RESEARCH

Invasive Non-native Plants Alter the Occurrence of Arbuscular Mycorrhizal Fungi and Benefit from This Association

by Sigurdur Greipsson and Antonio DiTommaso

ABSTRACT

We investigated the influence of three aggressive non-native invasive plants (pale swallow-wort [*Vincetoxicum rossicum*], kudzu [*Pueraria lobata*], and Chinese privet [*Ligustrum sinense*]) on arbuscular mycorrhizal fungi (AMF). Our findings from central New York and southeastern Alabama forest habitats confirm that each species formed symbiotic associations with native AMF populations. Mycorrhizal dependency of kudzu was high when grown in invaded (85 percent) and non-invaded (83 percent) soil. The results indicate that invasion into new areas by non-native plant species may alter the occurrence of AMF in the resident soil. Evidence for a possible alteration in the AMF community was obtained largely from a Mycorrhiza Infection Potential bioassay. The bioassay indicated that root colonization levels by AMF of bait plants grown in soil collected from areas where the non-native plants formed pure stands were in general significantly greater than root colonization levels in soils collected from adjacent areas where the invasive plants were not present. Furthermore, the number of AMF spores in soil collected from areas invaded by pale swallow-wort was significantly higher than for nearby non-invaded soil. High colonization levels of pale swallow-wort roots with hyphae were observed during most of the growing season. The presence of arbuscules in pale swallow-wort roots was most evident in July and corresponds with the pre-flowering period of this species.

Keywords: arbuscular mycorrhizal fungi, Chinese privet, kudzu, invasive species, *Ligustrum sinense*, mycorrhizal infection potential, non-native species, pale swallow-wort, plant-soil interactions, *Pueraria lobata*, *Vincetoxicum rossicum*

Degradation of ecosystems by invasion of non-native plants is one of the most serious threats to the function and integrity of native ecosystems (Pimentel and others 2000, Wilcove and others 2000). Invasive non-native plants alter not only the aboveground structure and function of ecosystems, they may also affect the corresponding soil microbial communities (Kourtev and others 2002), which may have important implications for the management and restoration of invaded habitats.

Arbuscular mycorrhizal fungi (AMF) are an important soil microbial community of abundant asexually reproducing organisms that form mutualistic symbioses with nearly 90 percent of flowering plants (Brundrett 1991, Sanders 1999). Recent studies using molecular techniques have shown that the AMF-host plant association is species-specific (Vandenkoornhuyse and others 2003). Reported ecosystem benefits of AMF associations include improved soil stabilization, increased nutrient cycling (Bethlenfalvay and Linderman 1992), increased plant diversity and productivity (Dhillion and Gardsjord 2004), and facilitated plant community succession (Janos 1980, Greipsson and El-Mayas 2000).

Our research focuses on three invasive perennial, non-native species of forest understory habitats in different eco-regions of the United States: pale swallow-wort (*Vincetoxicum rossicum*) in central New York, and kudzu (*Pueraria lobata*) and Chinese privet (*Ligustrum sinense*) in southeastern Alabama.

Pale swallow-wort is a vigorous vine that has been a major concern in natural areas of the Lower Great Lakes Basin of North America for several decades, largely because its aggressive growth and dominance threatens several unique or rare ecosystems (Sheeley and Raynal 1996, DiTommaso and others 2005). For instance, its invasion of alvar habitats (shallow limestone barrens) in NewYork's Great Lakes Basin threatens 54 rare species of plants, insects, birds, and land snails (Bonanno 1999). Recent studies have also examined the possible deleterious effect this plant has on monarch butterfly (*Danaus plexippus*) reproduction (DiTommaso and Losey 2003, Mattila and Otis 2003) and on arthropod communities (Ernst and Cappuccino 2005). Pale swallow-wort was first introduced into North America from the Ukraine and southwestern Russia in 1889 and was observed in Monroe and Nassau counties in New York in 1897. Although a perennial, reproduction occurs primarily by seeds that can give rise to one or more (up to six) genetically identical seedlings (polyembryonic seeds).

