

Shoot Demographics for Melaleuca and Impacts of Simulated Herbivory on Vegetative Development

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INTRODUCTION

The morphological development of vegetative and reproductive structures in most plants progresses in a stepwise fashion, beginning with a dormant bud and terminating in mature foliage or a propagule. In agricultural and forestry systems, this progression is well studied with regard to the influence of various environmental factors on plant development (Reddy et al. 1992, Olszyk et al. 1998, Zhang et al. 2001, Romero et al. 2004). In contrast, the morphological development of plants in natural areas is rarely subjected to detailed study, so the maturation patterns of their vegetative and reproductive structures are often unknown. Detailed knowledge of developmental patterns of invasive plants that cause ecological damage can enhance management efforts. Characterization of vegetative bud and shoot development, for example, can assist the accurate timing of control tactics so they coincide with vulnerable developmental stages of the target plant.

The broad-leaved paperbark tree, or melaleuca (*Melaleuca quinquenervia* [Cav.] Blake [Myrtaceae]) is a long-lived evergreen tree introduced into Florida from its native Australia by horticulturalists during the late 1800s (Dray et al. 2006). Currently melaleuca is highly invasive in the forested, graminoid, and herbaceous wetlands of south Florida (Turner et

al. 1998). More than 200,000 ha in the region was converted to closed-canopy melaleuca forests by the early 1990s, resulting in regional environmental and economical impacts (Diamond et al. 1991, Bodle et al. 1994, Laroche 1999). As part of an integrated approach to the management of this exotic tree, a classical biological control program was developed that led to the introduction of the Australian melaleuca snout weevil (*Oxyops vitiosa* Pascoe [Coleoptera: Curculionidae]) into south Florida (Center et al. 2000). The larvae of this insect feed exclusively on young, tender melaleuca foliage flushing at branch apices (Purcell and Balciunas 1994). Consequently, availability of suitable foliage appears to be the primary factor affecting weevil population dynamics (Center et al. 2000). Little is known, however, concerning the rate of shoot development and the variability in biomass allocation between apical stems. The purpose of this study was (1) to describe and quantify development of new shoots on mature melaleuca trees and (2) to quantify the effects of simulated herbivory on melaleuca shoot development. A better understanding of vegetative shoot development and demographics for melaleuca is also necessary to create predictive models of weevil population dynamics and their concomitant effects on melaleuca populations. Such models are valuable when planning weevil redistribution efforts to maximize impacts on melaleuca infestations.

MATERIALS AND METHODS

We established two study sites in south Florida during January 2002, one in West Palm Beach, Palm Beach County and

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the other in Miami, Miami-Dade County. Observations at the West Palm Beach site ended in May 2002 when site owners removed the trees. Data collection continued at the Miami site until May 2003. Both sites comprised less than one acre of dense, mature melaleuca trees growing in suburban landscaping. Six reproductive melaleuca trees were selected without bias at each study site. A healthy branch originating near the center of the canopy was selected from each tree, equidistant from the canopy crown and base. All apical buds were tagged, numbered, and their developmental stages determined on each experimental branch. Three of the six selected branches at each site were randomly designated as controls or assigned to a simulated herbivory treatment. The simulated herbivory treatment consisted of using microdissecting scissors to remove (at the petiole) all leaves produced by expanding shoots between each assessment interval. Assessments of all branches in both treatments were done semi-monthly to determine the developmental stage of each initial shoot. In addition, newly emerging shoots were counted, classified as apical or axillary, and the developmental stage of each assessed. The stem length for each shoot (progeny) was measured from the point of attachment on the parent branch to the stem apex.

Buds and shoots were classified based on six developmental stages (Figure 1). Dormant Stage 0 buds are brown and round with tightly appressed bud scales. Buds erupt and begin to elongate during stage 1 as the bud tip becomes green. The shoot has opened by stage 2 and newly developed leaves are discernible, although the stem axis at the point of leaf at-

tachment is not yet visible. Leaves continue to expand and the stem axis is visible by stage 3. Most leaves are light green and fully expanded at stage 4. Stage 5 shoots consist only of dark, mature, and lignified leaves. The developmental stage of a bud or shoot correlates with its susceptibility to *O. vitiosa*. Young, tender light-green leaves are suitable as food for *O. vitiosa* larvae (Wheeler 2001), so leaves from stages 2 and 3 are susceptible to damage, stage 4 leaves are partially susceptible, and stages 0, 1, and 5 are not susceptible.

