Effects of Arbuscular Mycorrhizal Fungi on the Exotic Invasive Vine Pale Swallow-Wort (*Vincetoxicum rossicum*)

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The ability of arbuscular mycorrhizal fungi (AMF) to influence the performance of nonnative invasive plants in their introduced range has received increasing attention. The dependence of the invasive nonnative vine pale swallow-wort on AMF was studied in three greenhouse experiments. The aims of the present work were to (1) determine AMF colonization levels of field-collected pale swallow-wort plants and several co-occurring native and nonnative plant species, (2) evaluate the growth response of pale swallow-wort to different components of the soil microbial community from an infested site, and (3) determine the growth response of pale swallow-wort when grown with a nonlocal AMF species. AMF root colonization was greater in pale swallow-wort (85, 98, and 50% arbuscules, hyphae, and vesicles, respectively) than in leek (72, 80, and 25%), a species that has been frequently used as a predictor of AMF density in soil. Root colonization of pale swallow-wort in the field was also greater than root colonization of common milkweed, a native herbaceous species often co-occurring in the same habitats, as well as two other herbaceous species, Canada goldenrod and blueweed. Survival of pale swallow-wort plants was significantly greater in soil collected underneath dense monospecific stands of pale swallow-wort in a Henderson Harbor, NY, field site than in sterilized soil. After 12 wk, plants grown in sterilized soil had a 33% survival rate, whereas all plants grown in the unamended soil, with an intact microbial community, were alive. Moreover, plants grown in the unamended soil were 130% taller, had 50% more leaves, and had 83% greater total biomass compared with plants grown in sterile soil. Plants grown in soil containing a *Glomus intraradices* isolate collected in Troy, AL, were 50% shorter and had 15% lower total biomass than plants grown in the unamended New York field soil. These pale swallow-wort seedlings also had a high mycorrhizal dependency of 93%. Plants grown in a sterilized soil that was reamended with an AMF-free microbial wash had significantly lower belowground and total biomass than plants grown in the unamended soil with the resident AMF community. There was a trend of decreasing height and biomass for plants grown in sterile soil relative to the unamended controls treatment. Plants grown in sterilized soil had significantly (28%) greater total biomass than plants reamended with the AMF-free microbial wash. These findings suggest that AMF occurring in invaded habitats have beneficial effects on pale swallow-wort survival and growth.

Nomenclature: Common milkweed, *Asclepias syriaca* L.; leek, *Allium ampeloprasum* L.; blueweed, *Echium vulgare* L.; Canada goldenrod, *Solidago canadensis* L.; pale swallow-wort, *Vincetoxicum rossicum* (Kleopow) Barbar.

Key words: *Cynanchum rossicum*, community composition, mycorrhizal dependence, nonnative, plant diversity.

Invasive species pose one of the greatest threats to native plant populations and diversity, second only to habitat loss (Wilcove et al. 1998). Determining factors that allow nonnative invasive plants to establish and develop high densities in new undisturbed habitats when populations in their native ranges occur at relatively low densities has been the focus of much recent research (Bell 2001; Blossey and Nötzold 1995; Davis et al. 2000; Goodwin et al. 1999; Hallett 2006; Levine et al. 2003; Lockwood et al. 2001; Mack et al. 2000; Richardson and Pyšek 2006). It is commonly recognized that higher plants are host to numerous species of AMF (Brundrett 1991; Sanders 1999). Although AMF are obligately dependant on plant hosts for photosynthetic carbon, plants may benefit from

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Interpretive Summary

Invasion by the introduced herbaceous perennial vine pale swallow-wort has resulted in a disconcerting transformation of the native plant and animal community over thousands of hectares in the Great Lakes Basin region of North America. This is home to the rare and unique Alvar habitats, very fragile ecosystems that occur on shallow soils overlying limestone or dolomite bedrock. Pale swallow-wort has colonized more than 800 ha in the Henderson Shores area of New York State alone and populations are expanding at an alarming rate. The production of large quantities of wind-dispersed seeds that have the ability to produce more than one seedling (polyembryony), lack of herbivores and pathogens, and ineffective management strategies to date have made the control of pale swallow-wort especially challenging. In an attempt to identify and/or develop more effective methods of control, additional information on the survival and growth of this species in colonized areas is needed as well as information on the possible role that soil arbuscular mycorrhizal fungi (AMF) play in the invasion process.

The potential key role of AMF in plant invasions has only recently been recognized. Our study demonstrated that field-collected plants of pale swallow-wort readily established associations with native AMF, indicating an active symbiosis and the potential for this plant to receive benefits from this association, including increased nutrient uptake, enhanced water relations, and possibly protection from pathogens. Although additional research is required to determine in more detail how this symbiotic association directly benefits these two partners, it is clear from our study that pale swallow-wort survival and growth are enhanced in the presence of resident soil AMF and that this feature may contribute to its invasive success. A better understanding of how specific AMF species interact with pale swallow-wort and native plants in the Great Lakes Basin region may facilitate the development of more effective control strategies and more successful restoration of invaded sites.

this association by enhanced uptake of immobile nutrients such as phosphorus and zinc, especially under limiting nutrient conditions (Howeler et al. 1982). Despite these potential benefits, the role that AMF play in nonnative plant species invasions has been largely overlooked (Fitter 2005; Wolfe and Klironomos 2005).