Kudzu is a mat-forming, woody leguminous, trailing or high-climbing vine. It

Ecological Restoration, Vol. 24, No. 4, 2006 ISSN 1522-4740 E-ISSN 1543-4079

was introduced into the United States from Japan and China in the early 1900s for soil erosion control and as livestock feed (Miller 2003). It can now been found along the eastern part of the United States from New York in the north to Texas in the south. Kudzu grows well under a wide range of conditions and in most types of soil. It colonizes sites by seeds dispersed by animals or water and also by vines rooting at nodes (Miller 2003). Kudzu spreads rapidly, especially from disturbed habitats, and can form extensive pure stands in forest understories and oldfields, often altering the light, water, and fire regimes of these ecosystems.

Chinese privet is a thicket-forming shrub first introduced into the United States from China and Europe in the early- to mid-1800s. It can now been found from Maryland to Texas (Miller 2003). Privet prefers damp habitats and usually grows in low woods, bottomlands, streamsides, and disturbed areas. It often forms dense thickets, especially in forests and bottomlands, spreading along fencerows and rights-of-way. As a shade-tolerant species, it outcompetes native herbaceous forest floor plants and prevents pine and hardwood regeneration. Colonization of new habitats occurs primarily by bird- and animal-dispersed seeds, although it can also spread by root runners (Miller 2003).

No studies to date have investigated possible changes in the occurrence of resident AMF soil populations following invasion by these three species. We hypothesize that: 1) these non-native species form effective symbioses with native AMF populations and 2) that invasion of habitats by these species modifies the occurrence of AMF in soil within affected sites. The main objective of the study reported here was to compare the occurrence of AMF in soils colonized by these three non-native species to their occurrence in adjacent non-colonized soils. We also briefly discuss the implications of the findings for invasive plant management and habitat restoration.

Methods

Study Sites

Our study areas are forests in central New York and southeastern Alabama. The New York site is located in Cayuga County. This site is a 5-acre (2-ha) mixed deciduous forest stand of eastern white cedar (*Thuja occidentalis*), shagbark hickory (*Carya ovata*), sugar maple (*Acer saccharum*), and several ash (*Fraxinus*) species. The understory vegetation consists primarily of mayapple (*Podophyllum peltatum*), Canada mayflower (*Maianthemum canadense*), fescue (*Festuca* spp.), red baneberry (*Actea rubra*), and sedges (*Carex* spp.). Dense stands of pale swallow-wort (1,938 shoots/ft^2, 178 shoots/m^2) are located in the forest understory, although some adjacent areas have not been colonized by pale swallow-wort to date. The site is located on a shallow (1 ft , 0.3 m) Lima silt loam soil with a pH of 6.6 and 5.2-percent organic matter content. Measured soil chemical parameters did not vary significantly between invaded and non-invaded areas of the forest understory.

The second study site is located within the 74-acre (30-ha) Troy University Arboretum in Troy, Alabama, some 210 miles (340 km) from the Gulf of Mexico. One of the two hardwood forest sites there was colonized by kudzu, the other by privet. The soil of the study area is a Lucy loamy sand—a deep (5 ft, 1.5 m), well-drained soil with a pH of 5.0, and a 0.9-percent organic matter content. Dominant forest canopy species include loblolly pine (*Pinus tadea*), laurel oak (*Quercus hemisphaerica*), water oak (*Q. nigra*), and sweetgum (*Liquidambar styraciflua*). The understory vegetation consists mainly of panicgrass (*Panicum* spp.), little bluestem (*Schizachyrium scoparium*), greenbrier (*Smilax* spp.), common persimmon (*Diospyros virginana*), dogwood (*Cornus florida*), and chain fern (*Woodwardia afeolata*).

