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MYCORRHIZAE DIFFERENTIALLY ALTER GROWTH, PHYSIOLOGY, AND COMPETITIVE ABILITY OF AN INVASIVE SHRUB

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Abstract. Mycorrhizae improve phosphorus availability to host plants and alter their morphology, physiology, and competitive ability. We examined how different isolates of arbuscular mycorrhizal fungi, soil-P, light, and competition affect the growth, physiology, and biomass allocation of seedlings of an exotic invasive shrub of the southeastern United States, *Ardisia crenata*, in two greenhouse experiments. When *Ardisia* seedlings were grown singly in pots without competition, soil phosphorus concentration and light had no effect on seedling growth. Relative growth rates (RGR) and leaf area ratio (LAR), however, were higher for seedlings inoculated with mycorrhizal fungi isolated from *Ardisia* roots than those inoculated with single-spore isolates and nonmycorrhizal controls. In the second experiment, an *Ardisia* seedling was grown in each pot in competition with another conspecific seedling or with a seedling of *Prunus caroliniana*, a native subcanopy tree. The identity of the competitor had little effect on seedling RGR of *Ardisia*, but LAR was significantly higher for seedlings in conspecific competition. Overall, *Prunus* seedlings had higher RGR than *Ardisia*, but RGR and survival of *Prunus* seedlings were significantly reduced in competition with *Ardisia* when mycorrhizal fungi were suppressed by benomyl. These results suggest that competitive interactions of exotic invasive plants with native plants are dependent on the isolates of mycorrhizae present.

Key words: Ardisia crenata; *biomass allocation; competition; host specificity; invasive plants; mycorrhizae; north-central Florida; origin of mycorrhizal strains;* Prunus caroliniana; *seedling growth.*

INTRODUCTION

Plants must overcome obstacles of dispersal, abiotic conditions, and competition with existing species in order to colonize and establish in a new geographical locality. Arbuscular mycorrhizal fungi can aid or hinder the establishment of a new species by ameliorating or intensifying the abiotic stresses encountered in the new range. Arbuscular mycorrhizae (AM) may improve phosphorus (P) availability and enhance leaf photosynthetic rates and growth rates of the hosts (Siqueira et al. 1998, Sharma and Adholeya 2000). Due to improved P nutrition, mycorrhizal plants may allocate proportionally less to roots while increasing leaf area ratio and specific leaf area (Son and Smith 1988, Berta et al. 1995, Lovelock et al. 1996, Gavito et al. 2000). Change in allocation patterns of the host, in turn, may affect its interaction with neighboring plants for light and soil nutrients. Because the response of plants to AM is dependent on both soil-P levels and light availability (Gavito et al. 2000, Graham et al. 1997, Peng et al. 1993), effects of AM fungi on plant invasion process must depend on these abiotic factors.

Although AM can infect a wide range of hosts from various geographical localities, the responses of host plants to mycorrhizae vary greatly depending on the combination of plant and fungus genotypes (Smith and Smith 1996, Johnson et al. 1997). Different fungal genotypes can have positive, negative, or little effect on the growth of the same host species (Boerner 1990, Monzon and Azcon 1996, van der Heijden and Kuyper 2001), because AM may differ in their ability to infect a given host, efficiency of P transferred to the host, carbon demand, soil adaptation, and host compatibility (Johnson 1993, Graham et al. 1996, Monzon and Azcon 1996, Johnson et al. 1997). Thus, assessment of the effects of AM on plant invasion must consider the genotype and source of fungal isolates.

Mycorrhizal fungi may alter competitive interactions between invading and local plants. Although AM have been largely ignored as a mediator of plant invasion (Richardson et al. 2000), they have been shown to increase the growth of an invasive plant species over natives and accelerate the process of invasion in a grassland ecosystem (Marler et al. 1999). More generally, differences in competitive ability under the influence of mycorrhizae can alter community composition by favoring mycorrhizal-responsive, inferior competitors (Hartnett et al. 1993, Moora and Zobel 1996, Smith et al. 1999) or causing competitive exclusion of nonresponsive dominants (Gange et al. 1999, Marler et al. 1999). Whether mycorrhizal fungi promote or inhibit the process of plant invasion must be

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determined by examining responses of exotic plants to multiple fungal genotypes with and without competition with native species that occupy similar ecological niches.

Here we report the results of two experiments that examined effects of fungal isolates and abiotic environment on growth and competitive interactions of an exotic invasive shrub. Specifically, we examined the effects of various isolates of AM fungi, soil-P, light, and competition type on the growth, physiology, and biomass allocation of *Ardisia crenata* Sims (Myrsinaceae, hereafter *Ardisia*). In the first greenhouse experiment, we examined the effects of light, soil-P content, and AM fungal isolates on growth, photosynthetic rates, and biomass allocation patterns of *Ardisia* seedlings grown singly in pots. We hypothesized that mycorrhizal plants would exhibit higher growth rates, invest more biomass aboveground, and maximize leaf area ratio compared to nonmycorrhizal plants, and plant response to phosphorus would vary among mycorrhizal isolates. In the second greenhouse experiment, we examined the effects of AM on inter- vs. intraspecific competition between seedlings of *Ardisia* and a native shade-tolerant subcanopy tree, *Prunus caroliniana* (Mill) Aiton (Rosaceae, hereafter *Prunus*). We hypothesized that *Ardisia* would be less affected by conspecific than heterospecific competition, particularly when mycorrhizal.

