

# No evolution of increased competitive ability or decreased allocation to defense in *Melaleuca quinquenervia* since release from natural enemies

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**Abstract** If invasive plants are released from natural enemies in their introduced range, they may evolve decreased allocation to defense and increased growth, as predicted by the evolution of increased competitive ability (EICA) hypothesis. A field experiment using the invasive tree *Melaleuca quinquenervia* was conducted to test this hypothesis. Seeds were collected from 120 maternal trees: 60 in Florida (introduced range) and 60 in Australia (home range). Plants grown from these seeds were either subjected to herbivory by two insects from Australia that have recently been released as biological control agents or protected from herbivores using insecticides. Genotypes from the introduced range were initially more attractive to herbivores than genotypes from the home range, supporting EICA. However, genotypes from the introduced and home range did not differ in resistance

to insects or in competitive ability, which does not support EICA. Plants from the introduced range had a lower leaf hair density, lower leaf: stem mass ratio, and a higher ratio of nerolidol: viridifloral chemotypes compared to plants from the native range. Plants with an intermediate density of leaf hairs and with high specific leaf area were more susceptible to herbivory damage, but there were no effects of leaf toughness or chemotype on presence of and damage by insects. Herbivory had a negative impact on performance of *Melaleuca*. Other than an initial preference by insects for introduced genotypes, there was no evidence for the evolution of decreased defense or increased competitive ability, as predicted by the EICA hypothesis. It does not appear from this study that the EICA hypothesis explains patterns of recent trait evolution in *Melaleuca*.

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## Introduction

Invasive species are considered to be one of the greatest threats to natural ecosystems (Cox 1999; Mooney and Hobbs 2000; Rossman 2001). One favored explanation for why some species become

invasive is that they have escaped natural enemies in their introduced range, leading to increased population growth in the absence of top-down regulation (Keane and Crawley 2002). In addition to a population response to enemy release, invasive species might also exhibit an evolutionary shift in resource allocation away from defense and toward increased growth or competitive ability, as postulated by the evolution of increased competitive ability (EICA) hypothesis (Blossey and Nötzold 1995). The EICA hypothesis thus makes two major predictions for a given invasive species: (1) genotypes from the introduced range are more susceptible and less resistant to herbivory than genotypes from the home range and (2) genotypes from the introduced range grow faster or exhibit other competitive ability traits to a greater degree than genotypes from the home range.

Previous studies of the EICA hypothesis have taken several different approaches depending in part on the component of the hypothesis under investigation. Some work has focused on testing the premise of the hypothesis that invasive species actually have been released from natural enemies. For example, Agrawal et al. (2005) compared levels of herbivory on 30 native and invasive congeneric plant pairs and found significantly more herbivory on the native species, supporting the enemy release hypothesis. In reviewing the literature, Colautti et al. (2004) found mixed support for the enemy release hypothesis, with studies that compared species in their home and introduced ranges generally supporting the hypothesis and studies comparing native and introduced species in the same community generally failing to support the hypothesis.

If individuals have been released from natural enemies, they may reduce allocation to defense and show decreased resistance to herbivores. In contrast to tolerance, which is the ability of an individual to maintain fitness despite damage, resistance is the ability of an individual to reduce the amount of damage inflicted by a natural enemy, and traits that confer resistance include chemical (terpenoids, phenolics) and morphological (spines, hairs) features. If these traits are costly in the absence of enemies, a shift in allocation may be expected. Studies testing this EICA prediction of decreased resistance in invasive populations have used a variety of methods, including comparing native and introduced plant populations (Willis et al. 1999), introduced plant

populations with different histories of herbivory (Daehler and Strong 1997), and native and introduced species in the same community (Agrawal and Kotanen 2003). These studies have produced conflicting results, with support from some studies (Daehler and Strong 1997; Rogers and Siemann 2004) but not from others (Willis and Blossey 1999; Willis et al. 1999; Agrawal and Kotanen 2003; van Kleunen and Schmid 2003). There have also been mixed results within individual studies. For example, Stastny et al. (2005) found that growth rates and damage levels for genotypes of the invasive plant *Senecio jacobaea* were consistent with EICA predictions but that the differences between the home and native range could not be explained by the putative resistance trait-levels of chemical defenses. In a review of the literature, Bossdorf et al. (2005) found that a nearly equal number of studies comparing native and introduced populations supported as failed to support the hypothesis of the evolution of decreased resistance in invasive genotypes.