Soil Collection for AMF Determination

At each site, we took three soil samples in both the invaded areas and the adjacent non-colonized areas. Samples were collected using a standard soil corer to a depth of about 6 inches (15 cm). We immediately placed the samples in plastic bags for transport to the laboratory where they were placed in a cooler at a temperature of 39°F (4°C). Soils were sampled on May 15, 2002 in New York and on May 20, 2002 in Alabama.

Estimation of AMF Spore Numbers in Soil

An intensive study on the number of spores of AMF was only carried out for the New York site. Spores of AMF were extracted from each of the soil samples collected from the New York site using a modified version of the decanting and wet sieving technique developed by Daniels and Skipper (1982). Soil samples were subjected to vigorous water injection blasts for 5 seconds intervals in order to separate the soil particles. Spores of AMF were flushed with water and transferred to a Petri dish and observed and counted under a stereomicroscope (50x). Empty spores were discarded. Three replicates were taken for each soil sample within each site. We compared the numbers of AMF spores between pale swallow-wort-colonized and adjacent non-invaded areas by using Student's t-test ($p < 0.05$).

Estimation of AMF Root Colonization

Roots of the invasive species were rinsed carefully with water and stored in 50-percent ethanol until required. Within a few days of field collection, roots were again rinsed with water and cleared using 10-percent KOH at 194°F (90°C) for 20 minutes. Roots were then rinsed five times using tap water and acidified by soaking in HCl (2.5 percent) for 30 minutes at room temperature. Roots were subsequently stained with Trypan blue (0.05 percent) for 30 minutes at 194°F and then de-stained in a solution of 50-percent glycerol and 50-percent water (Phillips and Hayman 1970). Roots were mounted on slides for observation using bright field microscopy.

The percentage colonization by AMF was estimated using the root-piece method (Johnson-Green and others 1995). Colonization of different AMF structures (hyphae, arbuscules, and vesicles) was estimated by scoring 0.4-inch- (1-cm-) long root pieces for the presence or absence of AMF structures under bright field microscopy. Twenty-five root pieces were scored from each plant. Percentage colonization of pale swallow-wort roots by AMF structures was compared using one-way ANOVA followed by SNK ($p < 0.05$).

AMF Root Colonization during the Growing Season

An intensive study on the AMF root colonization of pale swallow-wort from the New York site was conducted throughout the 2002 growing season. Roots of pale swallow-wort were collected on three sample dates (May 20, June 22, and July 24) from the New York site. Five replicated root samples (about 500 g fresh weight each) were carefully collected using a shovel.

Mycorrhizal Infectivity Potential of Soil

We also estimated the Mycorrhizal Infectivity Potential (MIP) of the soil collected from areas invaded by the three non-natives described above and from adjacent non-colonized areas. The MIP is a bioassay technique that measures AMF density in soil (Mooreman and Revees 1979). The MIP of the soil in each site was estimated using a bioassay technique where corn (*Zea mays*) bait plants were grown in diluted soil (1:5, soil:sand) from each site in three replicated 3 L pots for 30 days. Sterilized quartz sand was used for soil dilution. Plants were grown for four weeks in a partially shaded greenhouse. Pots were watered as needed but were not fertilized. Following this incubation period, the degree of AMF root colonization of bait plants was determined as for field samples and used as an index of the soil's MIP. Total root AMF colonization levels of bait plants grown in soil from invaded areas and adjacent non-invaded areas were compared using Student's t-tests ($p < 0.05$). Proportions were arcsine-square root transformed prior to analysis.

Growth Response of Kudzu to Native AMF

Kudzu seeds were germinated and grown in forest soil collected from 1) kudzu-invaded areas, 2) non-invaded adjacent areas, and 3) same as 2) but the soil was sterilized by autoclaving for 1 hour at 250°F (121°C) at 1.5 atmospheres. Plants were grown in three pots in an open growth chamber for two months. Abuscular mycorrhizal fungi root colonization was estimated by staining roots with Trypan blue. Mycorrhizal dependency (Md) was calculated according to the following equation:

$$Md = [1-[bn/\Sigma an]] \times 100\%$$

where a = mean plant dry mass of AMF plants and b = mean plant dry mass of non-AMF plants.