Materials and Methods

Species

Ardisia is a woody evergreen shrub that was introduced as an ornamental to the southeastern United States from east Asia ~100 years ago (Dozier 1999). *Ardisia* is actively invading mesic forest understory in Louisiana, Texas, Hawaii, and north-central Florida (Singhurst et al. 1997). *Ardisia* forms dense monodominant patches in the understory and suppresses local species richness and diversity of native understory plant species (A. Fox and K. Kitajima, *unpublished data*). The architecture of the plants creates strong self- and neighbor-shading, even when plants are not in dense clumps (K. Kitajima and M. Dooley, *unpublished data*). Growth of *Ardisia* seedlings in the field has been shown to be positively correlated with soil-P content (Dozier 1999), and *Ardisia* roots are highly colonized by AM in the field (S. Bray, *unpublished data*). *Prunus* was chosen as a heterospecific competitor in the experiment because its juveniles are abundant in forest understories where *Ardisia* typically invades (A. Fox and K. Kitajima, *unpublished data*). These species have similar seed size, and the juveniles of both species have evergreen leaves that are common under the partially deciduous canopy of mesic hardwoods forests in north-central Florida.

Experiment 1: effects of soil-P, light, and inoculum source on growth and allocation

Three inocula and a sterile control were used in this experiment. The inocula were *Glomus etunicatum* (S3029), *G. fasciculatum* (S3060), and host-associated fungi. S3029 has been maintained in pot culture for 15 years; S3060 was isolated in 1997 from a tomato field in north-central Florida (Sylvia et al. 2001). These isolates have caused positive growth responses in both agronomic and native woody plants (Sylvia 1990, Sylvia et al. 1993) and will be collectively referred to as standard inocula. The host-associated inoculum (HA) was composed of a corn trap culture initiated with washed *Ardisia* roots gathered from a north-central Florida hardwood forest (29°40′ N, 82°09′ W). Inocula were composed of soil, roots, and spores produced in the corn trap cultures. Infection potential of the inocula was determined by growing corn (*Zea maize*) with 5 g of inocula for 4 wk (Sylvia 1994). Infection potential rather than most probable number was used because we were interested in comparing inocula rather than determining absolute numbers of propagules. S3060 had the highest infection potential (51.7 ± 10.2%) followed by HA (46.7 ± 7.21%) and S3029 (33.0 ± 7.00%).

Ardisia seeds were gathered from four populations in Gainesville, Florida, mixed, cleaned of pulp, and stored in moist sand at 4°C for 24 wk. Seeds were germinated in petri dishes lined with moist filter paper at 26°C. Before leaf development, seedlings (~15 d after radicle emergence) were transferred to bleach-sterilized Deepots (6.2 cm top diameter ×24.5 cm, J. M. McConkey and Company, Sumner, Washington, USA) containing a 1:1:1 steam-pasteurized mixture of soil:sand:peat moss. The soil was collected from Austin Carey Forest (29°43′ N, 82°13′ W). The soil had a pH of 5.7 (2:1, H_2O:soil), 0.1% organic matter, and 1.6 mg Mehlich-I-extractable P/kg. The sand was acid washed to remove excess P, and rinsed until neutral pH was achieved. Phosphorus was added in the form of $KHPO_4$ at 0, 5, 30, or 60 mg P/kg soil. Pots were filled three-quarters full with the growth medium; 5 g of inoculum were added to inoculated treatments and thoroughly mixed with the growth medium. After adding seedlings of equal mass and remaining soil, pots were randomly assigned to shading treatments with one (moderate light) or two (low light) layers of shade cloth supported by PVC frames. These treatments created mean photosynthetic photon flux densities (PPFD) of 412 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (moderate) and 212 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (low) at midday. The shade treatments were randomly assigned to locations within each of three blocks along a greenhouse bench. Each block contained 3–4 plants per treatment group. For the control, S3060 and S3029 inoculum types, there were four P levels by two light levels by 10 replicates for a total of 240 plants. The HA inoculum was used only at the 5 mg/kg P level,

but had 10 replicates in each light treatment for 20 HA inoculated plants. Plants were watered when necessary and biweekly given a modified Hoagland's solution of 0.1× concentration of all nutrients, except P at 0.01× concentration (Sylvia et al. 2001).

The photosynthetic rates of the most recently fully expanded leaf were measured with a Li-6400 gas-exchange system (Li-Cor, Lincoln, Nebraska, USA) for three seedlings per light and inoculum treatment combination at the 5 mg/kg P level during the fourth month after planting. All measurements were taken between 0800 and 1200 hours. A CO_2 mixer unit maintained the CO_2 concentration of incoming reference air at 380 ppm. Temperature of the thermister block was maintained at 26°C. Flow rate was 250 mL/min. Light was supplied with a blue–red LED (LI6200-02B, Li-Cor, Lincoln, Nebraska, USA). Leaves were exposed to 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD for 15 min for photosynthetic induction, after which quasi steady state gas exchange rates were recorded at light levels of 800, 500, 300, 100, 60, 40, 20, 10, and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Plants were harvested after 258 d and leaf area was measured immediately with a portable area meter (Li-3000, Li-Cor, Lincoln, Nebraska, USA). Roots of five randomly selected plants within each treatment combination were weighed for fresh mass and set aside for analysis of AM colonization. Roots of the remaining plants along with stems and leaves of all plants were dried at 60°C until constant mass was reached. The dry mass of roots used for assessment of colonization was estimated from fresh:dry mass ratios of roots. To examine biomass allocation pattern, root:shoot ratio (R/S), specific leaf area (SLA, leaf area divided by leaf mass), net assimilation ratio (NAR, net carbon assimilation on leaf area basis), and leaf area ratio (LAR, leaf area divided by total mass) were calculated. Relative growth rate (RGR) was determined using the following equation:

$$\text{RGR } (\text{mg} \cdot \text{g}^{-1} \cdot \text{d}^{-1}) = [\ln(\text{seedling mass at harvest}) - \ln(\text{initial seedling mass})]/[\text{duration of study (d)}]$$