If individuals reduce allocation to defense as a result of release from natural enemies, they may be able to increase their allocation to growth or competitive ability. Studies testing this EICA prediction have again produced mixed results. Siemann and Rogers (2001) present the results from a 14-year common garden experiment that show that genotypes of the invasive plant *Sapium sebiferum* were larger than native genotypes. In contrast, other studies did not find increased size of invasive plants based on common garden experiments with single (van Kleunen and Schmid 2003) or multiple (Willis et al. 2000) species, on maximum plant heights based on published floras (Thébaud and Simberloff 2001), and in competition experiments (Vilá et al. 2003). For the tropical plant *Clidemia hirta*, DeWalt et al. (2004a) found support for the enemy release hypothesis, but (DeWalt et al. 2004b) found that release from natural enemies did not lead to increased allocation to biomass or growth in introduced populations in this species, which fails to support EICA. Bossdorf et al. (2005) also surveyed studies testing the growth hypothesis and found support in 18 studies, four studies with findings opposite predictions, and 13 studies in which growth rates of native and invasive species did not differ.

The EICA hypothesis thus remains controversial, and more studies testing the predictions of this

hypothesis are clearly needed. Systems in which natural enemies have been introduced into novel habitats to control invasive species are well suited for testing the EICA hypothesis and other hypotheses related to invasive species and population regulation (Franks et al. 2004). In such biological control systems, the histories of introductions of the invasive species and their natural enemies may be well known, and it may be possible to determine how long various invasive populations have been enemy-free and how great an impact natural enemies have when reunited with their hosts. Furthermore, it is especially important to know if the EICA hypothesis is supported for species that are candidate targets for biological control. If EICA is true, then the invasive species would have reduced defenses in the absence of natural enemies in the introduced range. When the enemies are reintroduced as biological control agents, the invasive species could evolve increased defenses, and biological control may decrease in effectiveness over time.

We tested the EICA hypothesis using the invasive plant *Melaleuca quinquenervia* (Cav.) Blake (hereafter referred to by genus) and two specialist herbivores recently introduced as biological control agents. Because these herbivores have well-known introduction histories (Dray 2003), we have more complete information on the amount of time the invasive species has been without natural enemies and thus how long it has had to evolve changes in defense and growth allocation patterns. *Melaleuca* has over 450 known specialist natural enemy species in its home range (Burrows and Balciunas 1999) and no known native specialist or generalist natural enemies in its introduced range (Costello et al. 2003), making it likely that release from natural enemies could have contributed to the success of this plant in its invaded range. The insects that have been recently released for biological control are specialists, and there is almost no herbivory by generalists in the introduced range of the plant, making the allocation shifts predicted by the EICA hypothesis more likely. We collected seeds of the plant throughout the ranges in which it is native and invasive and raised seedlings of known maternity and origin both with and without natural enemies under controlled conditions in the field. We measured growth rates, degree of insect damage, and levels of potential defensive traits. This allowed us to test both major predictions of the EICA

hypothesis: decreased allocation to defense and increased allocation to growth for genotypes from the introduced range.

## Methods

### Study species

We studied the invasive tree *M. quinquenervia* Cav. (Blake) (Myrtaceae), which is one of the most problematic invasive plants in the Florida Everglades ecosystem (Turner et al. 1998; Serbesoff-King 2003). Native to the east coast of Australia, this tree was introduced into southern Florida beginning in the late 1800s (Dray 2003). *Melaleuca* grows fast, produces copious seeds, and can form dense monocultures in areas that it has invaded (Turner et al. 1998; Serbesoff-King 2003). While individual trees can live over 100 years (Dray 2003), they can become reproductive within 1–2 years (Meskimen 1962; Vardaman 1994). So despite the longevity of individuals, there is continual seed rain of new genotypes into existing stands and into invasion fronts and ample opportunity for selection and evolution to act on the genetic variation present. In addition, *Melaleuca* grows in very crowded stands in Florida, with previous studies reporting densities of 8,000–132,000 trees per hectare in mature stands (Rayachhetry et al. 2001) and seedlings in densities of 500–2,250 individuals/m<sup>2</sup> (Franks et al. 2006).

Florida has no native specialist natural enemies of *Melaleuca*, and trees there experience very little generalist herbivory (Costello et al. 2003). In contrast, over 450 species of insects have been reported feeding on *Melaleuca* in its home range, causing visible and substantial damage to trees at all life stages (Burrows and Balciunas 1999). There is evidence for genetic variation in the introduced populations. For example, variation in chemical defense is evidenced by the fact that there are two dominant terpenoid chemotypes (chemical phenotypes) in Florida, nerolidol and veridifloral, and the nerolidol chemotype tends to be preferred by herbivores compared to the veridifloral chemotype (Dray et al. 2004). Thus, over the last 100 years *Melaleuca* is likely to have been released from natural enemy pressure in its introduced range and may have