Results

AMF Root Colonization

We observed high AMF root colonization in pale swallow-wort (100 percent), kudzu (70 percent), and privet (60 percent). We found low root colonization of arbuscules in kudzu (10 percent) and privet (13 percent), while root colonization levels of arbuscules in pale swallow-wort were higher (50 percent). Arbuscules are, however, ephemeral structures and their presence is dependent on the symbiotic relationship between the fungi and the plant.

Estimation of AMF Spores in Soil

We noted that soil from areas in the New York site invaded by pale swallow-wort had four times as many AMF spores than soil from adjacent non-invaded areas ($p < 0.05$) (Figure 1).

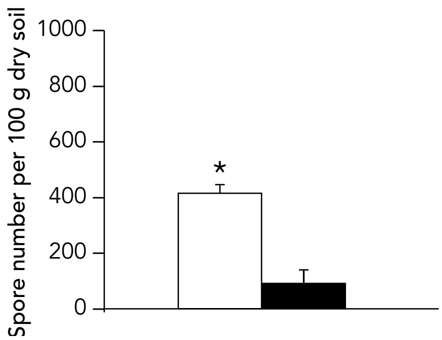

Figure 1. Mean number of AMF spores (± SE) from soil collected at the central NY State site. Soil was sampled from areas colonized by pale swallow-wort (□) and adjacent non-colonized areas (■). * indicates significant differences between values within site ($p < 0.05$).

AMF Root Colonization During the Growing Season

Pale swallow-wort maintained high (100 percent) hyphal root colonization during the growing season (Figure 2). High root colonization with vesicles was observed for the three sample dates, although root colonization with arbuscules was significantly greater for the June sample period compared with the May and July sample periods (Figure 2). The high percentage root colonization with arbuscules for the June sample period coincided with the start of the reproductive phase of pale swallow-wort at this site. Flower initiation in plants has previously been linked to additional uptake of phosphorus (Dunne and Fitter 1989). Uptake of phosphorus is, however, related to the life history of plants and can, therefore, occur at different times during the growing season (Mullen and Schmidt 1993). Consequently, peaks in root colonization of arbuscules have been observed early or late in the growing season (Reinhardt and Miller 1990) or throughout the season (Greipsson and others 2002). Few studies have followed AMF-plant root dynamics of specific fungal structures in wild plants through the growing season (Reinhardt and Miller 1990, Mullen and Schmidt 1993, Greipsson and others 2002, Ruotsalainen and others 2002). Additional experiments are needed to establish a link between phosphorus inflow during the reproductive phase of invasive species, such as pale swallow-wort, and the high occurrence of arbuscules within roots.

Mycorrhizal Infectivity Potential of Soil

Invasion by the three non-native plants increased the occurrence of AMF in resident soil relative to soil from adjacent areas where these plants were absent. We found significantly higher AMF root colonization for corn bait plants grown in soil collected from areas where pale swallow-wort was present than in soil collected from adjacent non-invaded areas (Figure 3a). Interestingly, the roots of bait plants grown in soil collected from areas not invaded by pale swallow-wort were not

