae) Saplings to Colonization by Specialist Herbivores

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Response of two chemotypes of *Melaleuca quinquenervia* (Myrtales: Myrtaceae) saplings to colonization by specialist herbivores

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**Abstract**

Two chemotypes of *Melaleuca quinquenervia* (Cav.) S. T. Blake (Myrtales: Myrtaceae) saplings were planted in a common garden under 2 water treatments and exposed to either restricted or unrestricted herbivory from 2 insect herbivores. Chemotypes consisted of either a predominately E-nerolidol terpenoid complex or one that consisted primarily of viridiflorol; both types had influenced the performance or preference of both insect herbivores in laboratory studies. The densities of the 2 specialist herbivores *Boreioglycaspis melaleucae* Moore (Hemiptera: Psyllidae) and *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae) were monitored regularly as they colonized the plantings, as were plant responses like leaf abscission and tree height. Neither the chemotype nor the water treatments influenced the densities of *B. melaleucae* and *O. vitiosa*. Trees subjected to unrestricted herbivory shed more leaf biomass than those protected by insecticides from herbivory. There was no relationship between the amount of biomass shed via abscission and the density of either herbivore despite a significant correlation with a damage rating developed for *O. vitiosa*. There was a chemotype response to herbivory whereby more leaf biomass was shed by the *E*-nerolidol chemotype than by the viridiflorol chemotype when subjected to unrestricted herbivory. Tree height was influenced by herbivory but not chemotype or water, although there were separate 2-way interactions between all factors. Thus, despite equal herbivore pressure, the response of young *M. quinquenervia* trees to abiotic and biotic forces diverged at the plant variant level.

**Key Words:** *Oxyops vitiosa; Boreioglycaspis melaleuca; E-nerolidol; viridiflorol*

**Resumen**

Dos quimiotipos de arbolitos de *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtales: Myrtaceae) fueron plantados en un jardín común bajo 2 tratamientos de agua y fueron expuestos a la herbivoría restringida o no restringida de 2 insectos herbivores. Los quimiotipos consistían de un complejo terpenoide *E*-nerolidol predominantemente o uno que consistía principalmente en viridiflorol; ambas clases habían influido el rendimiento o la preferencia de ambos insectos herbivores en los estudios de laboratorio. Las densidades de los 2 herbivores especializados *Boreioglycaspis melaleucae* Moore (Hemíptera: Psyllidae) y *Oxyops vitiosa* Pascoe (Coleóptera: Curculionidae) fueron controladas regularmente durante su colonización de las siembras, al igual que las respuestas de las plantas como la abscisión foliar y altura de los árboles. El quimiotipo y los tratamientos de agua no influyeron en la densidad de *B. melaleucae* y *O. vitiosa*. Los árboles sometidos a la herbivoría sin restricciones perdieron más biomasa foliar que los protegidos por los insecticidas de herbivoría. No hubo una relación entre la cantidad de biomasa perdida a través de la abscisión y la densidad de cualquiera de los herbívoros a pesar de una correlación significativa con una calificación de daño desarrollada para *O. vitiosa*. Hubo una respuesta quimiotipo a la herbivoría que resultó en la disminución de más biomasa de hojas por el quimiotipo *E*-nerolidol que por el quimiotipo viridifloró cuando fueron sometidos a la herbivoría sin restricciones. La altura del árbol fue influenciada por la herbivoría pero no por el quimiotipo o agua, aunque hubo diferentes interacciones de 2 vías entre todos los factores. Por lo tanto, a pesar de presión igual del herbívoro, la respuesta de los árboles jóvenes de *M. quinquenervia* a las fuerzas abióticas y bióticas divergieron a nivel del variante de planta.

**Palabras Clave:** *Oxyops vitiosa; Boreioglycaspis melaleuca; E-nerolidol; viridiflorol*
Oxyops vitiosa Pascoe (Coleoptera: Curculionidae) in 1997 and Boreioglycaspis melaleucae Moore (Hemiptera: Psyllidae) in 2002 (Center et al. 2000, 2006). Oxyops vitiosa is a defoliator whose larvae feed on both leaf surfaces and consume the tissue through to the cuticle on the other side, whereas B. melaleucae is a phloem feeder (Purcell & Balciunas 1994; Purcell et al. 1997). Both species have unique features, like O. vitiosa larvae that are covered in a viscous layer of essential oils that deters predators, whereas B. melaleucae nymphs secrete waxy filaments that form highly visible flocculent masses on leaves (Purcell et al. 1997; Wheeler et al. 2002).