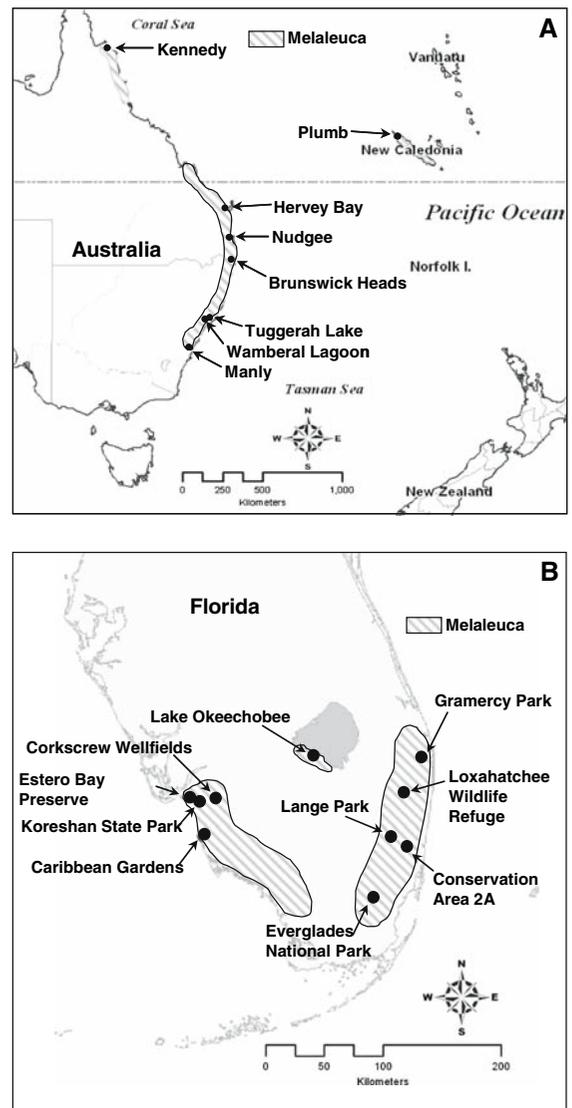
evolved a shift of resources away from defense and toward increased growth and competitive ability.

At the time of the study, two herbivorous insects from Australia had been released in an effort to control *Melaleuca*. *Oxyops vitiosa* (Pascoe) (Coleoptera: Curculionidae) are leaf-chewing weevils that were first released in Florida in 1997 (Center et al. 2000). *Boreioglycaspis melaleucae* (Moore) (Hemiptera: Psyllidae) are sap-feeding psyllids that were first released in Florida in 2002 (Pratt et al. 2004). Previous studies have shown that these insects can reduce performance and fitness of *Melaleuca* (Franks et al. 2006; Pratt et al. 2005).

### Experimental design

*Melaleuca* seeds were taken from trees in ten collection locations in the introduced range in Florida and eight collection locations in the home range of Australia and New Caledonia (Fig. 1) in the fall of 1999 through the spring of 2000 and stored in glass vials at 25°C until use. While the exact locations of the original seed collections for the introduction of *Melaleuca* into Florida are not known, the locations sampled likely encompass both source and non-source areas (Dray 2003). The collection locations represent groupings for the purposes of collecting, and the main question of interest was comparing the native and home range. For the experiment, we used seeds from 120 maternal trees (60 from Australia and 60 from Florida). In April 2003, seeds were germinated in seedling trays in the Invasive Plant Research Laboratory (IPRL) greenhouse in Fort Lauderdale, Florida. Trays were kept in standing water, and seedlings were thinned to two to three individuals per cell within the seedling tray.

In June 2003, seedlings were transplanted into a garden plot at the IPRL research station. At this site, the climate is mild and subtropical, with temperatures averaging around 19°C in January and 28°C in August and monthly precipitation approximately 30 mm in January and 270 mm in August (Chen and Gerber 1991), so the growing season is essentially year-round. The study site contained soil that was mainly composed of calcareous sand, and was manually cleared of weeds and tilled prior to the experiment. Plants were fertilized once with a



**Fig. 1** Seed collection locations. The countries are the native range of Australia and New Caledonia (a) and the introduced range in Florida in the US (b). The distribution of *Melaleuca quinquenervia* is shown with hatch marks. Collection sites are named on the maps. Between six and eight trees were sampled per collection location

controlled release fertilizer (Nutricote Total, Type 270, 13N:13P:13K, Chisso-Asahi Fertilizer Co., Ltd., Tokyo), watered regularly, and kept under 40% shade cloth for 4 weeks to reduce transplant shock. After this point, no supplemental water or fertilizer was added, and shade cloth was removed. If more than one seedling per plant position remained, all but one were removed within the first 2 weeks.