The invasive nonnative vine pale swallow-wort [*Vincetoxicum rossicum* (Kleopow) Barbar; dog-strangling vine] has become increasingly problematic in the Lower Great Lakes Basin of the northeastern United States and Ontario, Canada, since its introduction to North America from Ukraine and southwestern Russia in the late 1800s (Sheeley and Raynal 1996). This herbaceous perennial is well adapted to extremely shallow soils and is tolerant of a wide range of soil and environmental conditions (Sheeley 1992). Pale swallow-wort is increasingly problematic in mixed deciduous forest understories, Christmas-tree plantations, pastures, and possibly in no-till maize (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] cropping systems (Christensen 1998; DiTommaso et al. 2005). In some heavily infested sites of northern New York State, densities of nearly 5,000 seedlings/m^2 are common (Smith et al. 2006). Seedling survival and successful transition into reproductively mature plants in pale swallow-wort are remarkably high, particularly when compared with other co-occurring herbaceous plants (Ladd and Cappuccino 2005; Turnbull et al. 2000).

Pale swallow-wort plants can form associations with AMF in its introduced range and it has been suggested that pale swallow-wort colonization of new habitats is associated with a change in the composition of AMF communities in affected soils (Greipsson and DiTommaso 2006). These results are consistent with other studies showing that nonnative plant invasions can influence the soil microbial community (Hawkes et al. 2006; Levine et al. 2003; Stinson et al. 2006). Association of pale swallow-wort plants with AMF in its introduced range may be an important contributing factor to its rapid population expansion and invasiveness in the Lower Great Lakes region of North America over the past two decades. Lata et al. (2003) demonstrated that inoculation with different AMF species increased the growth rate of pale-purple coneflower (*Echinacea pallida* Nutt.) seedlings and increased seedling survival by 58 to 92% depending on the species of AMF used as inoculum. The development of a common mycorrhizal network by dense vegetation may also benefit seedling recruitment by facilitating the integration of the seedling root system into the mycorrhizal network and enhancing AMF effects on seedling nutrition (Francis and Read 1995; Zobel et al. 1997). Although quantification of AMF root colonization is not a measure of AMF function (McGonigle 1988; Smith et al. 2004), the ability of pale swallow-wort roots to exhibit high levels of AMF colonization may contribute to its invasiveness, especially because AMF can influence competitive outcomes between native and nonnative plants (Pedersen and Sylvia 1996; Reinhart and Callaway 2004; West 1996). The ability of invasive plants to 'cultivate' these beneficial relationships and outcompete native plants can lead to instability and decreases in native populations and points to the key role played by AMF in structuring plant communities.

The aims of the present work were to (1) determine AMF colonization levels of field-collected pale swallow-wort plants and several co-occurring native and nonnative plant species, (2) evaluate the growth response of pale swallow-wort to different components of the soil microbial community from an infested site, and (3) determine the growth response of pale swallow-wort when grown with a nonlocal AMF species.

Materials and Methods

Determining Root Colonization of Bait Plants. In early July 2003, approximately 2 kg (4.4 lb) of soil was removed from the top 5 to 20 cm (2 to 8 in) of soil in the 0.5-m (1.6 ft) boundary region surrounding each of 36 field plots. These 36 plots were part of a parallel field study that was initiated to determine the growth and reproductive potential of pale swallow-wort in a highly infested site (Smith et al. 2006). This 2-yr fully replicated study was established in May 2003 at Henderson Harbor, NY (43°51′N, 76°14′W), on a 5.7-ha (14 ac) field site dominated by pale swallow-wort (95% of ground cover) for at least 5 yr. Other plant species present included catchweed bedstraw (*Galium aparine* L.), common milkweed (*Asclepias syriaca* L.), garlic mustard [*Alliaria petiolata* (Bieb.) Cavara and Grande], blueweed (*Echium vulgare* L.) sulphur cinquefoil (*Potentilla recta* L.), *Solidago* spp., and *Carduus* spp. Tree species within the field and wooded areas included eastern red cedar (*Juniperus virginiana* L.), black locust (*Robinia pseudoacacia* L.), *Fraxinus* spp., and American elm (*Ulmus americana* L.). Soils at the site are classified as a Benson-Galoo complex (loamy-skeletal, mixed, active, mesic Lithic Eutrudepts; loamy, mixed, nonacid, mesic Lithic Udorthents) occluding Galway silt loam (coarse-loamy, mixed, superactive, mesic Typic Eutrudepts) with an organic matter content of 13.0% and pH 6.7. No treatments were applied in this field study. Soil collected from these plots was placed into a 10-cm-diam pot. Care was taken to minimize disturbance of the soil samples in order to preserve established mycorrhizal networks. In each pot, a single leek (*Allium ampeloprasum* L.) plant was grown from seed to serve as a bait plant to assess the colonization potential of AMF in the soil. Leek plants are considered excellent hosts for a broad range of AMF species (Brundrett et al. 1996) and also provide resistance against several pathogenic nematodes. Plants were grown in the greenhouse under a 14-h photoperiod and temperature range of 21–31 C (70–88 F). Plants were watered twice a day and were not fertilized during the study period. After 4 wk, roots were harvested from all pots and washed of soil, cleared with 10% KOH at 90 C and 2.5% HCl at room temperature, and stained with 0.05% trypan blue at 90 C. Roots were then cut into 1-cm segments and viewed using bright field microscopy (Phillips and Hayman 1970). The root-piece method was used to determine the percentage of root length colonized by AMF structures (Johnson-Green et al. 1995).

