Natural enemy density and soil type influence growth and survival of *Melaleuca quinquenervia* seedlings

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**Abstract**

Multiple insects are commonly introduced as biological control agents to reduce the performance of invasive plants. Little is known about the species-specific contributions of co-existing agents, and about the effects of insect density on the control of invasive plants. Several agents are expected to strengthen the control. The effects of insect type, density, and soil type on seedling survival and growth of the invasive tree *Melaleuca quinquenervia* were investigated in Florida. The biological control agents are *Oxyops vitiosa* (weevil) and *Boreioglycaspis melaleucae* (psyllid). A three-way factorial shade-house experiment was conducted, manipulating weevil density (zero/low/high), psyllid density (zero/low/high), and soil type (organic/sandy). Only high densities of one or both insects combined consistently reduced plant survival or growth; low densities did not. Overall, psyllids had stronger effects than weevils and for some performance parameters, the effects of one insect depended not only on its density but also on the density of the other insect. Interaction effects between insects were sometimes positive (i.e. seedling survival was lowest when both insects were in high densities). In other cases, high densities of only one agent were enough to reduce plant growth. Plants perform better on organic soils than on sandy soils. Weevils were more effective in reducing biomass on organic soils, while psyllids were more effective in reducing survival on sandy soils. Seedlings compensated for damage by producing more leaves, with the highest standing leaf crop at intermediate insect densities. These results show that the effectiveness of biological control on *M. quinquenervia* seedlings varies with insect species and depends upon insect density and soil type.

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### 1. Introduction

Biological control is the use of natural enemies to reduce the density and spread of invasive species (McFadyen, 1998), and it can be an effective method of suppressing invasive plants (Huffaker and Kennett, 1959; Osmond and Monro, 1981; Moran and Zimmermann, 1984). Biological control programs generally employ multiple species of natural enemies to control a single target pest. Quantifying species-specific contributions to the control of an invasive plant can be difficult when multiple co-existing agents have been introduced (McEvoy and Coombs, 1999a). A review of 26 biological control programs reported that control was attributable to only one agent in 81% of the case studies (Myers, 1985). However, other studies have reported that the cumulative effects of several agents were needed to control weeds (Schroeder and Goeden, 1986; Denoth et al., 2002; Millbrath and Nechols, 2004). Considering that the introduction of natural enemies is costly, time-consuming (McEvoy and Coombs, 1999a,b), and potentially risky (Simberloff and Stiling, 1996; Louda et al., 1997, 2003), evaluating the relative contributions of already-established agents prior to introducing additional natural enemies is useful to managers. Of particular interest is to determine whether the introduced natural enemies affect life stages or demographic processes (survival, growth, and reproduction) that result in marked reductions in population growth (Shea and Kelly, 1998; Buckley et al., 2004; Davis et al., 2006; Ramula and Buckley, 2008).

Two insects, native to Australia, have been introduced as biological control agents of *Melaleuca quinquenervia* Cav. Blake (Myrtaceae), a tree that is native to Australia but invasive in subtropical southern Florida. The insects are: the leaf-eating weevil *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae) and the sap-sucker psyllid *Boreioglycaspis melaleucae* Moore (Homoptera: Psyllidae). The weevil, *O. vitiosa*, was released in southern Florida in 1997 (Wineriter and Buckingham, 1997; Center et al., 2000). Larvae and adults feed on the newly developed foliage and expanding leaves at branch apices, producing paper-thin feeding trails (by larvae) and window-like feeding scars (by adults) (Pratt et al., 2004b). Weevils have four larval stages, and all of them are found on the plant. Since weevils pulate in the soil, their abundance is limited in permanently inundated areas (Center et al., 2000). Larval densities in Florida are highest during late fall and...
winter, and lowest in spring and summer, unless fresh leaf tissue is available (Pratt et al., 2004b). Adults are present throughout the year.

The psyllid, B. melaleucae, was released in southern Florida in 2002. Both nymphs and adults feed on sap from leaves and stems by inserting their stylets into the phloem. Most damage, however, is attributed to nymphs, which feed on tender, expanding buds and leaves, as well as mature foliage (Purcell et al., 1997; Pratt et al., 2004a). Psyllids cause leaf desiccation, curling, discoloration, premature leaf senescence, and ultimately defoliation (Morath et al., 2006). Psyllids have five nymphal instars. First instars are mobile but later stages are sessile and congregate on leaves or stems (Pratt et al., 2004a). Psyllid nymphs produce a white waxy flocculence that covers them, making it difficult to quantify density in the field, particularly on large adult trees. Psyllids are common on M. quinquenervia throughout the year, although the densities are highest from late fall through spring, before the start of the rainy season, which occurs in the summer in southern Florida.

