THE EFFECT OF MYCORRHIZAL INOCULUM 
ON THE GROWTH OF FIVE NATIVE TREE SPECIES AND 
THE INVASIVE CHINESE TALLOW TREE (SAPIUM SEBIFERUM)

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Abstract.—Mycorrhizal fungi may play an important role in plant invasions, but few studies have tested this possibility. Chinese Tallow (Sapium sebiferum) is an invasive tree in the southeastern United States. An experiment was conducted to examine the effects of mycorrhizal inoculation, fungicide application, and fertilization on the growth of Sapium and five native tree species (Liquidambar styraciflua, Nyssa sylvatica, Pinus taeda, Quercus alba, and Q. nigra) that co-occur in forests in the Big Thicket National Preserve in east Texas. Seedlings were grown in a greenhouse for twenty weeks under full factorial combinations of mycorrhizal inoculum, fungicide, and fertilizer. Mycorrhizal inoculation increased Sapium growth but caused zero to negative growth changes of the five native species. This suggests that Sapium may gain unusual benefits from mycorrhizal associations. Liquidambar styraciflua benefitted from mycorrhizal inoculation only in fertilized conditions which indicates that the potential advantage Sapium might gain from mycorrhizal associations may vary with native species and soil fertility.

Mycorrhizal fungi form close associations with roots of plants in which in exchange for fixed carbon, the fungi provide essential nutrients to the plant (N, P) and may protect the plant from pathogens, support helpful bacteria, enhance soil aggregation, assist in water transport and gain, and stimulate plant growth through auxin production; these associations can vary from mutualistic to parasitic depending on soil fertility levels (Harley 1968; Allen 1991; Johnson et al. 1997; Smith & Read 1997; Van der Heijden & Sanders 2002). It is possible that mycorrhizae play a key role in temperate forest dynamics and community responses by changing the outcome of competition and by influencing plant fitness (Johnson et al. 1997; Van der Heijden & Sanders 2002). Little attention has focused on how the existing mycorrhizal network of the introduced range may facilitate the invasion of exotic plant species.

Sapium sebiferum (L.) Roxb, a native to central China, was introduced to Georgia in the late 18th century (Bruce et al. 1997). Although present in Texas in the early 1900’s, Sapium did not become invasive until the middle of the century and has only rapidly increased abundance in the past two decades in mesic and hydric forests in the Big Thicket National Preserve (BTNP) in east Texas (Harcombe et al. 1999). Re-
cent studies have shown that Sapium benefits from low herbivore loads (Siemann & Rogers 2001; 2003ab; Rogers & Siemann 2002; 2003), but Sapium appears to have unusually high growth rates even after accounting for differences in aboveground herbivore impacts. Although a release from belowground pathogens could explain the high growth rates of Sapium, unusually large benefits from mycorrhizal associations are also a factor that may contribute to Sapium’s invasive success.

Generalist mycorrhizae with low host specificity may be able to form associations with invasive plants (Richardson et al. 2000). This association by itself would not create unusually high benefits, and thus could not be itself responsible for invasive success, unless the invader could utilize the mycorrhizae in a novel fashion (Richardson et al. 2000). The combination of potentially novel mycorrhizal utilization and the short co-evolutionary history exotic plants have with native mycorrhizal mutualists suggests that these plants could receive unusually high benefits or extremely high costs their introduced ranges (Richardson et al. 2000). Another way that exotic invaders could obtain benefits would be to usurp native species’ existing mycorrhizal network connections, or utilize neighbors’ nutrient pools with their own extraradical (soil exploring) hyphae, thus parasitizing neighboring competitors through enhanced nutrient uptake (Marler et al. 1999; Zabinski et al. 2002).

Only limited work to date has been done to examine how the existing mycorrhizal network of the introduced range may influence the competitive ability of exotic invaders (Bray et al. 2003). Understanding how Sapium utilizes mycorrhizal associations in its introduced range may help explain the mechanisms underlying its invasion in the BTNP and increase general knowledge of the role of mycorrhizae in affecting plant community dynamics.