In addition to recording developmental stages of shoots, emerging leaves were counted and classified as to whether they were small and tender enough to be suitable food for *O. vitiosa* larvae, large and suitable for larval feeding, or sufficiently lignified to be unsuitable for larval feeding. Average fresh and dry weights were determined for small suitable leaves and for large suitable leaves based on 10 representative leaves of each category. The number of suitable small and suitable large leaves produced per shoot and the corresponding average fresh leaf weights were used to estimate total foliar biomass available for consumption by *O. vitiosa* per stem apex.

We assumed that the effect of simulated herbivory on shoot dynamics approximated that of natural herbivory by *O. vitiosa*. While we recognize that this technique cannot precisely mimic the magnitude, timing, and distribution of *O. vitiosa* feeding damage, a mechanical approach allows for greater control over leaf removal. In addition, any indirect effects of natural herbivory, such as caused by larval saliva, for example, may not be represented. Several studies, howev-

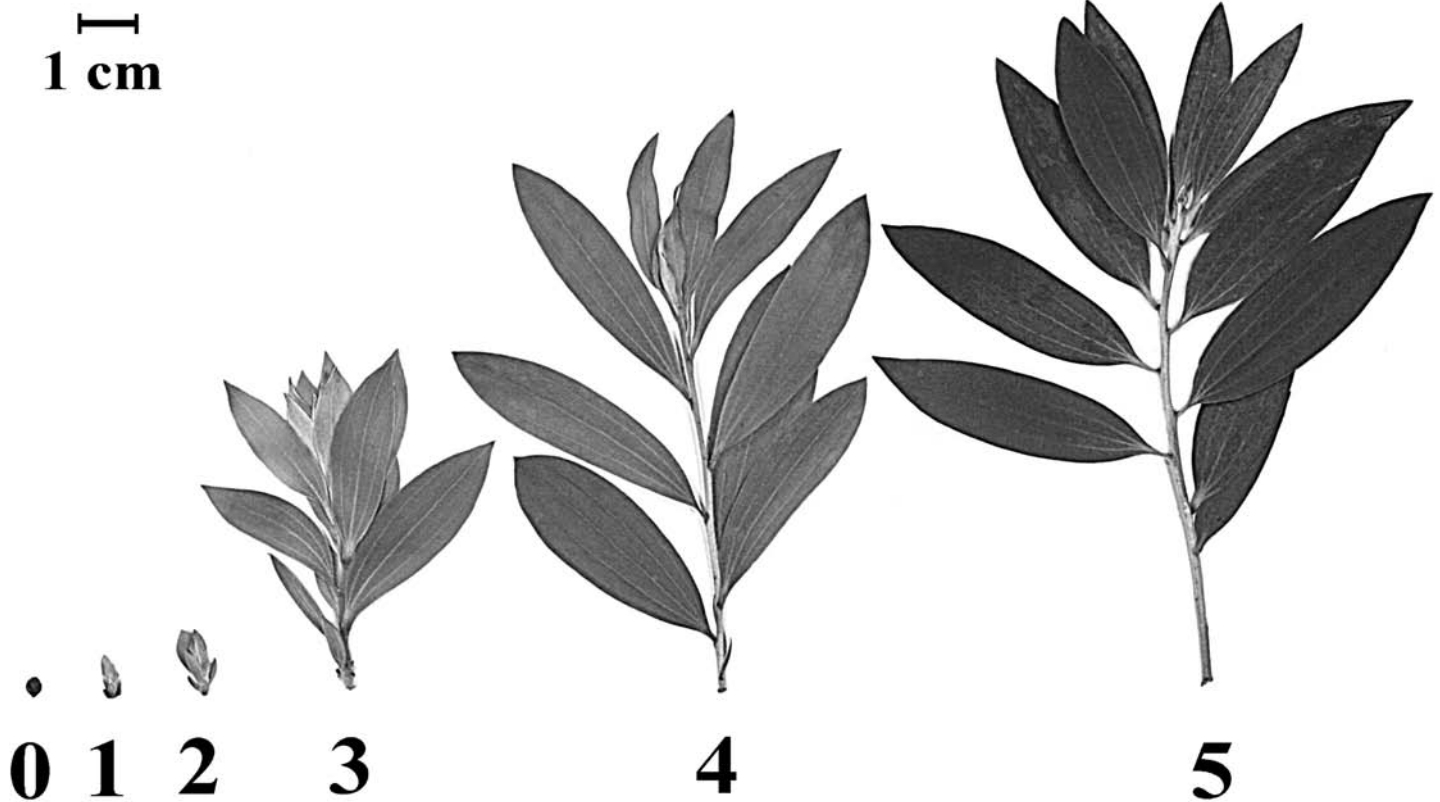


Figure 1. Developmental stages of *Melaleuca quinquenervia* foliage.

er, found the general trends revealed by simulated herbivory to be reliable reflections of the effects of natural herbivory (Landsberg 1990a, Gavloski and Lamb 2000, Hawkes and Sullivan 2001, Nykänen and Koricheva 2004), especially when looking at simpler forms of herbivory (e.g., leaf chewing) and plant responses (e.g., plant growth parameters; Lehtilä and Boalt 2004).

Analysis

We used observational data (stem lengths, biomass, leaf production, and development rates) from intact shoots (control treatment) to describe patterns of development and presented as \bar{x} (\pm SE). Data from the West Palm site were excluded when analyzing shoot production over the entire experiment because the study period at this site was truncated. The influence of shoot type (apical versus axillary) on measured parameters was analyzed with ANOVA and means were compared with a Tukey HSD test (SAS 1999). Logistic analysis of variance was used to compare the influence of simulated herbivory on bud dormancy, mortality and shoot type (PROC GENMOD; Littell et al. 2002). We used the inverse link function to convert the logit to the predicted probability and converted standard errors from the link function scale to the inverse link scale using the delta rule (Littell et al. 2002).