The plants were arranged in a split-plot design. Each whole plot contained 240 plants: two from each maternal family (60 families from the home range and 60 families from the introduced range). Whole plots were divided in half, with one plant from each maternal family in each half of a whole plot and each half of a whole plot randomly assigned an insecticide treatment (sprayed or not sprayed). Plants within a split plot were randomly assigned positions and were spaced approximately 5–10 cm apart, which is within the range found in natural populations in Florida (Franks et al. 2006) and close enough that fairly intense competition was expected. There were 12 plots containing 240 plants each, for a total of 2,880 plants.

Two to three weeks after planting, we applied acephate (8% active ingredient, Ortho, Columbus OH, USA) to plants in plots receiving the insecticide (herbivore exclusion) treatment. When applying the insecticide, we surrounded the plot on three sides with a plastic shield to minimize spray drift to other plots. The insecticide was applied at a concentration of 0.36 active ingredient (Tipping and Center 2002) every 2–3 weeks for the duration of the experiment, and any insects or eggs seen on the insecticide-plot plants were removed by hand. The no-insecticide plots were sprayed with water to control for the effects of spraying and of adding moisture to the insecticide plots. Previous studies showed that the insecticide itself, in the absence of insects, does not alter plant growth or performance (Tipping and Center 2002).

We assessed insect damage, plant resistance traits, and plant performance and fitness as follows. We recorded presence/absence and species identity of all insects on all plants every 2–3 months (approximately the same duration as the biological control insect life cycles) during the experiment. There was only variation in insect presence and damage levels in the unsprayed plots at the 4-month census, since there were very few insects recorded before this and afterwards nearly all plants had infestations. Thus we present data on attractiveness and resistance only from this 4-month census. We defined attractiveness to an insect species as the probability of a plant having that insect species present. We also recorded the presence/absence of herbivory damage by each insect species on all plants every 2–3 months. Resistance was defined as the probability of not

being damaged by a given insect species. We also estimated leaf area damaged divided into categories, but analyses of the categorical data showed little variation among damaged plants in damage classes and did not provide any more information than the more simple presence/absence data, so only the latter data are presented. The traits potentially related to resistance that we measured were leaf pubescence, leaf shape, leaf toughness and chemotype. Based on observations of variation in leaf hairs, we divided degree of leaf pubescence into three categories: low, medium and high, based on the density and length of hairs on several haphazardly chosen leaves per plant. We visually estimated pubescence for all plants and compared estimates to standards within the experimental population. Plants varied from having leaves that were nearly glabrous (hairless) to those with a thick covering of hairs. To assess leaf shape, we collected the first fully expanded leaf of all plants, scanned the leaves, dried them and recorded dried mass. We measured leaf length, width, and area using ImageJ software (National Institute of Health, Bethesda, MD, USA). Leaf shape was then analyzed in two ways: as length:width ratio and as area/mass, or specific leaf area (SLA). Leaf toughness and chemotype were measured for all available plants in three whole plots of the experiment ( $n = 323$  for toughness and 429 for chemotype). For these measurements, we used the third or fourth fully expanded leaf. Leaf toughness was estimated with a hand-held portable penetrometer with a 0.05 mm diameter flat-tipped needle (Wheeler 2001) as the force necessary to puncture a leaf and averaged over five puncture attempts for each leaf. Terpenoid chemotype was assessed with a gas chromatograph following the methods of Wheeler (2001), and plants were scored as either nerolidol or viridifloral chemotype (Wheeler 2001; Dray et al. 2004). Plant performance was measured as survival, growth, and biomass. Survival was assessed at the end of the experiment. Growth was measured as final height–initial height. At the time of harvest, all plants were cut at the base, the leaves and stems were separated and dried at 70°C to constant weight (at least 2 weeks), and biomass was measured as final aboveground dry weight. We did not attempt to harvest belowground biomass because it was not feasible to harvest and separate all roots from thousands of trees grown together in dense plots in the field. Also, while there may have been some

shifts in resource allocation, we expected that total aboveground biomass should still give a reasonable estimate of fitness, and previous studies of *Melaleuca* have shown strong correlations between aboveground biomass and reproduction (Rayachhetry et al. 2001). Reproduction was not measured because harvesting the plants as soon as flowering began was necessary to prevent pollen dispersal from the Australian genotypes. Harvesting took place in June and July 2004, which was 12 months after transplanting.

### Analysis

All analyses were performed with the SAS statistical software (version 8.0, the SAS Institute). To test the EICA predictions for competitive ability, we compared growth [ $\ln$  (final height - initial height)] and final aboveground biomass of Florida and Australia genotypes in the unsprayed treatment using the MIXED procedure. In the mixed model analysis of variance, source location (Australia and Florida) was a fixed effect and whole plot was a random effect. To test EICA predictions for resistance, attractiveness and resistance of unsprayed plants were analyzed with generalized linear mixed models with binomial error distributions and a logit link functions using the GLMMIX macro in SAS.