A greenhouse experiment was conducted to test the effects of mycorrhizal inoculation, fungicide application, and fertilization on the growth of Sapium and five tree species native to the BTNP. If mycorrhizae contribute to Sapium invasion, then the performance advantage of Sapium compared to natives should be greater with mycorrhizal inoculation than without. To potentially decrease the performance advantage of Sapium if mycorrhizal inoculation facilitates invasion, Rovral fungicide was applied (Gange et al. 1990; Ganade & Brown 1997). Fertilization is predicted to highlight plant alterations in mycorrhizal dependencies and mimic potential changes in field conditions. Fertilization is predicted to decrease the effect of mycorrhizae on plant performance...
because carbon costs are not offset by benefits of nutrient gathering in high fertility (Menge et al. 1978; Bualala & Goh 1981; Hetrick et al. 1988; Hetrick 1991; Johnson 1993; Peng et al. 1993) and additionally because the benefits plants receive from mycorrhizae may be less valuable in higher fertility conditions (Koide 1991; Johnson 1993; Johnson et al. 1997). In Flatland Hardwood Pine Forests of the Lance Rosier Unit in the Big Thicket, which are equivalent to Lower Slope Hardwood Forests found elsewhere, phosphorus tends to be in limited supply (Marks & Harcombe 1981; Knox et al. 1995; BTNP 2003) because of its difficulty to acquire at low levels and strong adsorption to soil particles (Nye & Tinker 1977; Read 1991). However, nitrogen deficiencies may limit growth of plants with non-mycorrhizal affiliations because they can only absorb simple forms of N (Chalot & Brun 1998). Together these predictions will begin to answer how mycorrhizae may promote or hinder Sapium’s invasibility and ultimately alter the surrounding native community.

METHODS

Seeds of five native tree species that are common in mesic and hydric forests in the BTNP and may potentially be outcompeted by Sapium sebiferum (Liquidambar styraciflua L. [sweetgum], Nyssa sylvatica Marsh [blackgum], Pinus taeda L. [loblolly pine], Quercus alba L. [white oak], and Q. nigra L. [water oak]) were purchased (Louisiana Forest Seed Company, Lecompte, LA) to ensure that seeds were from uniformly healthy trees. Sapium sebiferum seeds were collected at Armand Bayou Nature Preserve (Houston, TX). Stratification took place in a 21°C cold-room in January-February 2003. Germination of non-surface sterilized seeds occurred in an unheated greenhouse on the Rice University campus during March-May 2003. Germinated seeds were planted in 66 mL Conetainers (Stuewe & Sons, Inc., Corvallis, OR) filled with potting soil.

Forty-eight similarly sized seedlings of each species were selected approximately two weeks after germination. All of the plants within each species were randomly assigned to one of eight treatments in a full-factorial experimental design with inoculation (yes or no), fungicide (yes or no) and fertilizer (yes or no) for a total of six replicates per treatment. Roots were gently brushed free of soil and the soil was retained. Roots were then dipped in either “Silva Dip” (Reforestation Technologies International, Salinas, CA) which contained a total of eight general endo- and ectomycorrhizal species (Glomus intraradices, Glomus
aggregatum, Glomus mossae, Pisolithus tinctorius, and four species of Rhizopogon sp.) or distilled water. Excluding Rhizopogon sp., which is primarily found in the northwestern United States, at least one of the remainder of the endo- and ectomycorrhizal species listed would be encountered by the focal tree species of this study in the field (Keeley 1980; Black et al. 1981; McIntosh et al. 1985; Weber & Smith 1985; Walker & McLaughlin 1991; Metzler & Metzler 1992; Lewis & Strain 1996; Constable et al. 2001). After dipping, roots were covered with the retained soil and transplanted into 3.8 liter Treepots™ (Stuewe & Sons, Inc.) filled with a mixture of 2/3 potting soil and 1/3 perlite. Pots were placed within blocks grouped by species on plastic pallets on the greenhouse floor because of differences in germination times. Pots were watered as needed and periodically rotated within species blocks to minimize shading and location effects.