RESULTS

Experimental tree size at the West Palm site ranged from 4.8 to 17.1 cm diameter at breast height (DBH) and 2.4 to 5.0 cm DBH at the Miami site. Diameters of experimental branches at their points of insertion ranged from 1.2 to 2.8 cm at West Palm and 1.0 to 1.6 cm at Miami. Test branches possessed an initial 26 (\pm 4.58) buds, which increased to 142.67 (\pm 37.73) buds and shoots per branch by the end of the experiment.

Twenty-eight percent of all buds and shoots monitored throughout the study died or were aborted by the end of the study. Twenty-nine percent of the surviving buds had not initiated development by the end of the study and thus were considered dormant. Limited evidence suggests that the probability of breaking dormancy was slightly greater for buds on stems experiencing simulated herbivory compared to undamaged stems ($\chi^2 = 3.06$, $P = <0.080$).

Only 12 of the 1,775 shoots monitored during the study were reproductive. The resulting inflorescences were restricted to one tree in the undamaged treatment. Flowers required approximately two weeks to open and senesced before the subsequent sampling event. Inflorescence lengths ranged from 4 to 6.8 cm, averaging 5.3 (\pm 0.27) cm. The number of seed capsules derived from each infructescence averaged 41.5 (\pm 2.68) and ranged from 27 to 54. All but one of the inflorescences was derived from apical buds.

Biomass production differed by shoot type. Although the difference in the number of leaves produced per shoot was not highly significant (apical: $\bar{x} = 8.04$ [\pm 0.18]; axillary: $\bar{x} = 7.13$ [\pm 0.30]; $F = 3.73$, $P = 0.0550$), estimated fresh weight suitable for *O. vitiosa* feeding was greater for apical ($\bar{x} = 0.93 \pm 0.02$ g) than axillary shoots ($\bar{x} = 0.82 \pm 0.03$ g; $F = 3.94$, $P =$

0.0487). Apical shoots, however, experienced greater mortality than their axillary counterparts ($\chi^2 = 11.39$, $P = 0.0007$).