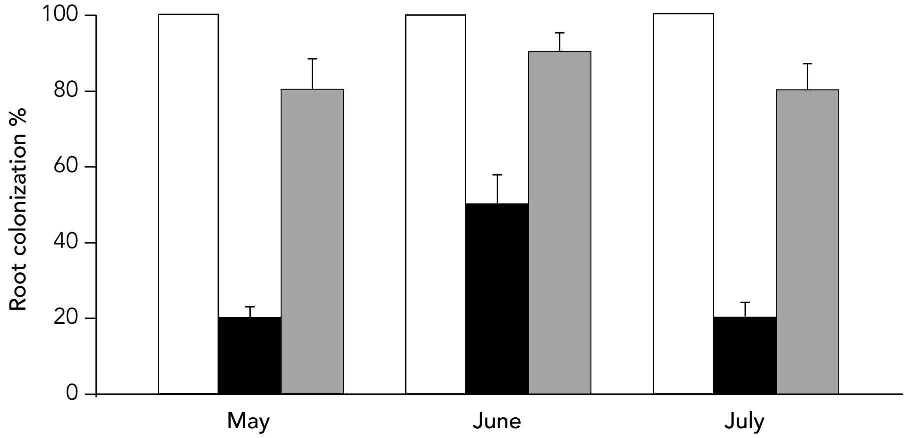

Figure 2. Root colonization percentage (± SE) of AMF structures: hyphae (□), vesicles (■), and arbuscules (■) in pale swallow-wort during the 2002 growing season. Plants were collected in central NY State. Means for columns having the same letter between sample periods are not significantly different ($p < 0.05$).

colonized by AMF (Figure 3). Similarly, significantly higher AMF root colonization of bait plants was observed in soil collected from privet-dominated areas compared with adjacent non-invaded areas dominated by the chain fern (Figure 3b). In soil collected from kudzu-dominated areas, root colonization of bait plants (17 percent) was also greater than for bait plants grown in soil from non-invaded adjacent areas (0 percent) (Figure 3c).

Growth Response of Kudzu to Native AMF

Kudzu grown in the sterilized forest soil grew poorly and showed signs of chlorosis and necrosis. Kudzu grown in soil collected from both invaded and adjacent non-invaded areas grew significantly better (40-percent AMF root colonization) than in sterilized soil (no AMF root colonization). Symbiosis with native soil AMF increased the total dry biomass of kudzu 412 percent compared with plants grown in sterilized soil in the absence of AMF. Similarly, kudzu plants had a significantly greater number of leaves when grown in soil collected from both invaded and non-invaded forest areas than for plants grown in sterilized soil (Figure 4a). Plants were also significantly taller when grown in soil collected from kudzu-dominated areas (Figure 4b). The mycorrhizal dependency of kudzu was high when plants were grown in soil collected from invaded (85 percent) and non-invaded (83 percent) areas.

Implications for Management and Restoration Efforts

Recent molecular work on the AMF-host plant symbiosis has revealed that this association is species-specific (Vandenkoornhuyse and others 2003). Therefore, each native plant species harbors a characteristic assembly of AMF species in its roots. Our study revealed that the invasive non-native plants we tested are able to establish associations with the native AMF and, furthermore, that the occurrence of AMF in the soil is altered. This alteration in the AMF community could affect restoration efforts. Restoration of invaded sites may not only require management and, ultimately, eradication of the non-native plants but the reintroduction or augmentation of AMF species that are associated with each of the native or naturalized species from the plant community affected.

We also found that kudzu has high mycorrhizal dependency (and this is now also known to be true for pale swallow-wort and privet), and we predict that these non-native invasive species also have a low mycorrhizal species sensitivity. That is, they are likely to exhibit low variability in their growth responses in the presence of different AMF species. We also predict that non-native plants tend to associate with fast-growing, opportunistic AMF species. Invasion by non-native plants may, therefore, lead to shifts in the AMF community. We are currently investigating these shifts in the AMF community following invasion of non-native plants. We suggest that eliminating AMF by sterilizing the soil at the invading fronts of noxious non-native plants could help control the spread of kudzu, and possibly other invasive species.