Populations of M. quinquenervia in Florida occur in 2 distinct chemical variants that are traditionally defined as chemotypes (Desjardins 2008). One chemotype (’E-nerolidol’) is characterized by acyclic foliar terpenes with high concentrations of the sesquiterpene E-nerolidol (74–95% of total oil), and the monoterpenes linalool (Ireland et al. 2002; Wheeler et al. 2007). The 2nd chemotype (‘viridiflorol’) contains high concentrations of cyclic foliar terpenes especially the sesquiterpene viridiflorol (13–66% of total oil) and the monoterpenes 1,8-cineole and α-terpineol (Ireland et al. 2002; Wheeler et al. 2007). Chemotype profiles in Florida populations of M. quinquenervia matched those in Australia (Padovan et al. 2010). Both chemotypes differentially affected the preference and performance of O. vitiosa and B. melaleucae in laboratory studies. For example, Dray et al. (2004) reported that both O. vitiosa larval survivorship and adult weight gain were greater on E-nerolidol compared with viridiflorol, whereas Wheeler (2006) found greater fecundity in O. vitiosa adults that were fed bouquets of E-nerolidol leaves compared with viridiflorol leaves. Wheeler & Ordung (2005) found that although B. melaleucae females oviposited more than twice as many eggs on the viridiflorol than on the E-nerolidol chemotype, overall performance was unaffected by chemotype. Wheeler et al. (2007) suggested that this information might guide the geographic deployment of the insects to infestations that consisted primarily of the E-nerolidol chemotype in order to maximize insect production in the field.

The primary objective of this study was to determine if the chemotypic profile of M. quinquenervia influenced the field colonization of a new population of plants by the 2 specialist herbivores. A secondary objective was to quantify various plant responses as influenced by chemotype, herbivory, moisture availability, and their interactions. Two null hypotheses were proposed; the 1st was that chemotype did not influence colonization by either herbivore, and the 2nd was that the plant’s response to herbivory was independent of chemotype and water availability.

Materials and Methods

Melaleuca quinquenervia saplings were propagated from cuttings of known chemotypes in a screen house and were planted into a common garden at the United States Department of Agriculture, Agricultural Research Service, Invasive Plant Research Laboratory in Ft. Lauderdale, Florida, on 4 Apr 2003, when they were 0.5 m tall. Plant chemotypes were determined by gas chromatography–mass spectrometry analysis of ethanol extracts from leaves as described previously (Wheeler 2006). The prevailing soil type at the field was a Margate fine sand, siliceous hyperthermic Mollic Psammamaquent, with less than a 1% slope. The experimental design was a complete 2 × 2 × 2 × 6 factorial randomized block with 2 herbivore treatments, 2 chemotype treatments (E-nerolidol or viridiflorol), 2 irrigation treatments, and 6 blocks, with the tree as the experimental unit located in the center of each square 56.25 m² plot. Each sapling was planted in the center of a 1 m² plastic mat which served to suppress weeds and catch leaves that were shed by the plant.

Herbivore treatments consisted of an herbivore control where herbivory by B. melaleucae and O. vitiosa was either restricted by regular applications of an insecticide or not restricted by applications of water. The insecticide was acephate (O,S-dimethyl acetylphosphoramidothioate) applied with a hand pressurized backpack sprayer at a concentration of 0.367% ai (v/v) until runoff. This systemic insecticide was applied as needed (every 3–6 wk) to prevent the insect populations from establishing on the plants. The insecticide concentration and application frequency neither inhibited nor stimulated plant growth (Tipping & Center 2002). Water availability was controlled with treatments that consisted of either natural rainfall or natural rainfall plus continuous irrigation using drippers that provided a mean flow rate of approximately 7.5 L h⁻¹ applied to a spot on the soil directly next to the trunk, resulting in continually saturated soils under the dripline of the tree.