Damage to M. quinquenervia by weevils and psyllids has been quantified in the field since their introduction. Herbivory on mature trees by weevils increased from 5% of leaves in 1999 to 45% of leaves in 2005 (Rayamajhi et al., 2006). Trees may compensate for weevil damage by replacing leaf-bearing branches that are attacked (Pratt et al., 2005; Silvers et al., 2008). However, Pratt et al. (2005) compared the incidence of inflorescence and capsule production between weevil-damaged and undamaged M. quinquenervia stands during spring 2000, and found that the probability of producing inflorescences/new capsules was 36 times higher in undamaged versus damaged trees. Damaged trees had 80% and 54% fewer inflorescences and capsules, respectively, than undamaged trees (Pratt et al., 2005). Center et al. (2007) observed psyllid damage on 43% of surveyed coppices in 2003 with between 50% and 85% of the leaves being damaged on affected coppices. Sustained damage by psyllids in abundance (>15 per plant for >35 d) caused a reduction in the height of seedlings (Center et al., 2007). However, psyllid-damaged seedlings compensate for herbivory by increasing lateral leaf production (Center et al., 2007). Melaleuca quinquenervia stands (individuals 1–33 cm diameter at 1.3 m height) with high levels of damage by both insects experience significant reduction (12%) in aboveground biomass (Rayamajhi et al., 2008). Insecticidal exclusion experiments revealed that weevils and psyllids together reduced growth rates, biomass, number of capsules, and seed production (Tipping et al., 2008; Franks et al., 2008a,b).

Although the effects of insect damage on survival and performance of M. quinquenervia have been studied in the field, the effects of different densities of each insect and the interaction between the insects have not been investigated under controlled combinations. Both insects have become widely naturalized throughout southern Florida (Center et al., 2000, 2006; Pratt et al., 2003), thus making it difficult to independently estimate the effects of either species under natural conditions (Rayamajhi et al., 2006; Tipping et al., 2008, 2009). One previous experimental field study (Franks et al., 2006) examined the effects of combinations of psyllids and weevils by manipulating the density of psyllids but not of weevils. At the time the study was conducted, weevils had only been observed to occur at the low rate of one larva per seedling (Wheeler, 2003; Franks et al., 2006). This study reported that: (1) high densities of psyllids (>15 nymphs per seedling) were required to reduce seedling survival, growth, and biomass; (2) weevils did not reduce survival, growth, and biomass of seedlings; and (3) the psyllids and weevils did not interact in their effects on seedlings. What remains unclear after this study is whether weevils might have a significant effect at higher densities than the density assayed. In recent years, up to three weevil larvae per seedling have been frequently observed in naturalized populations (L. Sevillano, personal observations).

Understanding the effects of multiple species and densities of herbivores on plant fitness is important both conceptually, in the framework of plant–insect interactions, and for management. The two insects introduced as biological control agents against M. quinquenervia have different feeding modes that may differentially affect plant survival and growth. Predicting which type of herbivore is most injurious is not easy. Damage inflicted by one herbivore may influence the vulnerability of plants being attacked by the other herbivore (Pilson, 1992; Karban and Baldwin, 1997). Multiple species of herbivores attacking a plant simultaneously can have additive or multiplicative effects on plant fitness (Strauss, 1991), but interaction effects between herbivores in general have rarely been investigated (but see Meyer, 1993). From a management perspective, multiplicative effects of biological control agents would be most effective in controlling invasive plants, provided the interaction is facilitative rather than interferential.

Plant response to herbivory may depend not only on the identity of the herbivore, but also on its density. Higher densities are expected to have a greater impact on plant fitness (Louda, 1982), although Meyer (1993) showed that a few individuals of one type of herbivore (spittlebugs) had a greater impact on plant fitness of goldenrod (Solidago altissima), than did hundreds of aphids or several leaf-beetle larvae. Insect density might determine the strength and extent of the impacts of herbivory on plant fitness. From a management perspective, knowing whether insect density influences control is relevant considering the economic and possible ecological implications of introducing biocontrol agents (Meyer et al., 1997).

The resilience of plants to herbivore-caused damage might also depend on the resources available after damage (Coley et al., 1985; Meyer and Root, 1993; Strauss and Agrawal, 1999; Hawkes and Sullivan, 2001). For example, according to the Resource Availability Hypothesis (Coley et al., 1985), plants in resource-abundant environments are expected to grow fast and easily replace lost tissues due to herbivory, while plants in resource-limited environments might be less able to replace lost tissue, and thus to allocate more resources to anti-herbivore defenses. Naturalized populations of M. quinquenervia in southern Florida inhabit sites with two different kinds of soil, nutrient-rich organic and nutrient-poor sandy soils. Plants growing in these two soil types would be expected to respond differently to herbivory. The predictions of the Resource Availability Hypothesis and other related hypothesis are still debatable (Hamilton et al., 2001; Wise and Abrahamson, 2007), and this study can help to address the debate.