Determining Root Colonization of Field-Collected Pale Swallow-Wort and Co-Occurring Species. Root samples of field-collected pale swallow-wort plants used to assess the level of AMF colonization were obtained from the 36-plot field site in Henderson Harbor, NY, described above. In mid-August 2003 and 2004, roots of at least 10 pale swallow-wort plants were collected from the perimeter of each of 36 (2.0 by 1.5 m) plots. Other plants adjacent to the experimental plots were also collected (10 plants of each species, including two native species—common milkweed and Canada goldenrod (*Solidago canadensis* L.)—and the nonnative blueweed) and analyzed for AMF colonization of roots. In the laboratory, roots were then washed of soil and stained using the same procedures described above. Roots of each species were then cut into 75 1-cm segments and viewed using bright field microscopy (Phillips and Hayman 1970). The number of AMF-related structures such as arbuscules, hyphae, and vesicles present in the roots was then estimated per 1-cm segment. AMF spores were also extracted from the soil of the pale swallow-wort study site. A modified version of the decanting technique from Daniels and Skipper (1982) was used in which samples were subjected to water injection blasts to separate soil particles. AMF spores were flushed with water and placed in a petri dish for observation and identification to genus or species using bright field microscopy.

Mycorrhizal Dependence Experiment: Plant Growth and Survival. A greenhouse study was established in 2004 to determine the effect of AMF on survival and growth of pale swallow-wort. The experiment consisted of five treatments—three control treatments and two active treatments—in a completely randomized design with six replicates. The five treatments consisted of different soil inocula added to pots used to grow pale swallow-wort plants. Treatment 1 (SC) soil consisted of a 2 : 1 sterilized sand : Cornell Soil Mix without soil inoculum. The Cornell Soil Mix is a 1 : 1 : 1 mixture of peat, perlite, and vermiculite without nutrients. Treatments 2–5 consisted of a 2 : 1 sterilized sand : Cornell Soil Mix and 110 g of soil inoculum layered approximately 6 cm in depth from the lip of pots. The soil inoculum for treatment 2 (+MAL) was produced from a *Glomus intraradices* Shenk and Smith isolate from Alabama and was obtained from S. Greipsson at Troy University, AL. Pale swallow-wort is currently not present in this region of the United States and did not occur at the *G. intraradices* collection site. The soil inoculum for treatment 3 (+MNY) was collected in October 2003 from the Henderson Harbor, NY, field site. Soil at this site is known to contain several species of AMF. Inoculum sources for both treatments 2 and 3 were collected and then amplified following the methods of Brundrett et al. (1996) to obtain greater soil volumes in pot cultures. The soil inocula for treatments 4 (NMAL) and 5 (NMNY) were the same as those used in treatments 2 and 3, respectively, but inocula were subject to steam sterilization before being added to the pots. Steam sterilization of inoculum was carried out for 1 h in an autoclave at 121 C and 138 kPa. Inoculum was then placed in plastic zip-lock bags and stored in a cool dark location for approximately 1 mo while seed germination and confirmation of AMF presence in the soil took place. It is recognized that autoclaving may have adverse effects on soil quality and bulk density, and may provide a nutrient pulse with the killing of the microbial community. However, the amount of the autoclaved soil inoculum was relatively small in proportion to the total volume of potting mix used to grow the plants.

Pale swallow-wort plants required for this experiment were grown from seeds collected in September 2003 from the Henderson Harbor, NY, field site. Seeds were stratified in the dark at 4 C on two layers of moist filter paper in petri dishes. After 2 wk, petri dishes were placed in a growth chamber with 14/10-h light/dark daily cycle and temperature cycle of 25/17 C light/dark. Only germinated single embryo seeds were used for this experiment. At the start of the experiment, a single germinated pale swallow-wort seed was sown at a depth of 1 cm in each of the 30 10-cm-diam pots. Pots were placed in a greenhouse with a 14-h photoperiod and temperature range of 21–31 C. Pots were rerandomized weekly. Three weeks after planting (WAP) only, pots received ~500 ml of 20–10–20 (N–P–K) because foliage appeared chlorotic early on in the growth of the plants. Every 2 wk thereafter plants received ~500 ml of 13–0–44 N–P–K fertilizer at concentrations of 300 ppm. This initial poor growth of plants was later determined to be due to the relatively high light intensities in the greenhouse. Plants of pale swallow-wort in the field have been observed to be shorter and sometimes chlorotic under high light conditions (Smith et al. 2006). At 16 WAP, above- and belowground materials were harvested; a small portion of fresh root material (0.25 g) was removed and the remaining portion was dried at 65 C for 72 h, and weighed to determine total biomass. The fresh root material was washed of soil and stained to determine the presence of AMF using the procedure outlined by Phillips and Hayman (1970) and described previously.

Mycorrhizal dependence (MD) was calculated using Equation 1 (van der Heijden 2002):

$$MD = [1 - [\text{mean total biomass of plants without AMF}/\text{mean total biomass of plants with AMF}]] \times 100\% \quad [1]$$