Every 2 to 3 d, the plastic mat around each tree was checked for abscised leaves, which were counted, weighed for fresh weight biomass, and then dried to a constant weight to obtain dry weight biomass. The tall height of the surrounding grass around the border of the plastic mat helped to prevent the abscised leaves from being blown off the mat. Every 30 d, the following measurements were taken: plant height, the numbers of small (instars 1–3) and large larvae (instars 4–5) and adults of O. vitiosa, the number of colonies of B. melaleucae as indicated by discrete flocculent masses, and a damage rating based on the percentage of leaves that showed O. vitiosa larval feeding, ranked as (0) no damage, (1) up to 25%, (2) 26–50%, (3) 51–75%, (4) 76–99%, and (5) 100% damaged. The duration of the experiment was from 30 May to 11 Aug 2003.

Repeated measures analysis of variance was used to examine the influence of the herbivory, chemotype, and water treatments and their interactions on plant and insect parameters. Variables like the number of insects per cm of tree height were calculated to take into account changes in tree growth. Two-sample t-tests were used to separate selected means post-hoc. All statistical analyses were conducting using SAS v 9.1 (SAS Institute 2004).

![Sample Date](image-url)

**Fig. 1.** Mean (± SE) dry weight biomass of leaves shed via abscission by saplings of 2 Melaleuca quinquenervia chemotypes subjected to 2 levels of herbivory by Oxyops vitiosa and Boreioglycaspis melaleucae.
Results

Plant chemotype did not influence the densities of either colonizing insect species or the damage ratings, thus supporting the 1st null hypothesis (Table 1). The 2nd null hypothesis was not supported because the amount of leaf biomass shed from trees via abscission was influenced by chemotype ($F_{1,35} = 9.3; P = 0.004$), albeit to a lesser degree compared with herbivory, with an herbivory × chemotype interaction whereby most leaf biomass was shed from the $E$-nerolidol chemotype when subjected to unrestricted herbivory ($T = 2.04; df = 41; P = 0.04$) (Fig. 1). Chemotype also indirectly influenced tree height through interactions with both herbivory and water (Table 1). For example, the chemotype × herbivory interaction was caused by a change in magnitude whereby trees with the viridiflorol chemotype grew taller when herbivory was restricted compared with unrestricted ($T = 2.30; df = 65; P = 0.02$), whereas tree height for the $E$-nerolidol chemotype was unaffected by herbivory ($T = 0.19; df = 61; P = 0.85$) (Fig. 2). The chemotype × water interaction with tree height was caused by a minor change in magnitude whereby trees with the viridiflorol chemotype responded more to irrigation than those with the $E$-nerolidol chemotype ($T = 1.70; df = 67; P = 0.09$) (Fig. 2).

Herbivory increased the amount of leaf abscission with a greater than 4-fold difference in biomass shed between restricted and unrestricted herbivory treatments (Fig. 3). Although there was a positive relationship between the damage caused by $O$. vitiosa and the amount of leaf biomass shed via abscission ($r = 0.32; P = 0.0002$), there were no correlations between the densities of $O$. vitiosa ($r = -0.001; P = 0.98$) or $B$. melaleucae ($r = -0.06; P = 0.45$) and the amount of leaf biomass that was shed. Water treatments had no effect on leaf abscission and there were no interactions with herbivory ($F_{1,35} = 0.29; P = 0.59$) or chemotype ($F_{1,35} = 0.13; P = 0.72$) (Table 1). Tree height was influenced primarily by herbivory but not directly by chemotype or water, although there were interactions between all 3 factors as mentioned above with chemotype (Table 1). The herbivory × water interaction was explained by a change in magnitude whereby non-irrigated trees were shorter when subjected to unrestricted herbivory compared with restricted herbivory ($T = 3.37; df = 59; P = 0.001$), whereas the mean height of irrigated trees was the same regardless of herbivory ($T = 0.72; df = 67; P = 0.47$). The chemotype × water interaction is explained in the above paragraph.