The current study sought to investigate the effects of density levels of both weevils and psyllids independently and interactively on survival and growth of M. quinquenervia seedlings. The study addressed the following questions: (1) How does the density of each of the herbivores affect survival and growth of M. quinquenervia seedlings? (2) Is the effect of psyllids on seedlings independent of the effects of weevils? and (3) Do the effects of the insects depend upon soil type? A three-way factorial experiment was conducted in a shade-house, manipulating weevil density (three levels), psyllid density (three levels), and soil type (sandy versus organic). We proposed the following hypotheses: (1) as insect density increases survival and growth of seedlings will decrease; (2) the effects of psyllids on plants will be independent of the effects of weevils; and (3) plants will be more susceptible to damage in sandy soils, which have fewer nutrients than organic soils.

2. Materials and methods

2.1. Study system

M. quinquenervia was introduced to southern Florida in 1886 (Dray et al., 2006), and is among the most invasive species in the
region (Bodle et al., 1994; Rayamajhi et al., 2002). Melaleuca quinquenervia populations are found in dry and flooded habitats throughout southern Florida. The species produces flowers year-round (Vardaman, 1994), which give rise to millions of wind-dispersed seeds that can be kept within serotinous capsules on the tree for several years. Massive seed releases and colonization events occur after vascular connections are disrupted by fire, mechanical damage, or herbicide applications, leading to the creation of dense monospecific carpets of M. quinquenervia seedlings (Meskimen, 1962; Woodall, 1982; Rayachhetry et al., 1998). Seedling establishment and growth are expected to be particularly susceptible to biological control (Turner et al., 1998; Center et al., 2000).

Populations of M. quinquenervia in southern Florida are common in coastal areas as well as in interior portions of the Florida Everglades (Turner et al., 1998). Soils on the east coast south of Lake Okeechobee are predominantly organic while on the west coast they are predominantly sandy. Although soil type has not limited the ability of M. quinquenervia to invade new areas in southern Florida (Bodle et al., 1994), the susceptibility of plants to biological control may differ with soil type.

2.2. Experimental design

This study was conducted in an ambient temperature (mean monthly temperature ranges from 26 to 33 °C over the entire year) outdoor growing facility at the University of Miami, Coral Gables, Florida. Melaleuca quinquenervia seeds were collected from five different populations on the east and west coasts of southern Florida. The seeds were germinated and six-month-old seedlings, 12 ± 3.5 cm tall (mean ± SE), were transplanted to 1.1 L pots randomly assigned to either organic or sandy soil (150 plants per soil type). Soils were collected from areas where there are naturalized forests of M. quinquenervia. Organic soil was collected in Holiday Park, Ft. Lauderdale, FL. Sandy soil was collected in the Picayu Strand State Forest, Naples, FL. General characteristics of the two soil types are presented in Appendix 1. At each site, soil was collected from several spots separated by 10–20 m, and was mixed thoroughly before filling the pots.

A 3 × 3 × 2 fully factorial experiment was conducted, with three levels of weevil density (zero, low, and high), three levels of psyllid density (zero, low, and high), and two levels of soil type (organic and sandy). Each of these 18 treatments was replicated five times. Each experimental unit (replicate) comprised three potted plants enclosed in a 25 × 50 × 75 cm PVC cage, covered with white mesh screen; a 25 × 50 cm tray was placed at the bottom of each experimental unit. The three plants within each experimental unit received the same treatment. To analyze survival, the experimental unit was every plant instead of the three plants within a tray (see below). To investigate if the cage with its white mesh covering had any effect on the response variables, five experimental units that had no cages and no insects for each soil type were included. In total, there were 100 experimental units, 90 with cages (18 treatments × five replicates per treatment), plus 10 with no cages (five per soil type), for a total of 300 plants.

The dependent variables were plant survival, growth, and biomass. Plant survival was recorded every two weeks. Plant size (height, diameter at 5 cm above the soil, and number of leaves) was measured at the beginning of the experiment and growth was estimated every two weeks as the change in the magnitude of each of these variables. Because many plants produced resprouting leaves on the main stem as a result of insect damage, new leaves emerging from the apical meristem (hereafter “primary leaves”) were distinguished from leaves produced through resprouting along the main stem, generally at the base of damaged leaves (“secondary leaves”). Secondary leaves were smaller than primary leaves, but this was only because they had not fully expanded and matured by the time the experiment was concluded. Biomass was quantified at the end of the experiment as the dry weights of shoots (stems + leaves) and roots of each plant.

