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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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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)