Discussion

The evidence for differential herbivore preference or utilization of chemotypes in the field is limited with results that frequently contrast with one another (Macel & Klinkhamer 2010). This may be a function of the temporal flux of the characteristic constituent compounds of

![Fig. 2. Final mean (± SE) height of Melaleuca quinquenervia saplings of 2 chemotypes (A: viridiflorol; B: E-nerolidol) after exposure to 2 levels of insect herbivory and 2 water treatments.](image-url)

![Fig. 3. Total mean (± SE) leaf biomass shed via abscission by Melaleuca quinquenervia saplings subjected to unrestricted or restricted herbivory by Oxyops vitiosa and Boreiglycaspis melaleucae. **: P = 0.01.](image-url)
chemotypes in response to climatic, edaphic, and biotic factors, as well as their interactions, which may moderate their influence (Perry et al. 1999; Pecetti et al. 2006; Szakiel et al. 2011). Laboratory studies that eliminate natural biotic and abiotic factors are likely to produce exaggerated results because the differences in insect fecundity, longevity, or preference evident in controlled laboratory settings may not translate to variable field population parameters like density (Zvereva et al. 2010). In a greenhouse study, Morath et al. (2006) also found no chemotype differences in the optical density of chlorophyll in _M. quinquenervia_ leaves following feeding by _B. melaleucae_.

Although both chemotypes were colonized equally by 2 specialist herbivores, the chemotypes reacted differentially with the _E_-nerolidol chemotype dropping more leaf biomass in response to herbivory than the viridiflorol chemotype. Premature abscission of leaves in response to herbivory is a common general response of _M. quinquenervia_ leaves following feeding by _B. melaleucae_.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chemotype (C)</th>
<th>Herbivory (H)</th>
<th>Water (W)</th>
<th>H × C</th>
<th>H × W</th>
<th>C × W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass shed (g)</td>
<td>df TSS (%)</td>
<td>df TSS (%)</td>
<td>df TSS (%)</td>
<td>df TSS (%)</td>
<td>df TSS (%)</td>
<td>df TSS (%)</td>
</tr>
<tr>
<td>Tree height (cm)</td>
<td>1 3.7*</td>
<td>1 17.6**</td>
<td>1 0.09</td>
<td>1 3.7*</td>
<td>1 0.04</td>
<td>1 0.003</td>
</tr>
<tr>
<td><em>O. vitiosa</em> cm ht⁻¹</td>
<td>1 0.02</td>
<td>1 3.1*</td>
<td>1 0.19</td>
<td>1 2.2</td>
<td>1 7.3**</td>
<td>1 3.0*</td>
</tr>
<tr>
<td><em>B. melaleucae</em> cm ht⁻¹</td>
<td>1 0.01</td>
<td>1 6.1**</td>
<td>1 0.2</td>
<td>1 1.6</td>
<td>1 0.3</td>
<td>1 0.03</td>
</tr>
<tr>
<td>Damage rating</td>
<td>1 0.1</td>
<td>1 15.5**</td>
<td>1 1.1</td>
<td>1 0.1</td>
<td>1 0.4</td>
<td>1 0.02</td>
</tr>
</tbody>
</table>

Presented are the degrees of freedom (df) and the rounded percentage of variance explained by a factor (TSS) calculated using the formula: TSS = 100 × (factor SS / total SS). There were no significant H × C × W interactions. Asterisks indicate significance levels, *: P = 0.05, **: P = 0.01.

These data also have particular relevance to the patterns of herbivore colonization. There is a paucity of data in the scientific literature that quantifies host plant selection at the earliest stage of patch colonization. Our findings indicate that selection of host plants by colonizing _O. vitiosa_ and _B. melaleucae_ adults and their resulting offspring are not influenced by chemotype or water availability. Colonization patterns among the tested treatments suggested that herbivory by the biological control agents is uniform, with no saplings escaping attack due to variation in preference. One limitation of this experimental design is the short duration that colonization can be measured. Dispersal of herbivores into a new habitat or host patch is inherently ephemeral, but is critical to understanding the realized host use patterns of intentionally introduced natural enemies.

The disparate results from laboratory, greenhouse, and field studies obscure a wider understanding of the role of chemotypes in _M. quinquenervia_ insect–plant interactions. Longer-term studies may provide additional insight by assessing how more mature trees of different chemotypes allocate internal resources while subjected to different and more extended levels of herbivory and resource availability.

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### References Cited


Tipping et al.: Colonization of Melaleuca quinquenervia chemotypes