Tissue phosphorous contents were determined after grinding the dried stems and leaves with a Wiley Mill with a 20-mesh screen (Thomas Scientific, Swedesboro, New Jersey, USA). Due to the small size of seedlings, two plants per block per treatment group at the 5 mg/kg P level were combined. Samples were ashed overnight at 500°C and digested with 12 mol/L HCl, followed by colorimetry methods of Murphy and Riley (1962) to determine tissue-P concentration and content per plant.

To quantify mycorrhizal colonization, *Ardisia* roots were cleared in 10% KOH at 80°C for 45 min, while corn roots used for determination of inoculum potential were cleared for 15 min. A longer clearing time was necessary for *Ardisia* roots due to their high tannin content. *Ardisia* roots were then rinsed and soaked in H_2O_2 at 50°C for 10 min for additional clearing. Roots were again rinsed and acidified in concentrated HCl (5 mL HCl/200 mL H_2O) for 5 min. The roots were then soaked overnight at room temperature in trypan blue stain, which had been used successfully to stain field-collected *Ardisia* roots (S. R. Bray, *unpublished data*). To estimate mycorrhizal colonization, 20 1-cm root fragments were mounted on microscope slides and examined at 100×. Roots were scored as mycorrhizal if they contained coils or arbuscules, spores, vesicles, or typical AM hyphae (aseptate, large diameter, angular branching).

Experiment 2: effects of mycorrhizae on seedling competition

As a competitor of *Ardisia*, we chose *Prunus*, a shade-tolerant subcanopy tree found in north-central Florida hardwood forests. *Prunus* seedlings with four to eight leaves and 7–12 cm height grown in soil-free medium were acquired from a commercial nursery (Urban Forestry Services, Micanopy, Florida, USA). Examination of cleared and stained roots of 10 seedlings revealed no mycorrhizal colonization. Although *Prunus* has been shown to be ectomycorrhizal in some cases (Smith and Read 1997), we found no evidence of ectomycorrhizae in *Prunus caroliniana*. *Ardisia* seedlings were collected from the same invaded forest as the inoculum in experiment 1. *Ardisia* seedlings had four to eight leaves and heights of 5–10 cm. Cleared and stained *Ardisia* roots revealed a total mycorrhizal colonization level of 52 ± 2%.

Prunus and *Ardisia* were grown in hetero- and conspecific competition with (AM) or without (NM) mycorrhizal inoculum. Two plants were potted in each 3.8-L pot containing the same medium as in experiment 1 with no additional P. Plants were paired according to height and number of leaves. Twenty pots contained two *Prunus* seedlings (*Prunus* conspecific competition), 20 contained two *Ardisia* seedlings (*Ardisia* conspecific competition), and 40 contained one *Ardisia* and one *Prunus* seedling (*Prunus* heterospecific competition and *Ardisia* heterospecific competition). Half of the pots were randomly assigned to the NM treatment and were drenched with 75 mg of benomyl (Benlate, DuPont, Wilmington, Delaware, USA) dissolved in 1 L of deionized water. Although benomyl may have phytotoxic effects in some species, no phytotoxic effects have been reported in either *Prunus* or *Ardisia*, and benomyl is commonly used to control fungal pathogens in horticultural nurseries growing *Prunus persica*, *Prunus dulcis*, and *Prunus serotina* (Stanosz 1992, Fontanet et al.1998). Plants in the AM treatment received 5 g of *G. fasciculatum* (S3060) inoculum to supplement indigenous AM fungi and one L of deionized water. In the AM treatments containing *Ardisia*, each pot contained S3060 and fungi already inhabiting the *Ardisia* seedling; treatments without *Ardisia* contained only

TABLE 1. ANOVA summarizing the effects of light and soil on LAR and R/S on *Ardisia* seedlings in experiment 1 (model $P < 0.05$).

Source	df	LAR		R/S	
		F	*P*	*F*	*P*
Model	7	4.51	0.0001	3.24	0.003
Light	2	9.24	0.002	0.20	0.66
P-level	4	3.11	0.03	3.94	0.009
Light × P-level	3	3.37	0.02	3.24	0.02
Error	192				

Notes: Fungal isolate effects (control, S3029, and S3060) were not significant and were pooled. Abbreviations are: LAR, leaf area ratio; R/S, root:shoot ratio; P, phosphorus.