We observed similar developmental rates and stem lengths among shoot types (apical or axillary, $P > 0.05$). Pooled data for both types show that leaves progressed from the small susceptible category to the large susceptible category in 3.36 (\pm 0.09) weeks, while development from large susceptible to large lignified leaf spanned 2.00 (\pm 0.06) weeks. Stems derived from elongating shoots measured 5.05 (\pm 0.19) cm. Average number of weeks spent in developmental stages 0 to 4 were 9.41 (\pm 0.02), 3.82 (\pm 1.21), 1.61 (\pm 0.56), 1.91 (\pm 0.06), and 3.35 (\pm 0.06), respectively. Average time spent in stage 5 before the next generation shoot began to elongate was 14.82 (\pm 0.11) weeks.

Shoots subjected to simulated herbivory produced more subsequent progeny than their undamaged counterparts ($F = 8.12$, $P = 0.0049$). Shoots in the control group produced an average of 2.32 ± 0.50 additional shoots during the study period (16 months), whereas 5.33 (\pm 0.82) new shoots were produced per initial shoot in the simulated herbivory treatment. There was also a significant difference ($\chi^2 = 40.97$, $P < 0.0001$) in the type of shoot produced, with individuals in the herbivory treatment more likely to produce axillary shoots than were those in the undamaged control group. Simulated herbivory also resulted in shorter shoot stem lengths ($F = 128.34$, $P = <0.0001$), with defoliated stems measuring 2.77 (\pm 0.10) cm versus 5.05 (\pm 0.19) cm for intact stems. Mortality was greater among defoliated versus control shoots ($\chi^2 = 45.32$, $P = <0.0001$). The odds that untreated shoots would survive were 2.4 times greater than those experiencing simulated herbivory (95% Wald confidence interval: 1.41 to 3.44).

DISCUSSION

Bud development patterns for melaleuca included an extended dormancy period, with 29% remaining dormant for over 16 months. Transition times between stages for those that did break dormancy were greatest for stages 0 and 5, intermediate for stages 1 and 4, and shortest for stages 2 and 3. Interestingly, stage susceptibility to herbivory by *O. vitiosa* correlated negatively with increases in stage duration. Stages 2 and 3, for instance, can be fully exploited by *O. vitiosa*, yet are the briefest temporal stages during shoot development. In total, the expanding foliage from a single new shoot should provide suitable food for *O. vitiosa* larvae for more than 5 weeks, enough time and biomass to support between one and two larvae through full development (Purcell and Balciunas 1994, Wheeler 2001, 2003).

Removal of expanding leaves to simulate *O. vitiosa* folivory led to a loss of apical dominance. This loss of apical dominance was most commonly associated with the differentiation of new axillary buds rather than the breaking of dormancy and expansion of existing axillary buds. Simulating herbivory prevented direct comparison of leaf biomass production or quality between treatments; however, descriptive data from the control treatment indicate apical shoots produce more leaf biomass than axillary, suggesting apical growth would support more *O. vitiosa* larvae per shoot. Higher mortality observed among apical shoots, however, may negate this apparent difference. In addition, the sheer increase in number of

axillary shoots from simulated herbivory (more than 2 to 1) likely overcompensates for their slightly lower biomass per shoot in terms of sustaining *O. vitiosa* populations. Pratt et al. (2005) found this to be the case in their study of melaleuca responses to long term, natural folivory by *O. vitiosa*. Trees subjected to multiple years of herbivory by *O. vitiosa* produced similar levels of foliar biomass but twice the number of terminal stems as trees without insect damage. In another myrtaceous species, *Eucalyptus blakelyi* Maiden, Landsberg (1988, 1990a, 1990b) found that leaves produced in response to loss of apical dominance had higher levels of nitrogen, water, and lower specific weights than the primary leaves, traits that signify enhanced photosynthetic ability but also increased dietary quality for herbivores. In a meta-analysis of 68 damage response studies in woody plants, Nykänen and Koricheva (2004) found elevated photosynthetic rates concomitant with elevated nitrogen in regrowth of damaged plants. The authors suggest increased shoot production by damaged plants creates more sinks, which in turn drive the need for greater photosynthesis as well as increased uptake and mobilization of nitrogen from roots. This did not translate into consistent or predictable effects on herbivore performance, however, although specialist herbivores appeared to fare better on damaged plants than did generalists.