The effects of the insecticide and plant source location (Florida or Australia) on plant growth, leaf toughness and final biomass were assessed using a mixed model analyses of covariance with initial plant height as the covariate. In the MIXED procedure, insecticide treatment and plant source location were fixed effects and whole plot was a random effect. The insecticide  $\times$  whole plot interaction was used as the error term for the insecticide effect to reflect the split-plot nature of the design. Growth was analyzed as the natural logarithm of the difference between final height and initial height. Final biomass was transformed with the natural logarithm to normalize the residuals. To analyze the effect of the insecticide on plant survival, we used a generalized linear mixed model with a binomial error distribution and a logit link function using the GLIMMIX macro in SAS.

We analyzed variation among plant traits as follows. To determine if the traits played a role in resistance, we examined the relationship between damage levels and chemotype, leaf toughness, leaf

hairs, SLA and attractiveness to the two insect species using generalized linear mixed models. Chemotype, leaf hairs, and the response variables were categorical, and leaf toughness and SLA were continuous. We used only the unsprayed plants for this analysis. To compare source locations for the traits, we used a generalized linear model with source location as a fixed effect, population within source as a random effect, and the insecticide spray treatment as a fixed effect. Both sprayed and unsprayed plants were used in this analysis.

We also ran all analyses comparing ranges (testing EICA) using collection location nested within range as the level of replication.

## Results

### Effects of herbivory

The insecticide was effective at reducing insect damage. There was a significant difference between insecticide sprayed and unsprayed treatments in damage due to insects ( $F_{1,11} = 47.22$ ;  $p < 0.0001$ ). After 4 months, 67.8% of the unsprayed plants had been damaged by insect herbivores while only 7.6% of the sprayed plants had experienced herbivory.

Although the magnitude of the difference was small, exclusion of herbivores significantly affected plant survival ( $F_{1,11} = 5.23$ ;  $p = 0.0430$ ), with 95.3% of plants in the pesticide treatment surviving and 92.8% of the plants in the unsprayed treatment surviving. Final biomass was also significantly affected by herbivore exclusion ( $F_{1,11} = 6.62$ ;  $p = 0.0260$ ), with plants in the sprayed treatment an average of 32.7 ( $\pm 2.25$ ) g and plants in the unsprayed treatment 24.5 ( $\pm 2.26$ ) g after 1 year. The effect of protection from herbivores on plant growth rate approached significance ( $F_{1,11} = 4.49$ ;  $p = 0.0576$ ), with sprayed plants growing 126.57 ( $\pm 4.89$ ) cm per year and unsprayed plants growing 119.29 ( $\pm 4.89$ ) cm per year.

### Evolution of competitive ability

Native (Australia) and introduced (Florida) genotypes did not differ in growth ( $F_{1,2683} = 0.35$ ,  $p = 0.554$ ), but native genotypes obtained greater biomass

( $F_{1,2657} = 9.76$ ,  $p = 0.0018$ ) (Fig. 2). Plants from the native habitat weighed an average of 33.80 ( $\pm 3.21$ ) g and plants from the introduced habitat weighed an average of 27.52 ( $\pm 3.21$ ) g. There was not a significant interaction between source and insecticide treatment on growth ( $F_{1,2683} = 0.01$ ,  $p = 0.963$ ) or biomass ( $F_{1,2657} = 0.06$ ,  $p = 0.804$ ) (Fig. 2).

### Evolution of defense

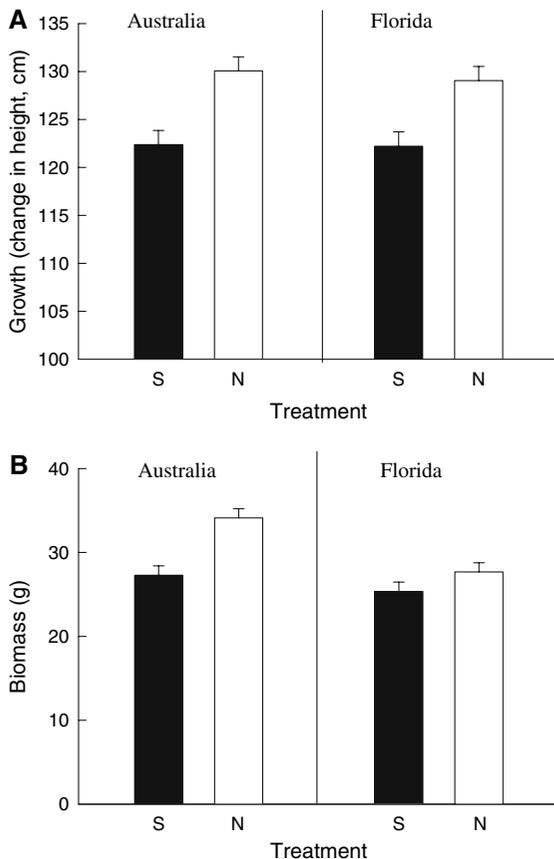
After planting and during the initial census, few plants had evidence of the presence of or damage by any insects. Four months after the start of the experiment, there were substantial numbers of insects present on the plants and there was evidence of