S3060. Pots were then randomly placed under a shade frame (~20% open-sky light) in the greenhouse. Pots were watered as needed and received the modified Hoagland's solution used in experiment 1 biweekly. The minimum and maximum temperatures averaged 18°C and 34°C, respectively. The average maximum PPFD in the greenhouse was 910 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

At 160 d after transplanting, growth analysis was conducted with one randomly selected plant per pot to ensure statistical independence. Root:shoot, LAR, SLA, and RGR were determined as in experiment 1. A subsample of each root system of six randomly selected individuals per treatment was used to estimate percentage colonization after determining fresh mass. Fresh : dry mass ratios of remaining roots were used to estimate dry mass of the subsamples used to determine colonization. Initial mass of seedlings was estimated through an allometric relationship of leaf number (*Ardisia*, 5.1 ± 0.24 leaves; *Prunus*, 5.7 ± 0.41 leaves; mean ± 1 SE) and height (*Ardisia*, 7.2 ± 0.23 cm; *Prunus*, 9.3 ± 0.35 cm) with the total dry mass for each species (*Ardisia*, 1.31 ± 0.05 g; *Prunus*, 0.46 ± 0.04 g). Percentage mycorrhizal colonization was determined as in experiment 1. Tissue-P content was quantified only for leaves with the same method as in experiment 1.

Statistical analyses

Allocation and growth data from both experiments were analyzed with factorial model fitting (JMP 4.0, SAS Institute 2000). For the analysis of the first experiment, the effects of soil-P, light, and inoculum type (control vs. standard inocula), and their interactions on R/S, LAR, SLA, NAR, and RGR were analyzed. Because the host-associated inoculum was given only at 5 mg/kg P level, the effects of inoculum type (control, S3029, S3060, HA), light, and their interactions at the 5 mg/kg P level were then examined. The results of experiment 2 were analyzed with a model that included the effects of species (*Ardisia* or *Prunus*), competitor (conspecific or heterospecific), mycorrhizal status (AM or NM), and their interactions. When treatment and interaction terms were not significant ($P \geq 0.1$), they were dropped from the model and the results of the analysis with the reduced model were reported. Tukey hsd at alpha = 0.05 was used to compare differences between means. Effects of LAR and R/S on RGR were examined with multiple regression analysis. Differences in survivorship among treatment groups were evaluated with logistic regression models in both experiments. Percentage colonization levels were converted by square root of the arcsine to achieve normality and analyzed by ANOVA.

RESULTS

Experiment 1: effect of light, soil-P, and inoculum type

In the first analysis, effects of the standard inocula (S3029 and S060) compared to nonmycorrhizal control were examined at factorial combinations of two light levels and four soil-P levels. Overall, there was no difference in RGR or biomass allocation patterns among three mycorrhizal treatments (S3029, S3060, and control) in any combination of light or soil- P. Thus, inoculum type was dropped from the ANOVA model. Neither soil-P nor light affected RGR, SLA, or NAR. Leaf area ratio and R/S, however, were affected by light and soil-P (Tables 1 and 2). Leaf area ratio was positively correlated with RGR and explained 37% of the variance in RGR ($P < 0.0001$) while R/S was negatively correlated with RGR and explained 15% of the variance ($P < 0.0001$). Leaf area ratio and R/S together explained 39% of the variance in RGR ($P < 0.0001$) in a multiple regression. A total of 27 seedlings of 260 died over the course of the experiment, but survivorship was not affected by treatments.

In the second analysis, the effects of HA inoculum on seedling growth and biomass allocation were compared to the standard inocula and control, at the 5 mg/kg P level (Fig. 1a–d). Relative growth rates of plants with HA inoculum were twice that of the standard inocula and control ($P < 0.0001$), while LAR was 2.5–3× that of the other treatment groups ($P < 0.0001$). For pooled data across treatments, LAR was positively correlated with RGR ($P < 0.0001$) and explained 35%

TABLE 2. Means of leaf area ratio (LAR) and root:shoot ratios (R/S) from all soil-P levels on *Ardisia* seedlings in experiment 1.

Light level	[P]	LAR (cm^2/g)	R/S	RGR ($mg \cdot g^{-1} \cdot d^{-1}$)
Moderate	0	20.5[a]	2.38[a]	5.05
	5	17.5[a]	2.24[a]	5.08
	30	22.5[a]	1.83[a]	6.05
	60	28.9[ab]	1.52[b]	6.62
Low	0	29.1[ab]	1.91[a]	5.77
	5	24.9[a]	2.12[a]	4.75
	30	38.0[b]	1.66[b]	7.02
	60	24.2[ab]	2.08[a]	5.18

Notes: Different letters in the same column signify significant difference in Tukey hsd values (alpha = 0.05). Abbreviations: RGR, relative growth rate; other abbreviations as in Table 1.