Microbial Wash Experiment: Plant Growth and Nutrient Analysis. The microbial wash experiment was initiated under greenhouse conditions in May 2005. The experiment consisted of a completely randomized design with three soil treatments each comprising 10 potted plants of pale swallow-wort. All soils were collected in early May 2005 from a pale swallow-wort–dominated field at the Henderson Harbor, NY, site. Treatment 1 (TSC), consisted of soil that had been autoclaved at 121 C and 138 kPa for 1 h and stored as described above for the mycorrhizal dependence experiment, and is considered the control. Treatment 2 (T − MF) soil was also autoclaved; however, this soil was then amended with a microbial wash 1 d after autoclaving. Soil treatment 3 (T + MF) consisted of the same field soil as for TSC and T − MF but was not autoclaved or amended. The microbial wash was prepared as follows: at the time of field soil collection in May 2005, pale swallow-wort plants including roots were harvested. Roots and attached soil were washed with deionized water, and the wash containing soil, water, and root fragments was collected in 500-ml beakers. This root wash was then poured through a #1 Whatman filter (11 µm)[1], and served as AMF-free microbial inoculum source (Eissenstat and Newman 1990). One day following filtration, 200 ml of the root wash was added to T − MF pots. The effects of autoclaving in these two soil treatments were carefully monitored. Equal volumes of soil were used in all treatments. The volume of soil did not decrease after autoclaving; therefore soil bulk density in these two treatments was not affected. Pots were also monitored in the greenhouse to ensure good water filtration. At no time did plants appear to be wilted or deprived of water due to soil quality. Seed collection and planting procedures for pale swallow-wort were identical to those described for the mycorrhizal dependence experiment. Pots were placed in a greenhouse with a 14-h photoperiod and temperature range of 21–31 C. Pots were rerandomized weekly. The height and number of nodes of all plants was measured every 4 wk. After 12 wk, plants were harvested and dried at 65 C for 4 d, before being separated into root and shoot tissue and weighed to determine biomass and root-to-shoot ratio. Prior to oven-drying plant material, approximately 0.25 g of pale swallow-wort root tissue was taken from each plant for staining and to determine the level of AMF colonization. The same procedures for staining roots and determining AMF colonization were used as outlined previously. Remaining root and shoot tissue from treatments TSC and T + MF was then sent to the Cornell Nutrient Analysis Laboratory for nutrient content analysis using HNO_3/N_2O_2 acid block digestion and analyzed using an inductively coupled plasma atomic emissions spectrometer.[2]

Statistical Analysis. Percentage root colonization data were arcsine transformed to normalize the data and data were analyzed using PROC MIXED (SAS 2002). Mean comparisons were performed using Tukey-adjusted Least Squared Means at the 95% level of confidence. Height, number of leaf nodes, biomass, and tissue nutrient content from the two greenhouse experiments were analyzed, after natural-log transformation to normalize data, using the PROC MIXED procedure in SAS. Orthogonal contrasts were used for multiple comparisons of means at the 95% level of confidence. Seedling survival was analyzed using the PROC LIFETEST procedure in SAS. In all cases, back-transformed means are presented and plotted using SigmaPlot version 8.0.[3]

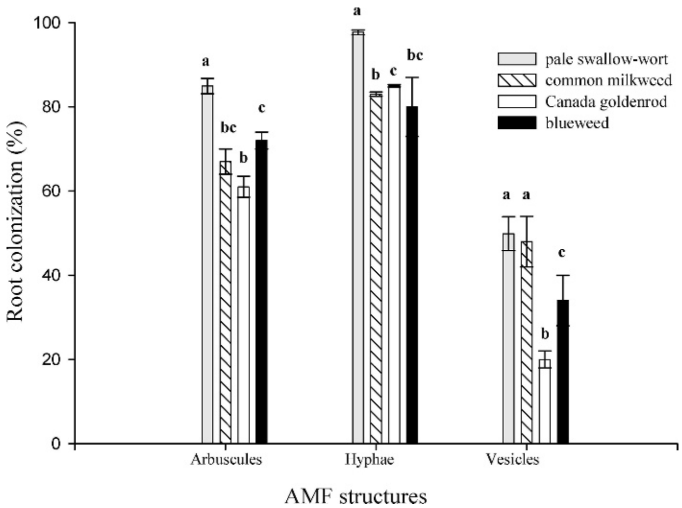

Figure 1. Percentage (± SE) of root colonization of pale swallow-wort and co-occurring plant species (common milkweed, Canada goldenrod, and blueweed) by various arbuscular mycorrhizal structures. Roots of pale swallow-wort and co-occurring species were collected in 2003 and 2004 from a heavily infested pale swallow-wort site in Henderson Harbor, NY. Means for plant colonization of arbuscular mycorrhizal fungi with the same letter are not significantly different according the Tukey adjusted Least Squared Means multiple range test at the level of ($P < 0.05$).