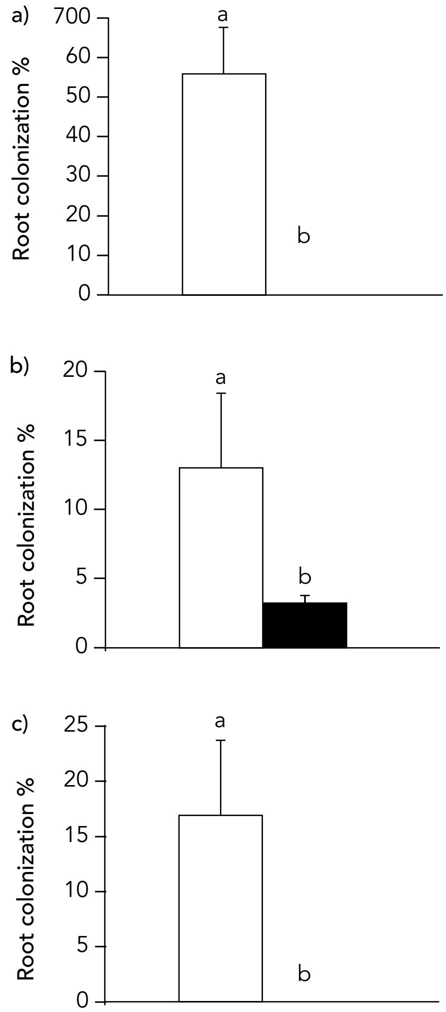

Figure 3. Root colonization percentage (± SE) of AMF in corn bait plants grown for 30 days in diluted soils (1:5, soil:sand). Soil was sampled from: a) invaded pale swallow-wort and adjacent non-invaded area, b) invaded privet and adjacent non-invaded area, c) invaded by kudzu and adjacent non-invaded area. Means for columns having the same letter between invaded and adjacent non-invaded areas are not significantly different ($p < 0.05$).

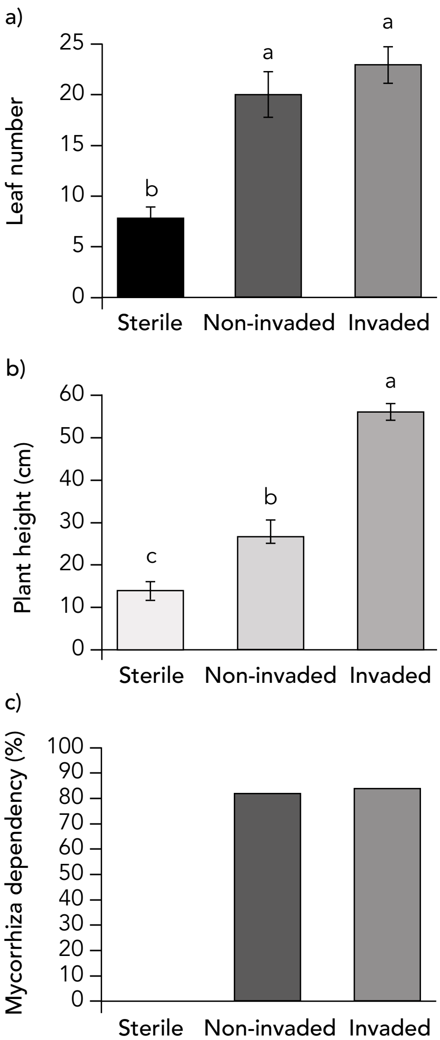

Figure 4. Growth response of kudzu to three soil types: 1) sterile forest soil, 2) non-invaded forest soil, and 3) invaded forest soil. Figure 4a is leaf number, Figure 4b shows plant height, and Figure 4c mycorrhizal dependency. Means for columns having the same letter are not significantly different ($p < 0.05$).

Further Research Needs

The long-term effects of altered AMF populations and activity in soil on ecosystem processes are not known. Recently, Castelli and Casper (2003) demonstrated that altered levels of AMF populations in a serpentine grassland resulted in changes in plant performance. It is possible that invasion and performance of AMF-dependent plant species may be enhanced by the increased occurrence of AMF in soil, especially given how quickly the three species used in this study displace resident vegetation in newly colonized habitats. However, additional data are needed to confirm this hypothesis.

The invasive success of non-native plant species can also be enhanced by AMF symbiosis through indirect effects. For instance, the rapid displacement of the native grass, Idaho fescue (*Festuca idahoensis*), from intermountain prairies of the United States by spotted knapweed (*Centaurea maculosa*) in the presence of AMF was not due to direct effects on either of these two species when grown separately, but through an increase in the negative effect of spotted knapweed on Idaho fescue when the species were grown together (Marler and others 1999). The possibility of indirect effects of AMF on competitive outcomes between the non-native plants used in this study and native or other introduced species has not been examined.