Adult weevils and psyllids were collected in the field as well as from the populations at the Invasive Plant Research Laboratory (USDA, Davie, Florida), and were placed in a 2 × 2 × 2 m enclosure where weevil larvae and psyllid nymphs were reared for the experiment. For the weevils, the low density treatment consisted of a single first or second instar larva per plant, and the high density treatment consisted of three first or second instar larvae per plant. For the psyllids, the low density treatment consisted of 1–5 first to third instar nymphs per plant, and the high density treatment consisted of 15–25 first to third instar nymphs per plant. Insect densities were chosen based on the published literature and on recent field observations of natural insect densities in seedling stands (Franks et al., 2006; Center et al., 2007; L. Sevillano, personal observations). Insects were added twice during the experiment: once at the beginning (March 20–25, 2007), and once half-way through (April 26–30, 2007). The second addition was done to maintain the prescribed densities. One or two days after each insect addition, the plants were surveyed to ensure that the required insect densities were reached, and more insects were added if necessary. Seedlings were checked twice a week to monitor insect density levels and contamination by other herbivores. Psyllid adults were removed to prevent oviposition on the plants and alteration of the prescribed density. One yellow sticky card was placed inside each experimental unit to trap occasional unwanted insects that appeared. In addition, prior to the start of the experiment and before the second insect addition, control plants (i.e. with no weevils or psyllids) were sprayed with insecticide (Ortho® Systemic Insect Killer; 8.0% acephate, 0.5% fenbutatin oxide). The rest of the plants (i.e. those with different combinations of insect densities) were sprayed with equal amounts of water to simulate the effects of the insecticide application. Previous studies have shown that acephate does not alter survival, growth, or biomass of M. quinquenervia seedlings (Tipping and Center, 2002).

Experimental units (three potted plants on a tray inside a cage) were placed on greenhouse tables and randomly arranged. The plants were protected from ambient rain by a clear plastic roofing material. Each plant within an experimental unit received 400 ml of water once a week, throughout the experiment. The plants were harvested on June 20, 2007 approximately three months after the experiment began. Stems and leaves were dried to constant weight (at 60 °C), and weighed. Roots were stored in a refrigerator until carefully rinsed to remove all the soil, dried at 60 °C, and weighed.

2.3. Statistical analyses

Caged and uncaged controls (zero density of weevils and psyllids) were pooled because no statistical differences were found between these treatments and measured response variables (survival; change in height, diameter, number of leaves; and biomass; t-tests, all P > 0.05).

A generalized linear model (R Development Core Team, 2008) was used to analyze the survival data as recommended by Crawley (2007) because the average value among the three plants within each tray is not a parameter with a continuous distribution and is not a very informative metric (there are four possible outcomes for each tray: 1, 0.67, 0.33, and 0). In such cases the unit of analysis should be each individual plant with a binomial response variable (alive or dead) (Crawley, 2007). The variation due to the tray is accounted for by incorporating the replicate number (tray number) as a random factor. The fixed factors are weevil density, psyllid density, and soil type. Generalized mixed models are used in cases where errors are non-normal, or in cases where the response
variable is binary with pseudoreplication (in this case, plants within each tray) (Pinheiro and Bates, 2000; Crawley, 2007); the error family is specified in the model (in this case, binomial).

Size and biomass measurements were made on the surviving plants and averaged in each experimental unit. The effects of the insects on the relative growth rates of seedlings were analyzed as follows. The dependent variable is a unit-less index of growth rate that controls for initial size ([final size − initial size]/initial size). For example, a relative growth rate of 1 means that a plant added an amount equivalent to its initial size during the study and a relative growth rate of 2 means that a plant added an amount equivalent to twice its initial size during the study. Similarly, a relative growth rate of 0.5 means that a plant added an amount equivalent to one-half its initial size during the study. Since these are unit-less measures, the effects of insects on growth rates of different aspects of plant size can be compared. Differences among treatments on relative growth, measured as relative change in height, diameter, or number of leaves were analyzed with a three-way analysis of variance (ANOVA) in which weevil density, psyllid density, and soil type were fixed factors (JMP 7.0).

Post-hoc Tukey tests were used to determine the statistical differences among levels of each factor (e.g., insect density). Final biomass of leaves, stems, and roots was natural log transformed, and was analyzed with three-way ANOVA (JMP 7.0).

3. Results

3.1. Survival

Both weevil and psyllid densities significantly affected survival (Table 1). In the controls (zero density of weevils and psyllids, caged and uncaged), all seedlings survived until the end of the experiment regardless of soil type. At high weevil density, only 77% of seedlings survived (Fig. 1a). At high psyllid density only 43% of seedlings survived (Fig. 1b). A significant interaction was observed between psyllid density and soil type (Table 1). At low psyllid density, seedling survival was higher in sandy as compared to organic soil (97% vs. 89%, respectively), but this trend reversed at high densities. While 53% of the seedlings harboring high psyllid density survived to the end of the experiment in organic soil, only 38% survived in sandy soil. Survival was lowest, only 27%, when both weevils and psyllids were at high density (Fig. 1c). Although the weevils × psyllids interaction was marginally significant ($P = 0.07$, Table 1), at high psyllid densities there was a much greater effect of adding weevils than there was when psyllids were at either zero or low density (Fig. 1c) which may be biologically significant.