Results and Discussion

Root Colonization of Leek, Field-Collected Pale Swallow-wort, and Co-Occurring Species. Root colonization of leek plants by AMF after 8 wk revealed that the mycorrhizal potential of the Henderson Harbor, NY, field site soil was high with 72% of roots containing arbuscules, 80% containing hyphae, and 25% of roots having vesicles (data not shown). This finding suggests that there is potential for active symbiosis between AMF and pale swallow-wort plants in the Henderson Harbor, NY, field site. Roots of pale swallow-wort collected from each of the 36 plots at the field site were also heavily colonized by AMF with, on average, 85, 98, and 50% of roots containing arbuscules, hyphae, and vesicles, respectively during the two growing seasons (Figure 1). Pale swallow-wort plants experienced the greatest root colonization of any of the four plant species sampled at the Henderson Harbor field site. Colonization of pale swallow-wort roots by arbuscules and hyphae was greater than for roots of common milkweed, blueweed, and Canada goldenrod (Figure 1). Only colonization levels of vesicles in roots of the native herb common milkweed (48%) were not different from levels found in pale swallow-wort roots (Figure 1). The high AMF colonization levels of pale swallow-wort plants in the field suggest that this species has a symbiotic relationship with AMF and that this association may aid in the expansion of this species in its introduced North American range. The level of colonization by AMF of pale swallow-wort roots was greater than for leek trap plants, a species that has been used in research as a measure of AMF density in soil (Mooreman and Reeves 1979). The relatively higher number of arbuscules, which typically supply nutrients and water, found in pale swallow-wort roots compared with the other native and nonnative species examined in the Henderson Harbor, NY, field site indicates that an active relationship may be occurring between this nonnative, invasive vine and resident AMF species. Although quantification of AMF root colonization is not a measure of AMF function (McGonigle 1988; Smith et al. 2004), the ability of pale swallow-wort to harbor elevated densities of AMF in its roots may contribute to its invasive abilities because AMF have been shown to influence competitive outcomes between native and invasive plants (Pedersen and Sylvia 1996; Reinhart and Callaway 2004; West 1996). Certainly, a direct test of this hypothesis should include amended and nonamended AMF treatments and plants subjected to different competitive environments, especially because it has been demonstrated in several studies that the response of host plants to AMF inoculation may vary depending on whether they are subjected to intra- vs. interspecific competition (Bray et al. 2003; Marler et al. 1999). Spores of several AMF species were identified from soil collected in pale swallow-wort colonized areas of the field site including *Glomus clarum, Glomus mosseae, Glomus etunicatum, Glomus caledonium, Glomus intraradices, Gigaspora gigantea, Acaulospora spinosa,* and *Scutellospora calospora*. Inferences about the functional role of specific AMF species have been made in several studies (e.g., Burleigh et al. 2002). For example, *G. intraradices*, which is known to proliferate in certain environments, was the only species to produce vesicles in the host plant, which may ensure that sufficient carbon is available for sporulation. Research by Smith et al. (2000) showed that *Scutellospora* species obtained phosphorus close to plant roots whereas a *Glomus* species obtained phosphorus from soil areas further from the plant roots. Therefore, different species of AMF appear to have specific biological roles and niches in soil (Burleigh et al. 2002).

Mycorrhizal Dependence Experiment: Plant Survival and Growth. In the mycorrhizal dependence trial, pale swallow-wort plants showed significant differences in survival and growth between the AMF and sterile soil treatments. All pale swallow-wort plants grown with unamended field-collected soil from Henderson Harbor, NY, (+ MNY) were alive after 16 wk of growth (Figure 2). Survival of plants grown in *G. intraradices*–amended pots (+ MAL) was 83%. Significantly more plants survived in the amended soil compared with the three sterilized control

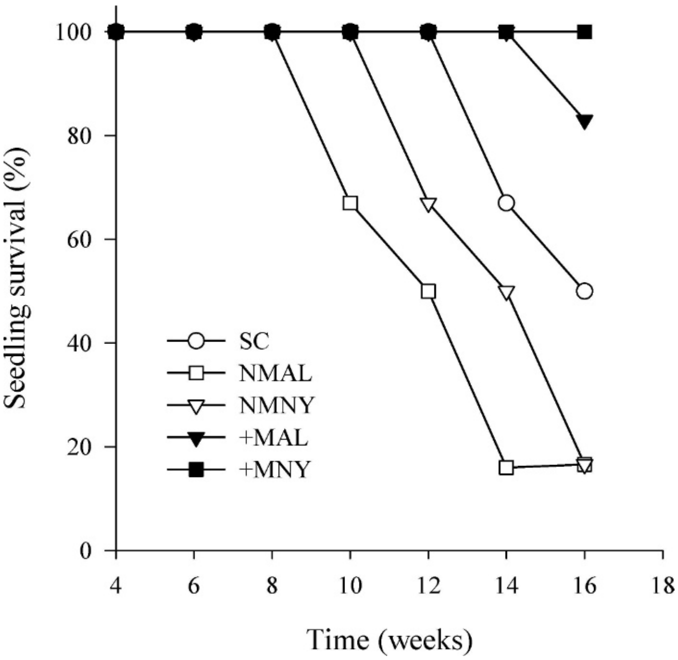

Figure 2. Seedling survival of pale swallow-wort under greenhouse conditions. Observations began 4 wk after planting. SC, autoclaved Cornell mix; NMAL, Cornell mix and autoclaved *Glomus intraradices*; NMNY, Cornell mix and autoclaved field soil; + MAL, Cornell mix and *G. intraradices* from Troy, AL; and + MNY, Cornell mix and arbuscular mycorrhizal fungi, locally collected at the Henderson Harbor, NY, field site.

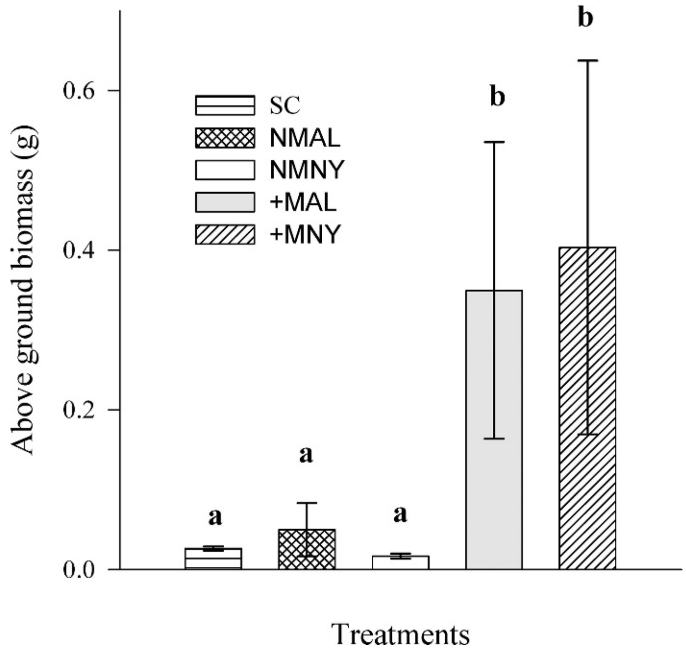

Figure 3. Mean (± SE) aboveground biomass of pale swallow-wort under greenhouse conditions. Observations began 4 wk after planting. Means for pale swallow-wort biomass with the same letter are not significantly different according the Tukey adjusted Least Squared Means multiple range test at the level of ($P < 0.05$). For treatment information, see Figure 2.