Invasive non-native plants that are not necessarily AMF dependent have also been found to change the occurrence of AMF in the soil. For example, declines of soil AMF populations were reported following invasion of Russian thistle (*Salsola tragus*) and garlic mustard (*Alliaria petiolata*)—two species that do not depend on AMF (Goodwin 1992, Roberts 1997). The eventual impact of invasion by non-native species on AMF communities and soil dynamics appears to be related to the AMF dependency (mycotrophy) of the invading plant species such that non-dependent species (for example, garlic mustard) reduce the activity of AMF in the resident soil whereas dependent species (pale swallow-wort, kudzu) increase the occurrence of AMF in the resident soil.

Acknowledgments

We thank Nathaniel Hubert, Joseph Dauer, Bradley Webster, and Larissa Smith for valuable field assistance and Jessica Weaver for help in typing the manuscript. We thank Drs. Daniel C. Brainard, Charles L. Mohler, and Maurice Vestberg for editorial suggestions on earlier drafts of the manuscript.

References

Bethlenfalvay, G.J. and R.G. Linderman. 1992. *Mycorrhizae in sustainable agriculture*. Madison: ASA Special Publication # 54.

Bonanno, S.E. 1999. Jefferson County Alvar Megasite Conservation Plan. Rochester: The Nature Conservancy.

Brundrett, M. 1991. Mycorrhizas in natural ecosystems. *Advances in Ecological Research* 21:171-313.

Castelli, J.P. and B.B. Casper. 2003. Intraspecific AM fungal variation contributes to plant-fungal feedback in a serpentine grassland. *Ecology* 84:323-336.

Daniels, B.A. and H.D. Skipper. 1982. Methods for the recovery and quantitative estimation of propagules from soil. Pages 29-35 *in* N.C. Schenck (ed.), Methods and principles of mycorrhizal research. St. Paul, MN: The American Phytopathology Society.

Dhillion, S.S. and T.L. Gardsjord. 2004. Arbuscular myhorrhizas influence plant diversity, productivity, and nutrients in boreal grasslands. *Canadian Journal of Botany* 82:104-114.

DiTommaso, A. and J.E. Losey. 2003. Oviposition preference and larval performance of monarch butterflies (*Danaus plexippus*) on two invasive swallow-wort species. *Entomologia Experimentalis et Applicata* 108:205-209.

DiTommaso, A., F.M. Lawlor and S.J. Darbyshire. 2005. The biology of invasive alien plants in Canada. 2. *Cynanchum rossicum* (Kleopow) Borhidi [= *Vincetoxicum rossicum* (Kleopow) Barbar.] and *Cynanchum louiseae* (L.) Kartesz & Gandhi [= V*incetoxicum nigrum* (L.) Moench]. *Canadian Journal of Plant Science* 85:243-263.

Dunne, M.J. and A.H. Fitter. 1989. The phosphorus budget of a field grown strawberry (*Fragaria* x *ananassa* cv. Hapil) crop: evidence for a mycorrhizal contribution. *Annals of Applied Biology* 114:185-193.

Ernst, C.M. and N. Cappuccino. 2005. The effect of an invasive alien vine, *Vincetoxicum rossicum* (Asclepiadaceae), on arthropod populations in Ontario old fields. *Biological Invasions* 7:417-425.

Fitter, A.H. 2005. Darkness visible: Reflections on underground ecology. *Journal of Ecology* 93:231-243.

Goodwin J. 1992. The role of mycorrhizal fungi in competitive interactions among native bunchgrasses and alien weeds: A review and synthesis. *Northwest Science* 66:251-260.

Greipsson, S. and H. El-Mayas. 2000. Arbuscular mycorrhizae of *Leymus arenarius* on coastal sands and reclamation sites in Iceland and response to inoculation. *Restoration Ecology* 8:144-150.