Fertilizer was applied four times in the course of the 20-week experiment in weeks 3, 7, 12, and 17. Application rates were equivalent to 4 g/m² each of N, P and K per application. This mimics field regulation standard rates. Nutrients were added as ammonium nitrate (N), superphosphate (P), and potash (K) dissolved in 40 mL of distilled water. Distilled water was added to non-fertilized controls.

Rovral® 4 Flowable Fungicide (Aventis CS, Bridgewater, NJ) was applied three times in the course of the 20-week experiment in weeks 4, 10, and 16. Rovral, active ingredient iprodione, has been shown to reduce mycorrhizal infection in plant roots and is a contact pesticide with no known systemic action (Gange et al. 1990; Ganade & Brown 1997). Application rates followed recommendations for controlling pathogenic root fungi (Aventis 2001).

Initial height of each seedling was measured. Initial heights were taken before seedlings were dipped into either inoculum or a distilled water control and as such did not require sterilization of equipment to pre-empt transfer of inoculum between sources. At the end of 20 weeks, roots, leaves, and stems were harvested and dried at 60°C for at least 72 hours before weighing.

An ANCOVA with starting height as a covariate was used to test whether final mass (log transformed to achieve normality) depended on experimental treatments in a model with all possible interactions among experimental treatments (SAS 8.2, SAS Institute, Cary, NC). Mass data were back transformed for graphical presentation. Single species ANOVAs were used to investigate significant interaction terms in the full
analysis and Fisher’s Least Significant Difference Test was used to test for differences between treatment means (Stat View 5.0, SAS Institute, Cary, North Carolina).

RESULTS

The percent of root mass was independent of all factors other than species ($F_{5.238}=119.80, P<.0001$). It was lowest for *Pinus* (29%) followed by *Liquidambar* (40%), *Q. nigra* (46%), *Sapium* (47%), *Nyssa* (53%) and *Q. alba* (73%). The contrasts among species were significant at $\alpha=0.05$ for all pairs of species except *Q. nigra* vs. *Sapium*. Because allocation patterns are independent of treatments (modeled as a percentage of belowground root biomass) and species is the only significant factor explaining the allocation pattern variance, the remainder of the analyses utilized total mass as the dependent variable.

Total mass varied among species (Table 1, Fig. 1) and the contrasts among species were significant at $\alpha=0.05$ for all pairs of species except *Q. alba* vs. *Nyssa* and *Liquidambar* vs. *Q. nigra*. No other main effect significantly affected mass in the ANCOVA (Table 1). Total mass depended on starting height in the ANCOVA (Table 1). The species which had significant correlations between starting height and log (final mass) in z-tests were *Q. alba* ($r=0.59, P<0.0001$) and *Nyssa* ($r=0.41, P<0.001$). Variation in mass depended on several interactions: species/inoculation, species/fertilization, species/inoculation/fertilization, and species/inoculation/fungicide. Since each interaction term had species as one of the factors, individual species ANOVAs were used to help identify the main factors influencing the interactions.

The significant effect of species/inoculation in the full model indicated that species differed in the direction or magnitude of their responses to inoculation. All five native species tended to have lower mass when inoculated but this difference was significant only for *Nyssa* ($P<0.01$) in single species ANOVAs. *Sapium* had significantly higher mass when inoculated ($P<0.01$). In a separate analysis with a two-level predictor that indicated whether a species was native vs. *Sapium*, the interaction of this term and inoculation was significant ($P<0.05$).