treatments. The aboveground biomass of pale swallow-wort plants grown in unamended Henderson Harbor soil was not significantly different than the aboveground biomass of plants grown in *G. intraradices*–amended soil (+ MAL) (Figure 3). Plants grown in both treatments containing AMF had significantly greater aboveground biomass than plants grown in the control treatments (Figure 3). The MD of pale swallow-wort plants grown in the + MNY soil was 93%, whereas the MD of pale swallow-wort plants in the + MAL soil was 92%. This high mycorrhizal dependence suggests that this species may derive greater benefits from AMF compared with other co-occurring species having lower dependence (van der Heijden et al. 1998).

The high mortality of pale swallow-wort plants deprived of AMF symbiosis in this study emphasizes the importance of AMF for establishment and survival of this plant species. Under the conditions of low phosphorus availability in this study, the presence of AMF greatly enhanced the survival of plants. Under field conditions, however, seedlings would likely be in close proximity to mature plants and therefore may be part of a common mycorrhizal network (Francis and Read 1984; Heap and Newman 1980). Seedling recruitment in areas of established vegetation may benefit from AMF by integrating the seedling root system into the mycorrhizal network, which can then more easily facilitate the supply of nutrients to newly establishing seedlings (Francis and Read 1995; Zobel et al. 1997). The results reported here support the view that AMF are beneficial for pale swallow-wort seedling survival and establishment and may explain the remarkably high seedling survival and successful transition to reproductively mature plants in this species, especially when compared with other co-occurring herbaceous plants (Ladd and Cappuccino 2005; Turnbull et al. 2000).

It would be valuable to repeat this experiment in a common garden setting at different planting densities to determine whether seedlings continue to benefit from AMF when grown in association with larger, well established pale swallow-wort plants (Facelli et al. 1999). Moreover, DNA-based characterization of which specific AMF species colonize the roots of pale swallow-wort and co-occurring native and nonnative plants present in our northern New York field site would also be valuable. The presence of AMF also had significant effects on plant height (Figure 4) and leaf number (data not shown). At the end of the trial, plants grown in local AMF from Henderson Harbor, NY, were taller than plants grown in the other treatments. Leaf production was significantly greater at the end of the 16-wk study for plants grown in local soil (~14 leaves per plant) relative to plants grown in the three sterile control

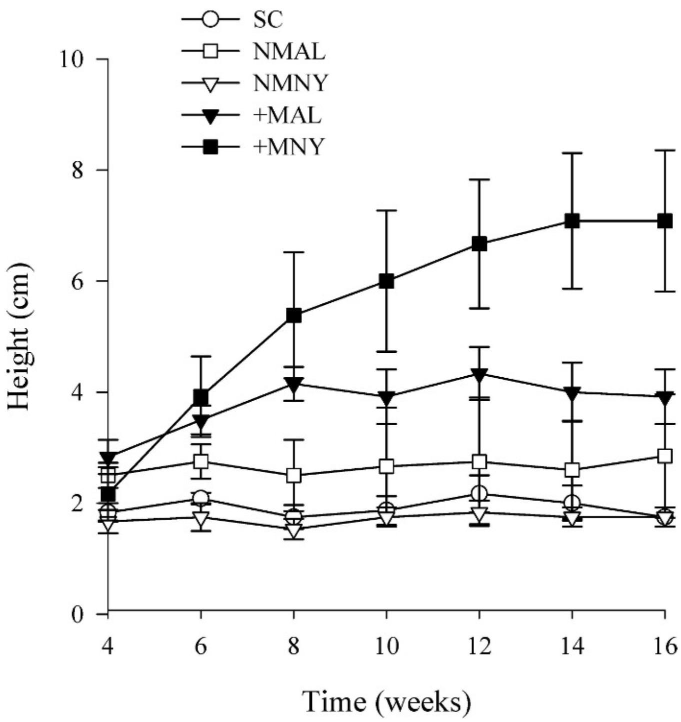

Figure 4. Mean (± SE) height of pale swallow-wort under greenhouse conditions. Observations began 4 wk after planting. SC, autoclaved Cornell mix; NMAL, Cornell mix and autoclaved *Glomus intraradices*; NMNY, Cornell mix and autoclaved field soil; + MAL, Cornell mix and *G. intraradices* from Troy, AL; and + MNY, Cornell mix and arbuscular mycorrhizal fungi, locally collected at the Henderson Harbor, NY, field site.

treatments (~six leaves per plant). However, there was no significant difference in the number of leaves produced by plants grown with the nonlocal *G. intraradices* isolate compared with the locally collected Henderson Harbor AMF.