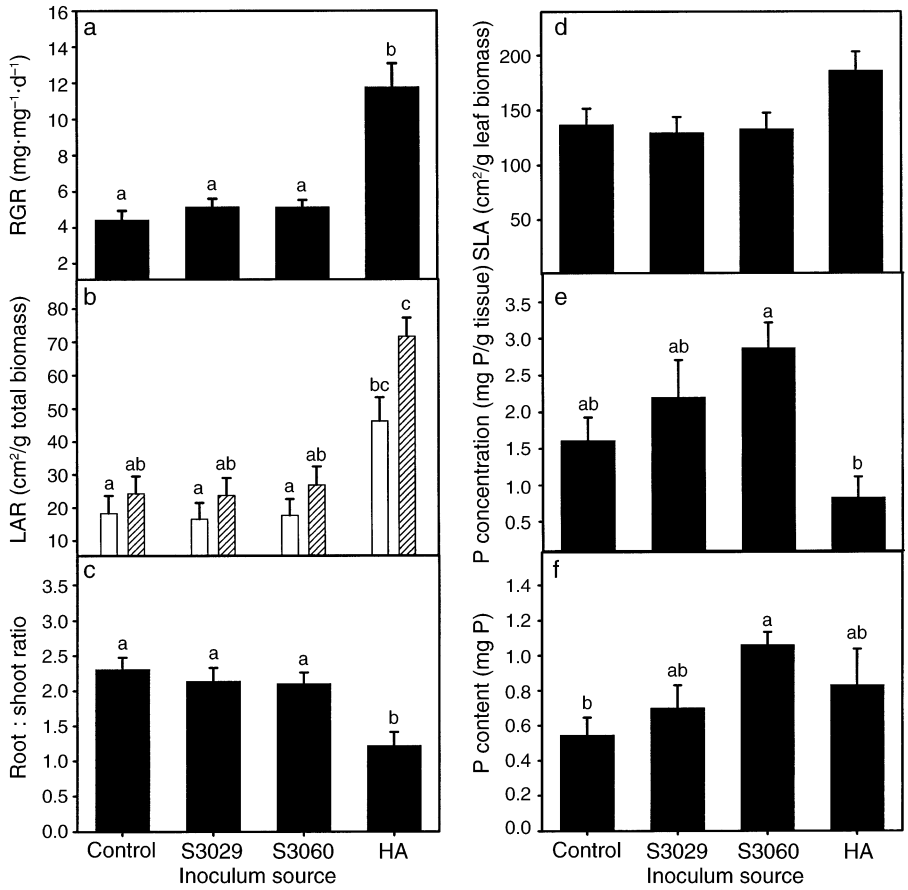

FIG. 1. Response of *Ardisia* to light and inoculum type at 5 mg/kg P. Light treatment is distinguished only when it had a significant effect ($P < 0.05$). (a) Relative growth rate in response to inoculum ($P < 0.0001$). (b) Leaf area ratio in response to light ($P = 0.003$) and inoculum ($P < 0.0001$); open bars, moderate light; hatched bars, low light. (c) Root:shoot ratio in response to inoculum ($P = 0.0006$). (d) Specific leaf area in response to inoculum ($P = 0.06$). (e) Shoot-P concentration (mg P/g tissue) in response to inoculum ($P = 0.005$). (f) Shoot-P content (mg P) in response to inoculum ($P = 0.04$). Error bars represent +1 SE; letters signify difference between Tukey hsd values at alpha = 0.05.

of the variance in RGR. Conversely, R/S of plants inoculated with HA were half that of the other inoculum treatments ($P = 0.0006$). Root:shoot ratio was negatively correlated with RGR ($P < 0.0001$) and explained 23% of the variance in RGR. Together LAR and R/S explained 39% of the variance in RGR ($P < 0.0001$). Leaf area ratio was the only morphological measure affected by light treatment, with plants in low light having higher values than those in moderate light ($P_{light} = 0.003$). Inoculum effects on SLA were nearly significant ($P = 0.06$); plants inoculated with HA had greater SLA (193.1 ± 17.49 g/cm^2 vs. 136.9 ± 14.47 g/cm^2) than controls and other inoculum sources. NAR was not affected by light or inoculum.

Both shoot-P concentration and content differed among treatments ($P = 0.005$ and $P = 0.04$, respectively). Plants inoculated with S3060 had the highest P concentration and content, while HA inoculum had the lowest P concentration and second-highest content (Fig. 1e, f).

Leaf gas exchange data showed similar trends to RGR in relation to inoculum type (Table 3). The maximum photosynthetic rate of plants inoculated with HA were approximately twice that of plants with S3060 and controls under moderate light. Due to the small size of plants and leaves inoculated with S3029 under moderate light, no gas exchange rates are available for this treatment group. Plants inoculated with HA had rates approximately twice that of controls in both light treatments. Dark respiration rates of all inocula were similar to the dark respiration rates of control plants.

Mycorrhizal colonization of plants mirrored the growth and biomass allocation differences among inocula ($P < 0.0001$); plants with HA inoculum were highly colonized (67 ± 7%, mean ± 1 SE) whereas standard isolates (S3029 and S3060) had low colonization rates (17 ± 7% and 7 ± 7%, respectively). Controls were virtually noncolonized (0.8 ± 0.8%). Septate hyphae, presumably saprophytic, were observed externally with many root samples, primarily concentrated

TABLE 3. Comparison of light saturated net photosynthesis rate (A_{max}) and dark respiration under moderate vs. low light treatments (means ± 1 SE) from gas exchange measurements of three individual *Ardisia* seedlings from experiment 1.

Treatment	A_{max}		Dark respiration	
	Moderate	Low	Moderate	Low
Control	2.02 ± 0.55	2.02 ± 0.53	−0.279 ± 0.062	−0.310 ± 0.045
S3029	NA	3.43 ± 1.19	NA	−0.307 ± 0.132
S3060	2.65 ± 0.38	2.19 ± 0.35	−0.382 ± 0.078	−0.253 ± 0.015
HA	4.04 ± 0.28	4.42 ± 0.78	−0.356 ± 0.046	−0.313 ± 0.065

Notes: No data were collected for the moderate light treatment of isolate S3029 due to small size of leaves in this group. NA, not available, HA, host-associated inoculum.

in plants inoculated with S3060 and S3029. Colonization levels were not related to original inoculum potential as determined by the corn assay.