Compensation by melaleuca for lost foliage may improve conditions for the flush-feeding *O. vitiosa* over time. Melaleuca in Florida tends to flush seasonally in late fall and winter (Center et al. 2000, Pratt et al. 2004) with *O. vitiosa* populations fluctuating in response to resource availability (Pratt et al. 2002, 2004). In our study, increased shoot production due to defoliation suggest that *O. vitiosa* feeding may increase the carrying capacity of its host plant, initiating a positive feedback loop by which folivory-induced flushes could support *O. vitiosa* populations through much of the year. The sustainability of multiple defoliation and refoleation events within a season remains unknown, but is expected to be short lived as stored reserves become limiting. Removal of juvenile leaves by *O. vitiosa* also alters leaf demographic patterns within the tree canopy. Studies conducted prior to the introduction of *O. vitiosa* determined that leaf longevity was about four years (Van et al. 2002). These data suggest that without new leaves to replace their older counterparts, defoliation may be exacerbated through natural senescence. Experimental evidence from other systems, however, has shown that leaf longevity of remaining foliage increases after bouts of partial herbivory (Hopkinson 1966, Hodgkinson 1974) and may serve as a possible mechanism for maintaining photosynthetic capacity.

Our study provided no conclusive data on the influence of folivory on melaleuca reproduction because only one branch in the study flowered. Inflorescence length and number of capsules produced were within the lower end of previously reported ranges (Meskimen 1962, Boland et al. 1987). Further, in accord with previous reports, most flowers were derived from apical shoots. Loss of apical dominance and increased axillary shoot formation following simulated herbivory, however, results in the replacement of damaged leaves, which draws on a limited resource pool. Pratt et al. (2005) reported a drastic decrease in melaleuca flowering in response to *O. vitiosa* herbivory, indicating a shift in above-ground resource allocation from reproduction to foliar

maintenance. Replacement of photosynthetic material at the expense of reproduction has also been observed in *E. blakelyi* (Landsberg 1988) and is common in woody plant species in general (Obeso 1993).

Simulated herbivory led to a marked change in growth characteristics that determine plant architecture. Increased branching through axillary shoot production and mortality in combination with decreased stem length promote a bushy habit. In a typical dense stand of melaleuca, competition for light interception and space produce a slender tree profile competitively superior to neighboring vegetation. The shift in tree architecture resulting from herbivory may therefore reduce competitiveness for these resources while simultaneously increasing costs associated with maintaining branch production, thereby destabilizing the benefit-cost ratio of growth (Küppers 1989).

Restricting the simulated herbivory treatment to one branch per tree may have artificially concentrated defoliation, and thereby enhanced the probability of breaking apical dominance, in a way actual herbivory by *O. vitiosa* in the field may not, particularly if natural larval feeding patterns are distributed more evenly through the canopy. However, Pratt et al. (2005) report that field populations of *O. vitiosa* defoliated an average of 75 to 100% of suitable expanding leaves thereby disrupting apical dominance, but acknowledge that the level of feeding pressure observed may not be consistent from season to season or year to year. Nevertheless, this suggests the level of defoliation imposed in our study is consistent with naturally occurring levels. In addition, the tender lateral regrowth that emerges after feeding damage to apical shoots is likely to attract and retain larvae already present. It may also attract additional oviposition, naturally concentrating *O. vitiosa* impacts on that same branch or area of the canopy. While caution should be exercised when using responses to folivory on a single branch to predict responses at the whole tree level (Haukioja and Koricheva 2000), the consistency of our results with those of Pratt et al. (2005) tends to validate our experimental approach. Pratt et al. (2005) investigated the effects of four years of natural *O. vitiosa* herbivory in the field on whole tree biomass allocation and found compensation for defoliation through increased stem production. Our study provides additional insight into the roles of shoot development and demography in growth responses of melaleuca to simulated *O. vitiosa* feeding damage.

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