The height and total biomass of pale swallow-wort plants were significantly increased in the presence of AMF. Roots of all pale swallow-wort plants in the two soil treatments containing AMF (+ MAL and + MNY) were colonized by AMF (data not shown). The biomass of plants from both AMF-containing treatments was greater than the biomass of all three treatments without AMF. However, the height of plants in the *G. intraradices* soil treatment (+ MAL) was no different than the height of plants that were grown in the sterile soil treatments (SC, NMAL, and NMNY). These plants were shorter than plants grown with the locally collected AMF inoculum (+ MNY) which likely contained multiple AMF species. The biweekly fertilization of plants should, however, be considered when interpreting these growth data. Although no P was added after the first application, subsequent additions of N and K can affect root growth which, in turn, could result in a larger root surface area for nutrient uptake.

Glomus intraradices is one of the more commonly occurring AMF species in soil (INVAM 2005; Stutz et al. 2000) and was also identified in the field associated with this pale swallow-wort population. The rationale for using inoculum from the *G. intraradices* isolate from Alabama was to determine whether pale swallow-wort had the ability to form an association with an AMF isolate that it had likely never encountered. Certainly, current climatic conditions in the southeastern United States do not appear suitable for pale swallow-wort establishment and growth and thus we are confident that pale swallow-wort is not present in this region. However, our findings using the *G. intraradices* isolate from Alabama suggest that despite these climatic limitations, pale swallow-wort is capable of forming symbiotic associations with this AMF isolate and of subsequently increasing its survival and growth. Although the Alabama isolate *G. intraradices* did not produce as significant effects as the + MNY treatment, the response might have been different had pale swallow-wort been grown in the presence of a mixture of AMF species from Alabama rather than in the presence of a single species.

AMF can exhibit both intra- and interspecific variation in response to different host plants (Munkvold et al. 2004; van der Heijden et al. 2003) and a decrease in AMF diversity in soil inocula can decrease the ability of these inocula to stimulate plant growth (van der Heijden et al. 1998). In our study, pale swallow-wort plants showed a significantly greater growth response to inoculum from resident AMF populations than to inoculum from the nonresident *G. intraradices* isolate alone. Importantly, it is not clear whether the growth response observed in pale swallow-wort was a function of the difference in diversity between the different inocula used or whether pale swallow-wort altered the AMF species composition of Henderson Harbor soil in a way that benefits its overall growth and fitness. Based on work by Greipsson and DiTommaso (2006) in other pale swallow-wort–dominated sites in central New York, this invasive species may be able to modify the species composition of resident soil AMF populations and possibly benefit from this change. These workers reported significantly higher spore densities of several AMF species in pale swallow-wort–infested areas compared with adjacent noncolonized areas. Recent work in other ecosystems also suggests that invasive species may alter species composition of resident AMF populations. For instance, identification of AMF species in the roots of native plants in California revealed that when grown in mixture with nonnative grasses, the AMF community in the roots of the native species was more closely related to that of the nonnative plants than to the AMF community in roots of native plants grown in pure stand (Hawkes et al. 2006). Mummey et al. (2005) reported similar results with the AMF community associated with a naturalized grass

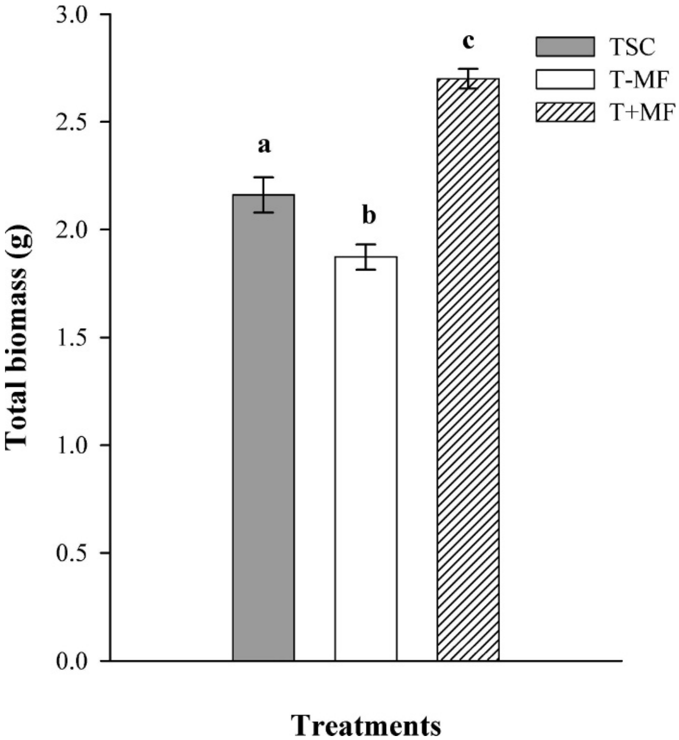

Figure 5. Mean (± SE) total biomass of pale swallow-wort plants after 12 wk of growth in a greenhouse. TSC, sterilized soil (without arbuscular mycorrhizal fungi [−AMF] and soil microbial community); T − MF, microbial wash (−AMF); T +MF; unamended (+ AMF and soil microbes). See microbial wash experiment for more details on treatments. Means for pale swallow-wort biomass with the same letter are not significantly different according the Tukey adjusted Least Squared Means multiple range test at the level of ($P < 0.05$).

species shifting to reflect the composition of the invasive perennial herb, spotted knapweed (*Centaurea maculosa* Lam.), when growing in its presence. Jin et al. (2004) indicated that in locations where the perennial herb Canada goldenrod had become invasive in China, the abundance of several AMF species increased with time since invasion. Clearly, more specific testing of pale swallow-wort plants to determine if diversity in general or a specific AMF species was responsible for the results we obtained in the current study is required, especially in light of the increasing number of studies revealing the importance of the functional diversity of individual AMF species. Furthermore, studies performed in the native range of pale swallow-wort could also provide additional insight about the role that AMF play in the invasiveness of this species in its introduced range (Hierro et al. 2005).