Experiment 2: effects of mycorrhizae on seedling competition

Mycorrhizal colonization was significantly lower in plants treated with benomyl (NM treatment) than AM plants of both species ($P < 0.0001$). Benomyl was more effective in reducing colonization in *Prunus* (AM = 73 ± 7%, NM = 9 ± 10%) than *Ardisia* (AM = 59 ± 1%, NM = 38 ± 6%; Table 4). Competition type had no effect on colonization levels.

Nine of 80 seedlings died over the course of the experiment. Mortality was significantly higher for NM *Prunus* seedlings (7 of 9 dead seedlings, log-likelihood $\chi^2 = 6.57$, $P = 0.038$, $P_{myc} = 0.037$) than AM *Prunus* seedlings.

Relative growth rates differed significantly between treatments ($P < 0.0001$) with *Prunus* having greater RGR than *Ardisia* ($P < 0.0001$, Fig. 2a). *Prunus* and *Ardisia* also responded differently to competition treatment (Table 4, significant species × competition interaction). While *Ardisia* had the highest RGR in heterospecifc competition, the RGR of *Prunus* decreased approximately by half when grown in heterospecific competition without mycorrhizae (Fig. 2a).

Leaf area ratio also differed among treatment groups as *Prunus* and *Ardisia* responded differently to mycorrhizal status ($P_{sp\times myc} = 0.015$, Table 4). Unlike experiment 1, *Ardisia* tended to have higher LAR when nonmycorrhizal in heterospecific competition, whereas *Prunus* had higher LAR with mycorrhizae in both competition types (Fig. 2b). *Ardisia* had a higher R/S than *Prunus* ($P < 0.0001$, Fig. 2c); however, R/S of *Ardisia* decreased under conspecific competition when mycorrhizal ($P_{comp\times myc} = 0.04$). Specific leaf area did not vary significantly among treatments and species ($P = 0.18$, Fig. 2d).

Leaf-P concentration and content differed among species and treatments (Table 5, Fig. 2e, f). *Ardisia* had lower P concentration than *Prunus*, and was not affected by competition or mycorrhizal treatment (Fig. 2e). *Prunus* had its highest P concentration in the NM, heterospecific competition treatment, while the other three treatments did not differ significantly from one another (Fig. 2e). *Ardisia* in the NM, conspecific treatment had a significantly lower total P content than the other three treatment groups (Fig. 2f). *Prunus* had lower P content in NM than AM treatments with no effect from competition (Fig. 2f).

DISCUSSION

Effect of inoculum source on Ardisia

Although all three types of inocula colonized *Ardisia* roots, they had strikingly different colonization levels and effects on biomass allocation and growth of the host. In experiment 1, only the HA inoculum isolated from field-collected *Ardisia* roots, but not the standard

TABLE 4. ANOVA summary of the effects of species, competition, and mycorrhizae on RGR, LAR, R/S, and colonization rates from seedlings in experiment 2 (model $P < 0.05$).

Source	RGR		LAR		R/S		Colonization	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Species (Sp)	29.1	<0.0001	0.129	0.73	62.5	<0.0001	2.03	0.16
Mycorrhizal colonization (Myc)	2.94	0.09	1.16	0.28	2.93	0.09	38.9	<0.0001
Competition (Comp)	3.19	0.08	2.20	0.14	0.059	0.81	NS	NS
Sp × Myc	1.10	0.30	6.31	0.01	NS	NS	12.0	0.001
Myc × Comp	0.481	0.49	NS	NS	4.54	0.04	NS	NS
Sp × Comp	11.9	0.001	3.36	0.07	NS	NS	NS	NS
Sp × Myc × Comp	3.78	0.06	NS	NS	NS	NS	NS	NS

Notes: Degrees of freedom were: RGR, 1,63; LAR, 1,65; R/S, 1,66; colonization, 1,67. NS indicates that the specified effect was not significant ($P > 0.1$) and was dropped from the model.