The significant effect of species/fertilization in the full model indicated that species differed in their responses to fertilization. In single species ANOVAs, *Pinus* ($P<0.01$) and *Liquidambar* ($P<0.05$),
Table 1. The dependence of log(final mass) on experimental treatments in an ANCOVA with starting height as a covariate. Significant terms are noted with an asterisk (*).

<table>
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<tr>
<th>Factor</th>
<th>df</th>
<th>SS</th>
<th>F-Value</th>
<th>P-Value</th>
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<td>48.2</td>
<td>157.7</td>
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<td>0.20</td>
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<td>Fungicide</td>
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<td>0.70</td>
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<td>0.35</td>
</tr>
<tr>
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<td>0.8</td>
<td>2.6</td>
<td>&lt; 0.05</td>
</tr>
<tr>
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but no other species, were significantly larger when fertilized (Fig. 1). *Pinus* had larger mass in fertilized controls and maintained this increase when inoculated. However, *Liquidambar*’s growth had significant mass increases with inoculation in the fertilized treatments only.

Single species ANOVAs show that the significant interaction of species/fertilization/inoculation in the full model was related to the idiosyncratic effect of these treatments on *Liquidambar* mass ($P < 0.01$, Fig. 1). Inoculation reduced *Liquidambar* mass in low fertility conditions but increased it in high fertility conditions.

The significant effect of species/inoculation/fungicide largely reflected the distinct responses of *Sapium* to fertilizer and fungicide since the interaction of these treatments was only significant for *Sapium* ($P < 0.01$) in single species ANOVAs. Submodels showed fungicide-non-inoculated plants to be significantly different from fungicide-inoculated plants and control (non-fungicided, non-inoculated) plants to be significantly different from fungicide-inoculated plants by Fisher’s Least Significant Difference Test, respectively ($P < 0.01$, $P < 0.05$). Specifically, *Sapium* mass was lowest in the fungicide only treatment (average = 7.8 g) followed by control (non-inoculated and non-fungicided), (15.1 g), inoculation only (15.1 g), and finally the combination of inoculation and fungicide (20.9 g).
Figure 1. The dependence of mass (g) of *Liquidambar styraciflua*, *Nyssa sylvatica*, *Pinus taeda*, *Quercus alba*, *Quercus nigra*, and *Sapium sebiferum* seedlings on fertilization (con = no fertilizer, fert = fertilized) and mycorrhizal inoculation after 20 weeks. Fungicide treatments are not shown. See Table 1 for statistical results.

**DISCUSSION**

*Sapium*'s striking positive growth response to mycorrhizal inoculation (65% increase) differed markedly from the neutral to negative responses of native tree species (Fig. 1). The magnitude of reductions in growth of the five native tree species in response to inoculation ranged from negligible (*Q. alba* = 1% reduction, *Q. nigra* = 6%) or minor (*Pinus = 17%, *Liquidambar* = 24%) to large and significant (*Nyssa* = 46%) but the direction of the response to inoculation was always negative. *Sapium* was clearly able to gain large benefit from mycorrhizal associations with a generalist mycorrhizal inoculum in conditions where natives could not. It appears that natives were unable to benefit from the generalist inoculum in this study suggesting that mycorrhizal specificity is important (Bever 2002; Klironomos 2003). The strains used in this study may not be beneficial in these conditions and may create an unnecessary obligate symbiosis with direct translations to decreases in growth (Hetrick et al. 1988; Hetrick 1991). This supports the hypothesis that unusual relationships between the exotic *Sapium* and North American mycorrhizae species, such as those in the inoculum, may contribute to *Sapium*'s success as an invader.
There are a number of explanations for why lack of specialist mycorrhizae (Bever 2002, Klironomos 2003), which was predicted to be beneficial, appeared to be especially detrimental in fertilized treatments for *Nyssa*, *Pinus*, *Q. nigra* and for the native species *Liquidambar* and *Nyssa* in unfertilized treatments in this experiment. First, carbon drain on host plants, which is well documented (Buwalda & Goh 1981; Hetrick 1991; Johnson 1993; Peng et al. 1993; Graham et al. 1996) may have exceeded the benefits of increased nutrients and/or water in these relatively fertile, well-watered greenhouse conditions. Second, mycorrhizae in this experiment may have used carbon from plants largely for respiration rather than increasing extraradical hyphae surface area and increasing nutrient absorption (Peng et al. 1993; Graham et al. 1996). Increases in maintenance respiration, as well as higher root construction costs due to high lipid vesicle allocation, has been shown in P addition experiments for *Citrus volkameriana* (Peng et al. 1993, Graham et al. 1996) and has been attributed to decreases in carbohydrate root exudates from plants in highly fertilized soils (Johnson et al. 1997).