Microbial Wash Experiment: Plant Growth and Nutrient Analysis. Height of pale swallow-wort plants was highly variable across the three soil treatments and therefore no differences were found (data not shown). Total biomass of plants grown in T + MF soil was greater than the total biomass of plants grown in soil treated with the microbial wash (T − MF) (Figure 5). Bright field microscopy revealed no AMF present in the sterile control or the microbial wash soil, but non-AMF fungi colonized 31% of pale swallow-wort roots in this microbial wash treatment. Interestingly, plants grown in soil with microbial wash had a 30% lower total biomass than plants grown in sterilized soil. Roots grown in the T + MF treatment did have extensive AMF colonization with 78% of the roots containing arbuscules, 87% containing hyphae, and 44% having vesicles. Plants grown in the presence of resident AMF (T + MF) had 44% greater total biomass than plants grown in the microbial wash of T − MF. The shoot biomass of plants grown in the microbial wash soil was lower than the shoot biomass of plants grown in the unamended soil (T + MF) and sterile soil (TSC). Significant root biomass differences were only found when combining plants from treatments TSC and T – MF, which lacked AMF and plants from the T + MF treatment, which contained AMF. No differences were found in the root-to-shoot ratio of plants between the three soil treatments. Nutrient analysis of the plant tissue revealed that phosphorus concentration was greater in pale swallow-

Table 1. Mean (± SE) phosphorus concentration (µg/g) and content (g/plant) of pale swallow-wort plants grown for 12 wk in a greenhouse. Soil was collected from a pale swallow-wort-dominated area and was either unamended (T + MF) or sterilized by autoclaving (TSC).

	Phosphorus[a]	
Treatment	Concentration	Content
	µg/g	g/plant
Sterile (TSC)	907(177)a	0.1904(0.1)a
No amendments (T + MF)	1,092(40)a	0.2948(0.0)b

[a] Means (± SE) followed by the same letters within each column are not significantly different according to the Tukey adjusted Least Squared Means multiple range test at the level of $P < 0.05$.

wort plants grown in unamended soil compared with plants grown in soil that had been sterilized (Table 1). However, no significant difference in the phosphorus content of plants subjected to these treatments was found (Table 1).

Results from the microbial wash experiment also support the view that AMF are beneficial to pale swallow-wort plants. Although plants were able to survive in the non–nutrient-limiting soil, their biomass was significantly reduced when grown in soil lacking AMF (TSC and T − MF). Therefore, pale swallow-wort may be obligately mycorrhizal under very low nutrient soil conditions, but is able to survive under elevated nutrient soil conditions at lower growth rates. Phosphorus content in pale swallow-wort tissue in this study was significantly different between the sterilized and unamended soils, but the phosphorus concentrations did not differ. Although the significant difference in phosphorus concentration between the two treatments appear to be an artifact of plant size, differences in phosphorus content may have been greater had the experiment been carried out for a longer period. Our findings from the microbial wash experiment are also supported by those of Klironomos (2002) showing that plants may exhibit signs of negative feedback from pathogenic soil microbes, especially in native soils, whereas invasive plants accumulate AMF, which promote growth. The overall composition of the microbial community in soil treatment T + MF may have benefited pale swallow-wort and AMF growth. AMF have been shown to influence bacterial communities in the soil (Andrade at al. 1998), which in turn, may promote AMF development (Garbaye 1994) as well as promoting the mineralization and solubility of nutrients (Perotto and Bonfante 1997). Although the root wash procedure results in an AMF-free treatment, the filtering process may also remove other members of the soil microbial community. Therefore, to accurately assess the role of the other components of the soil community relative to that of AMF, additional treatments are required (e.g., autoclaved soil amended with bacterial wash and AMF). The large discrepancies in biomass between T − MF and the other two treatments may in part be explained by the microbes included in the wash treatment causing a drain on photosynthates even if the microbes added were not specific pathogens of pale swallow-wort. It is difficult to determine if a species such as pale swallow-wort, which likely has few, if any, pathogens in its introduced range (DiTommaso et al. 2005) would be negatively affected by an AMF-free soil microbial community as experienced in this study. In general, however, our findings suggest that pale swallow-wort may receive additional benefits from its relationship with AMF other than just increased nutrient availability, especially in non–nutrient-limiting soils. Newsham et al. (1995) have described numerous other benefits from plants by associating with AMF including limiting the uptake of toxic heavy metals and increased resistance to root-infecting pathogens.

In summary, our research demonstrates that pale swallow-wort has a beneficial relationship with AMF. Whether this invasive plant forms specific associations with AMF in invaded soils or performs best in the presence of a diverse group of resident AMF species remains to be determined. However, any benefit a nonnative invasive plant receives from AMF can contribute to its invasibility, especially if this symbiosis enhances seedling survival and establishment (e.g., Fumanal et al. 2006) as appears to be the case with pale swallow-wort. Future research should aim to determine how this association between AMF and a nonnative invasive vine such as pale swallow-wort may be altering other components of the soil microbial community in heavily infested sites, particularly those which may feed back to native plants or ecosystem functioning.

Sources of Materials

[1] Whatman #1filter (11 μm), Whatman Int., Maidstone, U.K.

[2] Inductively coupled plasma atomic emissions spectrometer, Spectro Cirosccd, Spectro Analytical Instruments, GmbH & Co. KG, Kleve, Germany.

[3] SigmaPlot v. 8.0, Systat Software Inc., San Jose, CA.

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