Greipsson, S., H. El-Mayas, M. Vestberg and C. Walker. 2002. Arbuscular mycorrhizal fungi in sandy soils in Iceland. *Arctic, Antarctic and Alpine Research* 34:419-427.

Janos, D.P. 1980. Mycorrhizae influence tropical succession. *Biotropica* 12:56-64.

Johnson-Green, P.C., N.C. Kenkel and T. Booth. 1995. The distribution and phenology of arbuscular mycorrhizae along an

inland salinity gradient. *Canadian Journal of Botany* 73:1318-1327.
Kourtev, P.S., J.G. Ehrenfeld and M. Häggblom. 2002. Exotic plant species alter the microbial community structure and function in the soil. *Ecology* 83:3152-3166.
Marler, M.J., C.A. Zabinski and R.M. Callaway. 1999. Mycorrhizae indirectly enhances competitive effects of an invasive forb on a native bunchgrass. *Ecology* 80:1180–1186.
Mattila, R. and G.W. Otis. 2003. A comparison of the host preference of monarch butterflies (*Danaus plexippus*) for milkweed (*Asclepias syriaca*) over dog-strangler vine (*Vincetoxicum rossicum*). *Entomologia Experimentalis et Applicata* 107:193-199.
Miller, J.H. 2003. Nonnative invasive plants of southern forests. USDA, General Technical Report SRS-62.
Moorman, T. and F.B. Reeves. 1979. The role of endomycorrhizae in revegetation practices in the semiarid west. II. A bioassay to determine the effect of land disturbance on endomycorrhizal populations. *American Journal of Botany* 66:14-18.
Mullen, R.B. and S.K. Schmidt. 1993. Mycorrhizal infection, phosphorus uptake, and phenology in *Ranunculus adoneus:* Implications for the functioning of mycorrhizae in alpine systems. *Oecologia* 94:229-234.
Phillips, J.M. and D.J. Hayman 1970. Improved procedure for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55:158-161.
Pimentel, D., L. Lach, R. Zuniga and D. Morrison. 2000. Environmental and economic costs of nonindigenous species in the United States. *BioScience* 50:53-65.
Reinhardt, D.R. and R.M. Miller 1990. Size classes of root diameter and mycorrhizal fungal colonization in two temperate grassland communities. *New Phytologist* 116: 129-136.
Roberts, K.J. 1997. An examination of the interactions between garlic mustard (*Alliaria petiolata* (Beib.) Cavara & Grande) and vesicular-arbuscular mycorrhizal (VAM) fungi (*Gigaspora rosea*). Ph.D. thesis, Illinois State University, Normal.
Ruotsalainen, A.L., H. Vare and M. Vestberg. 2002. Seasonality of root fungal colonization in low-alpine herbs. *Mycorrhiza* 12:29-36.
Sheeley, S.E. and D.J. Raynal. 1996. The distribution and status of species of *Vincetoxicum* in eastern North America. *Bulletin of the Torrey Botanical Club* 123:148-156.
Vandenkoornhuyse, P., K.P. Ridgway, I.J. Watson and A.H. Fitter. 2003. Co-existing grass species have distinctive arbuscular mycorrhizal communities. Molecular Ecology 12:3085-3095.
Wilcove, D.S.D., J. Rothstein, A. Dubow Phillips and E. Losos. 2000. Leading threats to U.S. biodiversity: What's threatening imperiled species? Pages 239-255 *in* B. Stein, L.S. Kutner and J.S. Adams (eds.), Precious heritage: The status of biodiversity in the United States. Oxford: Oxford University Press.

Sigurdur Greipsson is an associate professor in the Department of Biological and Environmental Sciences, Troy University, Troy, AL 36082; 334/670-3663, Fax: 334/670-3662, greipsso@ troy.edu.

Antonio DiTommaso is an associate professor in the Department of Crop and Soil Sciences, 903 Bradfield Hall, Cornell University, Ithaca, NY 14853.