herbivory damage by both insect species. At the time of this 4-month census, genotypes from the introduced habitat were more attractive to insects than home range genotypes. The plants from the introduced habitat were more likely to have *Boreioglycaspis* ( $F_{1,1388} = 4.46$ ,  $p < 0.05$ ; Table 1) and *Oxyops* ( $F_{1,1389} = 4.49$ ,  $p < 0.05$ ; Table 1) present than plants from the home range. However, this difference decayed over time. At harvest, all plants had evidence of insect presence, and there was no difference between introduced and home range genotypes in resistance to insect damage (Table 1).

### Defensive traits

There was no relationship between insect presence or damage and plant chemotype or leaf toughness (Table 2). Variation in SLA was not associated with attractiveness but was associated with resistance (Table 2); leaves with lower SLA were more resistant to herbivory. The degree of leaf pubescence influenced attractiveness to *Oxyops* and resistance (Table 2). Plants of intermediate pubescence were more attractive to *Oxyops* and less resistant to damage than plants with low or high pubescence.

Plants from the two source locations differed in chemotype, degree of leaf pubescence, and ratio of leaf to stem mass (Table 3). Genotypes from the home range were 27.5% nerolidol and 72.5% viridiflora, while genotypes from the introduced range



**Fig. 2** The effect of source genotype (native versus introduced) on growth (a) and final biomass (b) of *Melaleuca quinquenervia* plants in the insecticide sprayed (S; black bars) and no insecticide (N; white bars) treatments. Shown are means  $\pm$  1S.E

**Table 1** Comparison of the native (Australia) and introduced (Florida) range in plant defense, competitive ability and fitness

Trait	NDF	DDF	F
Attractiveness to <i>Boreioglycaspis</i>	1	1,388	4.46*
Attractiveness to <i>Oxyops</i>	1	1,389	4.49*
Resistance	1	1,356	0.18
Plant survival	1	1,275	2.39
Plant growth	1	1,372	0.19
Biomass	1	1,308	4.34*

Shown is a summary analysis of variance table of the effects of plant source location (native versus introduced genotypes) on defense (attractiveness and resistance to insect herbivory) and competitive ability (survival, growth rate and final biomass). Only plants not sprayed with insecticide were included in this analysis

\*  $p < 0.05$

**Table 2** Relationship between plant morphological and chemical traits and defense

Trait	Attractiveness and resistance	NDF	DDF	F
Chemotype	Attractiveness to <i>Boreioglycaspis</i>	1	420	0.21
Chemotype	Attractiveness to <i>Oxyops</i>	1	421	1.58
Chemotype	Resistance	1	418	0.22
Leaf toughness	Attractiveness to <i>Boreioglycaspis</i>	1	346	0.34
Leaf toughness	Attractiveness to <i>Oxyops</i>	1	346	0.12
Leaf toughness	Resistance	1	330	0.21
Specific leaf area	Attractiveness to <i>Boreioglycaspis</i>	1	1,362	0.02
Specific leaf area	Attractiveness to <i>Oxyops</i>	1	1,363	2.54
Specific leaf area	Resistance	1	1,340	13.07***
Hairs	Attractiveness to <i>Boreioglycaspis</i>	2	1,380	2.35
Hairs	Attractiveness to <i>Oxyops</i>	2	1,381	3.61*
Hairs	Resistance	2	1,346	5.11**