The unexpected results for fungicide and inoculation combinations, in particular the effects on *Sapium* mass, were inconsistent with the expectation that seedlings in the two treatments, non-inoculated fungicide only and inoculation plus fungicide, would be identical in size. This suggests that fungicide applications were not an effective method of fungal control. One possible explanation is that non-spore ingredients in the mycorrhizal inoculum had phytotoxic effects on seedling growth in the presence of fungicide. The reduction of *Sapium* mass by fungicide application (without inoculum) might indicate that beneficial microbes (phosphate-solubilizing microbes and plant growth-promoting bacteria) were present in the potting soil which were killed by the fungicide (Allen 1992). Alternatively, it might indicate direct toxic effects of fungicide on *Sapium*. The recovery of *Sapium* growth with inoculation in fungicide treatments suggests that the mycorrhizal inoculum was not effectively suppressed and that mycorrhizae may be acting synergistically with microbes in the fungicided soil that were not effective or prevalent in the non-fungicided soil. One goal of this greenhouse experiment was to develop methods that could be applied in field experiments. Further work with direct assays of mycorrhizal and non-mycorrhizal fungi in experiments with *Sapium* is needed to complete the identification of reliable field methods and identify the cause of the seemingly anomalous inoculation and fungicide result.
The prediction of decreased response of all species to mycorrhizal inoculation in high fertility environments was based on the assumption that mycorrhizal carbon costs are not offset by the benefits of nutrient gathering in conditions in which nutrients are abundant (Menge et al. 1978; Buwalda & Goh 1981; Hetrick et al. 1988; Hetrick 1991; Johnson 1993; Peng et al. 1993). The positive response of Liquidambar to mycorrhizal inoculation only in fertilized conditions was opposite the prediction that the benefit of mycorrhizal associations would be lower in more fertile conditions (Fig. 1). Indeed, the reverse pattern observed here suggests that there may be potential for strong competition for nutrients between mycorrhizae or other soil microbes and plants in low fertility environments that may counteract the potential benefit of mycorrhizal associations in these conditions (Bardgett et al. 2003).

The strong benefit of mycorrhizal inoculation for Liquidambar in some conditions (Figure 1) indicates that the competitive advantage Sapium might gain from mycorrhizal associations may vary with native species and soil fertility (Marler et al. 1999).

One theory explaining the success of invaders in their introduced range is the Enemy Release Hypothesis. It predicts that invasives experience a release from the pressures of the natural enemies in their native range and can therefore allocate additional resources to growth and reproduction (Alpert et al. 2000; Maron & Vila 2001; Keane & Crawley 2002; Mitchell & Power 2003). However, little attention has been given to belowground enemies. This experiment raises the possibility that the large size of Sapium in all conditions, although doing better with inoculum than natives, (Figure 1) reflects presence of belowground pathogenic fungi that more readily attack native tree species.

The results reported here would be more compelling with confirmation of mycorrhizal colonization and dependence by direct examination. Further, it is imperative that these results be verified in field trials as well as in experiments including competitive interactions between species. Such experiments are currently underway to rigorously test the preliminary conclusion presented here that interactions with soil microbes play a role in Sapium invasions in east Texas forests.

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NIJER, ROGERS & SIEMANN

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