3.2. Growth rates

Seedlings increased in height and diameter most rapidly in the controls (zero density of weevils and psyllids, caged and uncaged) and least rapidly when the insects were at high densities (Fig. 2). In contrast, the number of leaves increased most rapidly at intermediate levels of insect density (Fig. 3).

Weevils and psyllids significantly reduced the rate at which seedlings grew in height during the experiment (Table 2). Seedlings in the controls (zero density of weevils and psyllids, caged and uncaged) increased in height by 201% with respect to initial size, compared to only a 115% increase in height of seedlings with psyllids but without weevils (zero weevil density) and a 167% increase in height of seedlings with weevils but without psyllids (zero psyllid density) (marginal means in Appendix 2A). When weevils were at low density, seedlings increased in height by 95%; and when weevils were at high density seedlings increased in height by only 53% (not shown). Similarly, when psyllids were

| Table 1 Generalized mixed model of the effects of weevil density, psyllid density, and soil type on the survival of M. quinquenervia seedlings. Bold indicates $p < 0.05$; italic indicates $p < 0.10$. |
|---------------------------------|-----|-------|---|
|                                | df  | F     | P  |
| Weevil density                 | 2   | 6.5771| 0.0022 |
| Psyllid density                | 2   | 77.7330| <0.0001 |
| Soil type                      | 1   | 0.1281| 0.7213 |
| Weevil × Psyllid               | 4   | 2.2403| 0.0717 |
| Weevil × Soil                  | 2   | 0.7901| 0.4572 |
| Psyllid × Soil                 | 2   | 3.6035| 0.0316 |
| Weevil × Psyllid × Soil        | 4   | 0.6540| 0.6257 |

![Fig. 1. Survival of M. quinquenervia seedlings exposed to different densities of weevils (a) and psyllids (b). Bars correspond to the least square means ± 1 standard error. Different letters indicate significantly different means according to the Tukey post-hoc test. (c) Interaction effects of weevil and psyllid densities on seedling survival. Points correspond to the least square means ± 1 standard error in each of the nine weevil × psyllid treatment categories. Lines connect the means of each weevil density level, and psyllid density levels are shown on the x-axis.](image)
at low density seedlings increased in height by 79%, and at high density they increased in height by only 17% (not shown). The influence of density levels of psyllids on growth in height was greater than that of density levels of weevils, at least according to the density levels defined in this study. Comparing the growth rates in the zero versus the high density category, there was a 10-fold difference in relative growth rates for psyllids, and only a twofold difference for weevils. However, this result does not compare the per-capita effects of the insects, since the “high” density treatment was only three individuals for weevils, but 15–25 individuals for psyllids. Soil type also had a significant effect on growth in height (Table 2); seedlings in organic soil grew 21% more quickly than seedlings in sandy soils (not shown).

Weevils and psyllids interacted significantly in their effects on diameter growth (Table 2). The level of psyllid density affected the variability in growth rate among weevil density levels. At zero psyllid density, there was a fourfold difference in growth rate among weevil levels and at high psyllid density, there was only a fractional difference in growth rates among weevil levels (Fig. 2 and Appendix 2B), indicating that the reduction in growth rate due to high density of psyllids was less in the presence than in the absence of weevils. The lowest growth rate in diameter was for seedlings where there was a high density of psyllids and a low density of weevils; in this category plants shrank slightly (by −2%). Seedlings with high densities of both insects did only slightly better, they grew by 1%. The effects of soil type and weevil × soil type interaction on diameter were marginally significant (Table 2). Despite the significant weevil × psyllid interaction, the overall effects of the density of each insect on diameter growth were also significant (Table 2). Seedlings in the controls (zero density of weevils and psyllids, caged and uncaged) increased in diameter by 121% (Appendix 2B), compared to only a 73% increase in diameter of seedlings with psyllids but without weevils, and a 75% increase in diameter of seedlings with weevils but without psyllids (main effects not shown). Once again, the influence of density levels of psyllids on growth in diameter was greater than density of weevils, according to the density levels defined in this study. Comparing the growth rates in the zero versus the high density category, there was a 16-fold difference in relative growth rates for psyllids, and only a fivefold difference for weevils.

The three-way interaction among weevil density, psyllid density, and soil type significantly influenced the rate at which the standing crop of leaves increased (No. of primary + No. of secondary leaves) (Table 2). The highest rate of increase in leaves was not for seedlings with no insects, but rather for seedlings with low densities of both weevils and psyllids; they increased in leaf number by 320% in both soil types (Fig. 3, Appendix 2B). Seedling response to the weevil × psyllid interaction differed between soil types. Seedlings in the controls (zero density of weevils and psyllids, caged and uncaged) increased in leaf number by only 122%, with a slightly higher increase in the organic soils (Appendix 2B). The lowest rate of increase in standing crop of leaves was for seedlings with high density of both weevils and psyllids in sandy soil where increase in leaf number was about 100% (Fig. 3). Seedlings with low density of weevils and high density of psyllids had a higher rate of increase in sandy than in organic soils, although in most other comparisons, seedlings added leaves more quickly in organic soil.