Shown is a summary analysis of variance table of the effects of defensive traits on attractiveness and resistance to insect herbivore damage. See text for explanations of traits. Only plants not sprayed with insecticide were included in this analysis  
 \*  $p < 0.05$ , \*\*  $p < 0.01$ ,  
 \*\*\*  $p < 0.001$

were 41.6% nerolidol and 58.4% viridifloral. Genotypes from the home range were 25.5% low pubescence, 54.3% medium pubescence, and 20.2% high pubescence. Genotypes from the introduced range were 22.0% low pubescence, 73.4% medium pubescence, and 4.6% high pubescence. The average ratio of leaf to stem mass was 0.43 for native genotypes and 0.38 for introduced genotypes. Source locations did not differ in leaf toughness, leaf shape, or SLA (Table 3). There was significant variation among sites (populations nested within source locations) for chemotype ( $F_{16,851} = 9.34$ ,  $p < 0.0001$ ), leaf toughness ( $F_{16,685} = 5.12$ ,  $p < 0.0001$ ), leaf

shape ( $F_{16,2739} = 25.52$ ,  $p < 0.0001$ ), SLA ( $F_{16,2665} = 2.67$ ,  $p < 0.001$ ), and leaf to stem mass ( $F_{16,2636} = 16.14$ ,  $p < 0.0001$ ). Thus there were greater differences in traits among populations within continents than between continents.

#### Collection locations

When the collection location nested within range was used as the level of replication, there were no differences between the native and home ranges in growth, biomass, survival, attractiveness to either insect species, resistance, chemotype, or leaf pubescence (all  $p > 0.05$ ).

**Table 3** Comparison of the native (Australia) and introduced (Florida) range in morphological and chemical defense traits

Trait	NDF	DDF	F
Chemotype	1	851	13.28**
Leaf pubescence	1	2,792	27.81***
Leaf:stem mass	1	2,636	22.93***
Leaf toughness	1	685	0.11
Leaf length:width ratio	1	2,739	0.15
SLA	1	2,665	0.06

Shown is a summary analysis of variance table for the effect of source location on plant traits. See text for explanations of plant traits

\*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

#### Discussion

In our study, we found that herbivory significantly depressed *Melaleuca* performance. Plants that were protected from herbivory experienced less damage, were more likely to survive, grew faster and attained greater biomass after 1 year than plants that were exposed to herbivores. Since damage caused by herbivores could be attributed almost exclusively to the two introduced biological control insects, we can conclude that these insects are likely to affect

*Melaleuca* performance in the field and aid in the control of this invasive species. This result is consistent with other studies showing impacts of these insects on plant performance in both laboratory and field settings (Franks et al. 2006; Pratt et al. 2005). However, the magnitude of the insect impacts varied with the performance measure. Herbivory caused a 2.5% reduction in survival and a 25% reduction in biomass. To determine the effects of these reductions on overall population dynamics, it is necessary to use population modeling (Doak 1992; Shea and Kelly 1998; Caswell 2001). We are currently conducting studies of the sensitivity of *Melaleuca* population dynamics to herbivore impacts at different life stages.

The EICA hypothesis predicts that genotypes of invasive species in a new, enemy-free habitat should grow faster and be more competitive and more attractive to, and less resistant to natural enemies than home range genotypes (Blossey and Nötzold 1995; Keane and Crawley 2002). Four months after planting, *Melaleuca* genotypes from introduced populations were more likely to have insect infestations than plants from native populations, a result consistent with EICA predictions. However this difference quickly disappeared, and source populations ultimately did not differ in herbivory or rate of height increase. While there was a difference in final biomass between the source populations, the plants from the home range were larger, which is the opposite of the EICA prediction. In addition, there was no interaction between the insecticide treatment and source location in growth and final biomass. This finding again contrasts with expectations of the EICA hypothesis, which predicts that genotypes from the introduced habitat should perform better in the absence of insects (insecticide treatment) than genotypes from the native habitat and that genotypes from the native habitat should perform better with insects present (no insecticide treatment) than genotypes from the introduced habitat, which would be indicated by a significant source location by insecticide treatment interaction. Using the collection location level analyses, there were no differences between the ranges for any trait. We obtained these negative result despite having a large sample size of 2,880 plants which were obtained from seeds collected from throughout the home and introduced ranges and grown together under controlled conditions in the field.

There are several possible reasons that the Florida *Melaleuca* populations we sampled do not conform to the EICA predictions. First, although we expect that high seedling densities in natural populations (Franks et al. 2006) should create intense selection for faster growth, individual trees can live over 100 years (Dray 2003), and there may not have been sufficient time for evolutionary changes to have occurred since *Melaleuca* was introduced to Florida in the late 1800s. However, it is important to note that trees can become reproductive within 1–2 years, meaning that recruitment episodes have been occurring throughout the over 100 year history of the species in Florida, giving selection ample time to alter gene frequencies in natural populations. Second, population bottlenecks could occur during species introductions, and introduced *Melaleuca* populations may have lacked sufficient genetic variation in traits related to growth rate or competitive ability. This explanation, however, seems unlikely for *Melaleuca* because previous studies have found high diversity in both genetic markers, with many polymorphic allozyme loci (Dray 2003), and in quantitative traits, with genetically-based variability in responses to water and pH levels (Kaufman and Smouse 2001). Third, because we used field collected seeds, we can not rule out the possibility that maternal effects contributed to any differences or lack of differences between the home and introduced range genotypes. This is especially a concern because the environments experienced by the maternal trees in the home and introduced ranges are likely to be different. However, the longevity of the tree made producing multiple generations impractical. Furthermore, *Melaleuca* has tiny seeds, with maternal allocation to resources for each seed very small, and seedlings that were small or inviable were not used. Thus all of the plants should have started with basically the same resource allocation in the maternally inherited seed resources, and genetic differences detected are more likely due to additive genetic variance rather than maternal effects (Kaufman and Smouse 2001). Finally, our results for *Melaleuca* may not have supported the EICA hypothesis because there may be genetic constraints to selection, such as trait correlations, that would hinder the EICA or decreased herbivory resistance. Such evolutionary constraints have been demonstrated in other systems. For example, Etterson and Shaw (2001) found antagonistic trait correlations that