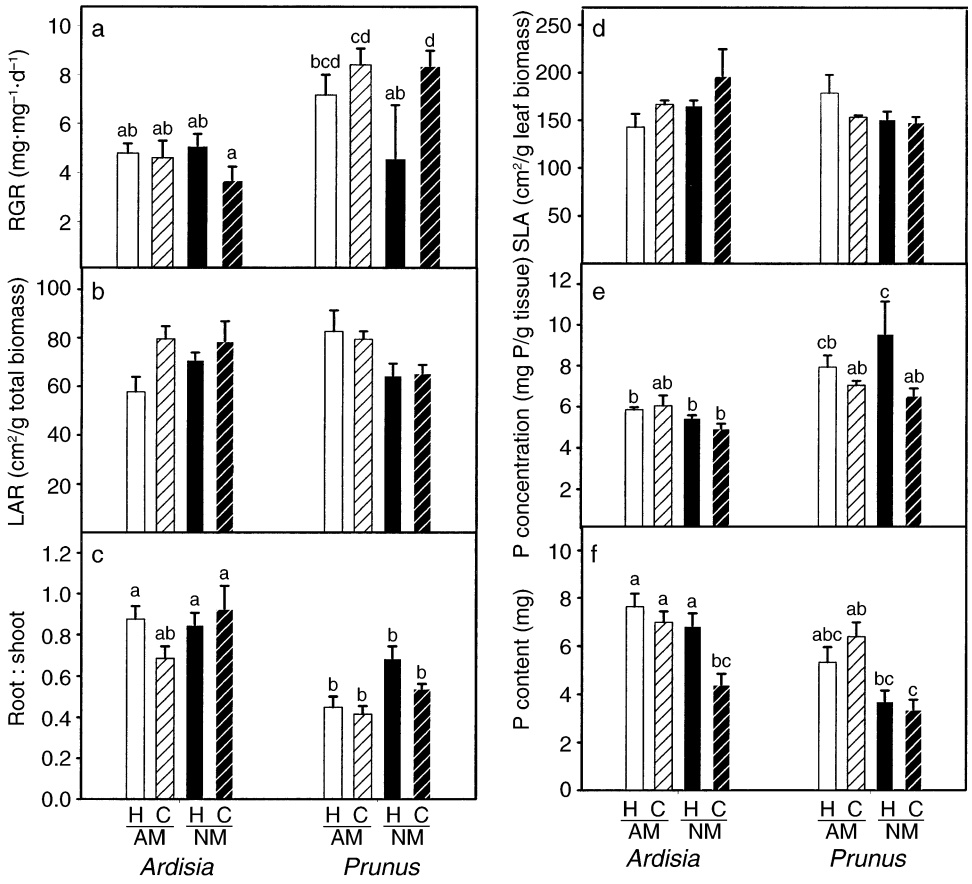

FIG. 2. Response of *Ardisia* and *Prunus* to heterospecific or conspecific competition and mycorrhizal status: (a) relative growth rate ($P < 0.0001$); (b) leaf area ratio ($P = 0.04$); (c) root:shoot ratio ($P < 0.0001$); (d) specific leaf area ($P = 0.18$); (e) leaf-P concentration (mg P/g tissue) ($P < 0.0001$); and (f) leaf-P content (mg P) in response to inoculum source ($P < 0.0001$). Error bars represent +1 SE. Letters signify difference between Tukey hsd values at alpha = 0.05. Abbreviations are: H, heterospecific competition; C, conspecific competition.

inocula, improved seedling RGR over the nonmycorrhizal control. As *Ardisia* had no response to soil-P nor was higher RGR accompanied by an increase in P content or concentration, it appears that the benefit of the HA mycorrhizae was mediated through changes in allocation and physiology rather than improved P nutrition. Plants inoculated with HA inoculum had less relative investment in roots, greater leaf area, and higher A_{max} (net photosynthesis rate) than the control. HA inoculum phenotypically altered LAR of *Ardisia* seedlings from a low value typical for shade-tolerant species to a higher value. LAR is generally thought to be the primary determinant of RGR both across and within species (Poorter and Remkes 1990) and in this study

TABLE 5. ANOVA summary of the effect of species, competition, and mycorrhizae for P concentration and total P content from the competition study.

Source	P concentration (mg P/g tissue)		Total P content (mg P)	
	F	*P*	*F*	*P*
Species (Sp)	43.6	<0.0001	19.1	<0.0001
Mycorrhizae interaction (Myc)	0.086	0.70	24.9	<0.0001
Competition (Comp)	9.6	0.003	2.57	0.11
Sp × Myc	3.81	0.06	NS	NS
Myc × Comp	4.15	0.05	4.51	0.04
Sp × Comp	7.06	0.01	5.27	0.02

Notes: Degrees of freedom were P concentration, 1,64; total P content 1,65. The three-way interaction was dropped due to lack of significance in both tests, as was the species × mycorrhizae interaction in the total P content test.

accounted for 35–37% of the variation in RGR. In a study by Lovelock et al. (1996), shade-tolerant seedlings of *Beilschmiedia pendula* also increased its RGR through an increase in LAR when mycorrhizal and its morphology became more similar to that of more light-demanding plants.

The lack of positive effects on morphology and growth by the standard inocula despite their positive effects on tissue phosphorus concentration was surprising, especially given that they have been shown to increase the biomass of several species, including woody, native plants (Sylvia 1990, Sylvia et al. 1993, 2001). Possibly the costs of the two *Glomus* species were greater than their benefits at the light levels used in this experiment. Mycorrhizal fungi can demand up to 20% of the total C budget of a plant in extreme cases (Peng et al. 1993), and carbon costs can vary widely among fungal genotypes (Graham et al. 1996). The differences between inoculum types may also be mediated by fungal diversity. Isolates S3029 and S3060 represent single-spore cultures, while the HA inoculum was likely composed of multiple fungal species and strains. At least one of these may have been more effective than the two *Glomus* species. Greater numbers of fungal species have been shown to increase the productivity of grass macrocosms (van der Heijden et al. 1998).

In contrast to the strong effect of HA inoculum in experiment 1, suppression of mycorrhizal fungi in experiment 2 had no effect on RGR of *Ardisia* seedlings, even though colonization rates were reduced from 59% to 38%. These seedlings were collected from a dense *Ardisia* population in the field and were presumably colonized with mycorrhizae similar to HA. The magnitude of reduction of mycorrhizal colonization (33% reduction) in this study was comparable to the reductions in many other studies, (Moora and Zobel 1996, Smith et al. 1999, Kahiluoto et al. 2000). However, it has been suggested that the relationship between mycorrhizal colonization and plant benefit is curvilinear with benefit to the plant eventually reaching a plateau at some colonization level (Gange and Ayres 1999). *Ardisia* may have reached its maximal benefit at or before a colonization rate of 38%, as found in the benomyl treatments.