3.3. Number of leaves and biomass

The standing crop (total number) of leaves at the end of the experiment exemplified the plant’s ability to compensate for mild attack by herbivores (similar to the results for the relative change in the number of leaves); it was higher when just a few insects were present, than it was when either zero or many insects were present (Fig. 4). Seedlings in the controls (zero density of weevils and psyllids, caged and uncaged) had produced on average 44 primary leaves and one secondary leaf, by the end of the experiment. In comparison, seedlings with no weevils produced on average 31 primary and 32 secondary leaves, and seedlings with no psyllids

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**Fig. 2.** Effects of weevil density across psyllid densities on growth, measured as the relative change in diameter, of *M. quinquenervia* seedlings. Points correspond to the least square means ± 1 standard error in each of the nine weevil × psyllid treatment categories. Lines connect the means of each weevil density level, and psyllid density levels are shown on the x-axis.

**Fig. 3.** Interaction effects of weevil and psyllid densities on growth estimated as the relative change in total number of leaves of *M. quinquenervia* seedlings growing in (a) organic and (b) sandy soil. Points in each figure correspond to the least square means ± 1 standard error in each of the nine weevil × psyllid treatment categories. Lines connect the means of each weevil density level, and psyllid density levels are shown on the x-axis.
Fully crossed ANOVA of the effects of weevil density, psyllid density, soil type, and all interactions on the growth of *M. quinquenervia* seedlings measured as relative change in height, diameter, and number of leaves. Factors in bold face are statistically significant.

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<tr>
<th></th>
<th>df</th>
<th>Height</th>
<th>Diameter</th>
<th>Total no. of leaves</th>
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<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
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<td>21.62 &lt;0.0001</td>
<td>4.17 0.02</td>
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<td>29.26 &lt;0.0001</td>
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</table>

**Table 2**

![Diagram](image)

**Fig. 4.** Total number of leaves (primary + secondary) produced by *M. quinquenervia* seedlings exposed to different densities of (a) weevils and (b) psyllids. The total number of leaves is the result of adding primary leaves (produced at the apical meristem of the plants), and secondary leaves (produced through resprouting along the main stem). Different letters indicate significantly different means according to the Tukey post-hoc test.

produced on average 36 primary and 17 secondary leaves (Appendix 2C). The number of primary leaves decreased as insect density increased; but the number of secondary leaves increased in the presence of insects (Fig. 4). Although total number of leaves was highest in plants with few insects (weevils or psyllids), leaf biomass of primary and secondary leaves decreased as insect density increased (Fig. 5), indicating that the photosynthetic tissue in plants with insects decreased as insect density increased. Leaf biomass of seedlings without insects was on average 0.73 g, compared to 0.54 g for plants with no weevils and 0.5 g for plants with no psyllids.

Weevil density, psyllid density, and soil type all significantly affected the total plant biomass (final weight) (Fig. 5). While all three factors also affected shoot biomass, only the insects and not the soil type affected root biomass (Table 3). Overall, seedlings in the controls (zero density of weevils and psyllids, caged and uncaged) reached greater biomass than those with insects. Total seedling biomass in the absence of herbivory was on average 1.57 g while seedlings with psyllids but zero weevils weighed on average 1.13 g and seedlings with weevils but zero psyllids weighed 1.06 g (Appendix 2C). Seedlings with low and high densities of weevils weighed 29% and 57% less, respectively, than plants without weevils (Fig. 5a). Similarly, seedlings with low and high densities of psyllids weighed 21% and 59% less, respectively, than plants without psyllids (Fig. 5b). Seedlings in organic soil weighed 20% more than plants grown in sandy soil (Fig. 5c). Also, soil type significantly interacted with weevil presence in its effects on plant biomass (Table 3): the influence of soil type disappeared when weevils were introduced into the system (not shown).

**4. Discussion**

Herbivory by weevils and psyllids reduced survival, growth, and biomass of *M. quinquenervia* seedlings. While previous studies have shown that herbivory can impact *M. quinquenervia*’s seedling survival and growth, this study shows that the magnitude of these negative effects is density dependent. High density levels of either weevils or psyllids consistently reduced seedling performance (survival, growth, and biomass), while low density levels did not. These results are consistent with the previously reported field studies in that psyllids attacking *M. quinquenervia* seedlings or coppicing stumps at high densities (>15 nymphs per plant) markedly reduce survival by >50% (Franks et al., 2006; Center et al., 2007). For some of the plant performance parameters, the effects of one insect depended not only on the density of that insect, but also on the density of the other insect. The marginally significant weevil × psyllid interaction suggests that survival is particularly affected when both insects are present. These results indicate that although psyllids are effective at high densities, both insects in concert will most effectively reduce seedling survival. In this sense, it would be relevant to investigate the population dynamics of both species of biological control agents to understand how insect densities fluctuate over time in the field, and predict the consequences of such fluctuations for the plant.