would be expected to constrain the evolution of adaptation to global climate change in the annual plant *Chamaecrista fasciculata*.

Although there were not overall differences between home and introduced ranges in the attractiveness and resistance of *Melaleuca* to insect damage, there was variation among plants in the amount of herbivores and herbivory, indicating the potential for selection to act on this variation. This variation could potentially be explained by one or more morphological and chemical defense traits that plants are known to possess (Karban and Myers 1989; Fritz and Simms 1992). We found correlations between resistance to herbivory and two morphological traits: leaf pubescence and SLA, but not with leaf toughness or terpenoid chemotype. Plants with intermediate pubescence were more susceptible to *Oxyops* and were more damaged by herbivores than plants with low or high levels of leaf pubescence. Plants with high leaf pubescence may have been better protected against herbivores than plants with intermediate pubescence as a direct result of the hairs deterring insect attack, as has been found in other studies of leaf hairs and trichomes (Levin 1973; Doss et al. 1987; Schoener 1988). It is unclear why plants with low hair density suffered less damage than the intermediate leaf hair density plants, but this could be due to the fact that plants with low hair density also tended to be shorter and were possibly less apparent to herbivores. Alternatively, there may be other defensive traits that are correlated with leaf hairs which produced this result. Ezcurra et al. (1987) found that for the Mexican madrone *Arbutus xalapensis*, leaf chewing insects were less abundant on pubescent than on glabrous leaf morphotypes, but that sap feeding insects were more abundant on pubescent than glabrous trees. In contrast to this result, we found that the leaf chewing insect, *Oxyops*, was most abundant on leaves of intermediate pubescence and that the sap feeding insect, *Boreioglycaspis*, did not differ in abundance among leaf types (Table 3).

Herbivory resistance was also associated with low SLA in our study. SLA is often closely linked with plant water status and water use efficiency patterns (Begum and Paul 1993; Liu and Stutzel 2004). Plants with low SLA may have had less water available in their leaves, which could make them less appealing to both sap feeding and leaf chewing insects. We found

no relationship between resistance and leaf toughness. This contrasts with other studies which found correlations between leaf toughness and herbivory for *Melaleuca* in particular (Wheeler 2001) as well as for other species (Choong 1996; Kudo 2003). One possible reason for this discrepancy is that in the Wheeler (2001) study, leaf toughness measurements were made among leaves within a plant. This produced the result that for a given plant, *Oxyops* preferred leaves of lower toughness. In our study, we examined a standard leaf on all plants, and there appears to be much more variation in toughness among leaves within a plant (with older leaves being tougher) than among plants for a given leaf position. Thus the insects may choose leaves within a plant rather than choosing among plants based on leaf toughness. In addition to the lack of an effect of leaf toughness on herbivores, there was also no relationship between resistance and terpenoid chemotype in our study. This result is surprising since previous studies showed that the veridifloral chemotype tends to be more resistant than the nerolidol chemotype to herbivory damage by *Oxyops* (Dray et al. 2004).

In summary, we found that herbivory by two insects introduced into Florida for biological control reduced survival, growth, and performance of *Melaleuca* plants. There was no difference between genotypes from the introduced versus home range in resistance to herbivory or in growth rate in a common garden experiment, which does not support the EICA hypothesis. If the EICA hypothesis were supported, this would mean that invasive species evolved reduced defenses in the absence of natural enemies, and that the evolution of increased defenses would be expected to occur when natural enemies are introduced as biological control agents. If this were the case, we might expect biological control efforts to decrease in effectiveness over time. In contrast, our results suggest that herbivory by these insects is likely to aid in efforts to control *Melaleuca*, and that the plants do not appear to have evolved increased competitive ability or decreased allocation to defense since their introduction into Florida.

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