That *Ardisia* has a differential response to different inoculum types may have important implications for its invasive ability. In heavily invaded areas, *Ardisia* is already associating with effective mycorrhizal fungi that alter its morphology and physiology to that of faster growing plants. The main mode of resource competition by *Ardisia* is through casting dense shade to its neighbors. Increased LAR, enabled by the reduction in R/S due to mycorrhizae, must enhance *Ardisia*'s competitiveness for light in the forest understory.

Competitive interactions

Ardisia seedlings grew better in heterospecific competition with *Prunus* seedlings than in conspecific competition. Conversely, *Prunus* seedlings had lower survival and growth with *Ardisia* seedlings than with conspecific seedlings, especially in nonmycorrhizal treatment (Fig. 2a). The architecture of *Ardisia* results in a higher amount of self- and neighbor-shading than that of *Prunus* (K. Kitajima, *unpublished data*). Hence, each *Ardisia* seedling is more shaded by a conspecific neighbor than a heterospecific neighbor. In conspecific competition, *Ardisia* responded with greater phenotypic plasticity of increasing LAR than in competition with less shade-casting *Prunus*.

Prunus seedling growth and survival was reduced to a greater extent by heterospecific competition in nonmycorrhizal treatment than in the mycorrhizal treatment. The presence of HA and S3060 inocula lessened the negative effect of interspecific competition on *Prunus*, as is often observed in more mycorrhiza-dependent species (Hartnett et al. 1993, Moora and Zobel 1996, Smith et al. 1999). More mycorrhiza-dependent species often have a low total investment in roots (Jakobsen 1991). *Prunus* had overall higher RGR than *Ardisia*, and this difference was associated with inherently higher LAR and lower R/S of *Prunus* (Fig. 2a–c). Mycorrhizae apparently allowed *Prunus* seedlings to invest less in roots and more to leaf area, and enabled them to compete more effectively with *Ardisia* seedlings for light. This finding of an apparent greater AM dependency by a faster growing species in a competitive regime is interesting because often the opposite trend has been found in the absence of heterospecific competitors (e.g., Janos 1980, Zangaro et al. 2000).

Different plant species in the same community have been shown to support different mycorrhizal communities in their rhizosphere (Bever 1994) and cause differential rates of sporulation (Bever et al. 1996). A high percentage of *Ardisia* roots are colonized by AM fungi in the field (S. R. Bray, *unpublished data*), likely dominated by preferred mycorrhizal fungi. The field-collected *Ardisia* seedlings in experiment 2 had been colonized by mycorrhizal fungi, some of which remained after the benomyl treatment. These fungi colonized *Prunus* seedlings at a low level (9%), but they did not benefit growth of *Prunus* seedlings in the heterospecific competition treatment. The community composition of mycorrhizal fungi may be highly modified in the dense clump of *Ardisia* in the invaded forest. If *Ardisia* does in fact alter the composition of AM, it may be even more difficult for *Prunus* and other native understory plants to compete with *Ardisia*.

Implications of effects of mycorrhizae on exotic species invasion

As a new colonist in Florida, *Ardisia* is apparently not limited by the lack of potential mutualists and in fact benefits from the local mycorrhizal fungi. Unlike typical weeds, *Ardisia* is highly shade tolerant and has low RGR. Many other exotic species that have a higher RGR than *Ardisia* have been shown to have a negative

or no response to mycorrhizae when they are grown alone (Marler et al. 1999, Richardson et al. 2000, Philip et al. 2001). This appears to be the first study to document a positive response of a slow-growing exotic to native mycorrhizae. It is likely, however, that further study will show that there is no link between life history and mycorrhizal dependency in exotic species as has been found in the mycorrhizal literature as a whole (Janos 1980, Allsopp and Stock 1992, Smith and Smith 1996, Zangaro et al. 2000). Until a greater predictive framework for mycorrhizal response is developed, invasive plant response must be examined on a species-by-species basis.

The results of our study suggest that it is difficult to predict how competitive interactions between exotic and native plants are modified by mycorrhizae. The exotic plant's response in isolation does not necessarily predict its response to mycorrhizae in a competitive environment. Another study of competition between an exotic forb and native grass found that neither species' biomass was altered by mycorrhizae when grown in isolation, but when grown in a competitive environment, mycorrhizae increased the growth of the exotic plant to the detriment of the native (Marler et al. 1999). Our results also suggest that the type of mycorrhizal inoculum must be considered in evaluation of mycorrhizal effects on competitive interaction between native and exotic plants. The results would be different whether the fungal inoculum is the one preferred by the native or the exotic. Studies should be designed considering both the plants and the mycorrhizae of the ecosystem invaded by exotic plant species.

The role of AM in mediating plant invasions and competitive interactions needs to be examined carefully. The response of exotic plants to mycorrhizae is highly variable depending on genotype interactions both in isolation and in competitive environments. Understanding how native and exotic plants respond to the local microbial community will be important for understanding the mechanisms and impacts of community invasion. Similarly, it is also imperative that we determine how exotic species potentially alter the microbial community and its ecosystem functions.

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