Soil type affected growth (measured as height), and biomass of *M. quinquenervia* (organic > sandy), and in some cases, the effects...
of insect herbivory depended on soil type. Sandy soils where *M. quinquenervia* lives in southern Florida are poor in terms of nutrient content and organic matter, and have low water holding capacity compared to organic soils (Appendix 1). Plants in organic soil would be more likely to overcome herbivory by replacing lost tissue, than those in sandy soil (Resource Availability Hypothesis, Coley et al., 1985). Although final biomass was higher in plants growing in organic soil, the results of this study indicate that plant responses to herbivory under different resource conditions also depend on insect density/damage intensity. For example, only seedlings with high psyllid density had higher survival in organic than in sandy soil. Although the significant psyllid × soil interaction suggests that plants growing in sandy soils can also overcome insect damage and keep growing, after a certain threshold (high density of psyllids) plants can no longer compensate for losses due to herbivory, and survival is lower. Overall *M. quinquenervia* seedlings growing in sandy soils would be expected to allocate more resources to plant defense than seedlings in organic soil. It would be relevant to study whether these two biocontrol agents can naturally achieve higher densities in sandy than in organic sites. Regardless of soil type, high density of insects impact *M. quinquenervia* seedling performance more efficiently.

Franks et al. (2006) did not find a significant interaction between weevil and psyllid damage in the field, although only the effects of low density of weevils across different levels of psyllids were tested. They suggested that the effects of the insects were additive but our experiment found a significant interaction effect of weevils and psyllids on relative growth (i.e. diameter). The negative impacts of psyllids observed on plants with zero or low density of weevils were diminished when weevils were present in high densities. This is what would be expected if the insects were competing with each other. Competition for resources between weevils and psyllids was not assessed in this study, but it is likely that some level of competition exists (Strauss, 1991; Denno et al., 1995; Paynter and Hennecke, 2001), particularly on seedlings or saplings in which the amount of leaf tissue is limited compared to larger plants. For adult *M. quinquenervia* trees, it has been suggested that defoliation produced by psyllids promotes the production of new foliage that, in turn, attracts weevil larvae (Center et al., 2006), further increasing the vulnerability of plants to damage (Pilson, 1992; Karban and Baldwin, 1997). Despite the strong effects of the weevils and psyllids on seedling growth and survival, a previous study (Franks et al., 2008b) showed that there was no selection for increased resistance to herbivory in the presence of the insects. Fortunately for biological control efforts, this indicates that the strong effects of herbivory shown in the present study are not likely to lead to *M. quinquenervia* evolving increased defense, at least over the relatively short term.

The interaction effects between weevils and psyllids have different consequences for survival than they do for growth of *M. quinquenervia* seedlings. For survival, the effects of psyllids at

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**Fig. 5.** Effects of weevil density (a) and psyllid density (b), and (c) soil type on total biomass (grams) of *M. quinquenervia* seedlings. Biomass is partitioned into root, stem, and leaf biomasses. Least square means ± 1 standard error of the total biomass are shown.

**Table 3**

<table>
<thead>
<tr>
<th>df</th>
<th>Total biomass</th>
<th>F</th>
<th>p</th>
<th>Shoots</th>
<th>F</th>
<th>p</th>
<th>Roots</th>
<th>F</th>
<th>p</th>
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<tr>
<td>Weevil density</td>
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<td>27.61</td>
<td>&lt;0.0001</td>
<td>27.42</td>
<td>&lt;0.0001</td>
<td>20.68</td>
<td>&lt;0.0001</td>
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<td></td>
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<tr>
<td>Psyllid density</td>
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<td>26.27</td>
<td>&lt;0.0001</td>
<td>28.11</td>
<td>&lt;0.0001</td>
<td>10.89</td>
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<td>Soil type</td>
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<td>0.001</td>
<td>0.65</td>
<td>0.42</td>
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</tr>
<tr>
<td>Weevil × Psyllid</td>
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<td>1.62</td>
<td>0.18</td>
<td>1.18</td>
<td>0.32</td>
<td>3.28</td>
<td>0.02</td>
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<tr>
<td>Weevil × Soil</td>
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<td>5.11</td>
<td>0.008</td>
<td>4.99</td>
<td>0.009</td>
<td>4.39</td>
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<td>2.11</td>
<td>0.13</td>
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</tr>
<tr>
<td>Weevil × Psyllid × Soil</td>
<td>4</td>
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<td>0.09</td>
<td>1.96</td>
<td>0.11</td>
<td>1.45</td>
<td>0.23</td>
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</tbody>
</table>
high density levels are enhanced when weevils are also present at high density levels. However, for growth, the effects are not enhanced when both insects are present but, on the contrary, having both insects is not as effective in reducing growth, as is having only one type of insect at high density levels.

*M. quinquenervia* seedlings that experienced herbivory produced secondary leaves, revealing that one of the mechanisms that these plants use as defense against herbivory is to compensate for defoliation by replacing damaged leaves (Rosenthal and Kotanen, 1994; Mauricio et al., 1997; Agrawal, 1998). Compensation is a commonly observed mechanism that plants use to tolerate insect herbivore damage (Trumble et al., 1993; Rosenthal and Kotanen, 1994; Dhileepan et al., 2000) and has been demonstrated for *M. quinquenervia* previously (Pratt et al., 2005). The magnitude of the compensatory response to herbivory can vary depending on the intensity of herbivory (Marquis, 1984; Gadd et al., 2001; Wurfl, 2006). Herein for instance, compensation was higher in seedlings receiving moderate levels of herbivory (low density of either weevils or psyllids) than high levels (Fig. 4). Understanding the mechanisms of defense against herbivory is particularly important for systems in which insects are used as biological controls of invasive plants. Small to moderate losses of plant tissue do not always have measurable impacts on plant performance, suggesting that below a threshold, compensatory responses might mitigate the effectiveness of biological control agents (Karban, 1993). Research should concentrate on testing if invasive plants have the ability to compensate or overcompensate for the effects of herbivory by growing or reproducing more than undamaged plants (Belski, 1986; Strauss and Agrawal, 1999). The results of this study indicate that *M. quinquenervia* seedlings can compensate for insect damage by producing new leaves (secondary leaves) but these leaves are much smaller initially and have less photosynthetic capacity. Some of those secondary leaves developed into new shoots, which in turn altered plant architecture. This mechanism has been observed in other plants as a response to leaf-beetle herbivory (Nozama and Ogushi, 2002; Schat and Blossey, 2005). Although the production of new leaves as a consequence of herbivory could be beneficial for *M. quinquenervia* seedlings in the short term, it can also induce new events of herbivory (Center et al., 2007), because both weevils and psyllids are particularly attracted to young tissue. Newly developed leaves were commonly damaged by both biological control agents, particularly on plants in which most primary leaves had already been damaged. This observation suggests that damage by weevils or psyllids increases the vulnerability of plants being further attacked by either (or both) insect, and thus having both insects in the same area will increase, at least at the seedling stage, the effectiveness of the biological control program.

The compensatory mechanism observed in growth did not result in an increase in seedling biomass. Both insects significantly reduced above and belowground biomass. Although seedlings that received relatively low herbivory had more leaves than those with no or high herbivory by the end of the experiment, they did not have higher leaf biomass. Indeed, leaf biomass followed the same trend as that observed for survival and growth (relative change in height and diameter): as insect density increased, leaf biomass decreased. This trend was also observed for root and stem biomass, and thus for total biomass (Fig. 5). Psyllid impacts on *M. quinquenervia* seedling biomass have also been reported in the field (Franks et al., 2006; Center et al., 2007), and this study shows that weevils and soil type can also influence biomass.

The results of this study showed that independently or in concert, both insects introduced as biological control agents against the invasive tree *M. quinquenervia* in southern Florida can reduce survival, growth, and biomass of seedlings. Psyllids are generally more effective than weevils, and the effects of the insects are consistently stronger when they are in high densities (except for the change in number of leaves). Interaction effects between biological control agents are in some instances positive in that the impacts on *M. quinquenervia* seedling survival are greater when both weevils and psyllids are in high densities than when only psyllids are in high densities. In other instances, the presence of only one agent in high densities (i.e. psyllids) is enough to reduce growth. Biological control agents were introduced with the expectation that their primary contribution to the reduction of the growth and spread of *M. quinquenervia* populations would be through the reduction of seedling and sapling survival, and seed production (Turner et al., 1998; Center et al., 2000). This study shows that both insects affect the seedling–sapling transition of the life cycle of this plant by reducing seedling survival and performance, and thus the number of plants that reach the next life cycle stage, or the rate at which they reach such stage (saplings). Other studies have shown that seedlings, and other stages of the *Melaleuca* life cycle, can be negatively affected by weevils and psyllids (Pratt et al., 2005; Franks et al., 2006, 2008a,b; Center et al., 2007; Rayamajhi et al., 2008; Tipping et al., 2008). The next step in understanding the effectiveness of *Melaleuca*’s biological control program is to study if the negative impacts of the biocontrol agents at the individual level translate into a reduction of the population growth rates and rates of spread of *M. quinquenervia*. We are developing mathematical models of spatial population dynamics for this tree to address this question.

Studies of the impacts of biological control agents should concentrate now on understanding the role that the agents play on the population dynamics of invasive plants, which is fundamental to improving weed biocontrol. Successful suppression of invasive plants requires that biological control agents, or any other control strategy, alter stage transitions that are important for plant population growth.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biocontrol.2010.01.006.

References


