Mycorrhizal Colonization of *Hypericum perforatum* L. (Hypericaceae) from Serpentine and Granite Outcrops on the Deer Isles, Maine

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Abstract - Given the paucity of literature on plant-fungal interactions on serpentine soils and limited investigation of serpentine geoecology in eastern North America, we examined mycorrhizal colonization of *Hypericum perforatum* from adjacent serpentine and granite outcrops on the Deer Isles, ME to determine whether plants were differentially colonized based on substrate. We coincided our sampling with three phenologic stages of *H. perforatum* (preflowering, flowering, postflowering) to determine possible differences in colonization based on plant phenology. The levels of mycorrhizal colonization in *H. perforatum* were not significantly different between serpentine and granite sites, while levels of colonization in postflowering plants were significantly higher than in those at preflowering and flowering stages.

Introduction

Serpentine soils are derived from igneous or metamorphic rocks consisting of at least 70% ferromagnesian minerals (Kruckeberg 2002) and are generally characterized by low levels of plant macronutrients; elevated levels of heavy metals such as Ni, Cr, Cd, and Co; and Ca:Mg ratios of less than one (Brady et al. 2005). Serpentine outcrops worldwide commonly display reduced plant productivity, high rates of endemism, and vegetation markedly different from that of surrounding areas (Whittaker 1954). For example, while only 1% of the state of California is made up of serpentine rocks, 12.5% of the state's endemic flora is restricted to serpentine (Brady et al. 2005, Safford et al. 2005). Due to their distinct characteristics and patchy distributions, both throughout the world and on regional scales, serpentine outcrops are model settings for studies of ecology and evolution (Boyd et al. 2009, Harrison and Rajakaruna 2011).

Much is known about vascular plants from serpentine habitats (Brady et al. 2005, Harrison and Rajakaruna 2011, Kruckeberg 2002), but the literature on plant-fungal interactions on serpentine is limited (Branco and Ree 2010, Casper and Castelli 2007, Doubková et al. 2012, Rajakaruna et al. 2009). Because of the reduced availability of plant macronutrients (especially phosphorus) on serpentine soils, plants on serpentine outcrops form mutualistic associations with root symbionts, such as arbuscular mycorrhizal (AM) fungi, to overcome the hardships presented by the soil (Strauss and Boyd 2011). AM fungi are members of the fungal phylum Glomeromycota; they obligately associate with the

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roots of angiosperms, gymnosperms, and pteridophytes, exchanging nutrients with their hosts (Smith and Read 2008). They are widely distributed and are major contributors to nutrient cycling in ecosystems (Read and Perez-Moreno 2003, Redecker 2008). Most higher plants participate in some kind of mycorrhizal association, and plants that form mycorrhizal symbioses occur in twice as many habitat types as those that do not form such associations, i.e., mycorrhizal fungi appear to increase the niche widths of plants (Wilkinson and Dickinson 1995). Despite their ecological importance and ubiquity, few studies have examined AM fungi in metal-enriched serpentine soils (Doherty et al. 2008, Schechter and Bruns 2008). Given the varied and inconsonant data available on the effects of toxic metals on AM symbioses (Pawlowska and Charvat 2004), it is important to further investigate the status of these symbioses in metal-enriched soils, particularly in light of the relevance of such studies to ecological restoration efforts (O'Dell and Claassen 2009) and emerging bioremediation technologies (Pilon-Smits and Freeman 2006).

While much effort has gone into elucidating the influence of ultramafic bedrock on associated organisms, such efforts in North America have largely been carried out in the western portion of the continent, leaving the serpentine geoecology of eastern North America largely unexplored (Boyd et al. 2009, Rajakaruna et al. 2009). Ultramafic rocks occur discontinuously along the eastern edge of the Appalachian mountain range from Alabama to Newfoundland (Tyndall and Hull 1999). For recent reviews of the serpentine geoecology of eastern North America, see Alexander (2009) and Rajakaruna et al. (2009).

In this study, we examined the extent of mycorrhizal colonization between specimens of *Hypericum perforatum* L. (Common St. John's Wort; Hypericaceae) collected from adjacent serpentine and granite outcrops on the Deer Isle complex, ME to determine whether plants were differentially colonized by AM fungi based on substrate. Furthermore, we compared different phenologic stages of *H. perforatum* in order to determine any possible differences in mycorrhizal colonization based on plant phenology. Previous research on these geologically distinct sites revealed stark differences in assemblages of bryophytes (Briscoe et al. 2009) and vascular plants (Pope et al. 2010). Furthermore, significantly higher levels of Mg, Fe, Ni, Zn, and Cd, as well as significantly lower Ca:Mg ratios, were documented in the serpentine soil compared to the granite soil, and significantly higher Ni concentrations in *H. perforatum* growing on the serpentine site was also shown (Pope et al. 2010).

Materials and Methods

Hypericum perforatum is a perennial that flowers from June to September (Gleason and Cronquist 1991). In April, July, and October of 2009, a total of 89 specimens of *Hypericum perforatum* were collected at different phenologic stages (preflowering, flowering, and postflowering). We define preflowering as plants exhibiting emerging young shoots in the spring, flowering as plants with

at least one flower in anthesis, and postflowering as plants senescing after the flowering period. Plants were collected from Pine Hill and Settlement Quarry on Little Deer Isle and Deer Isle, respectively, in Hancock County, ME. Pine Hill is a former peridotite (serpentine) quarry on Little Deer Isle, and Settlement Quarry is a former granite quarry on adjacent Deer Isle. For detailed descriptions and histories of Pine Hill and Settlement Quarry, see Harris et al. (2007) and Briscoe et al. (2009), respectively. For additional information on soil chemical and physical features at these sites, see Pope et al. (2010).

At each site, collections were made randomly using a line transect. A compass was placed roughly center in a collection area, and a transect was laid along a randomly generated degree corresponding to a direction on the compass. Plants were collected at predetermined, randomly generated intervals along this transect. The transect was walked from end to end. This process was repeated until at least 10 plants were collected for each unique combination of effects (phenology × substrate).

Excess soil was manually cleaned off of roots and shipped in paper bags to Dr. Efrén Cázares (Oregon State University, Corvallis, OR; www.mycoroots.com) for assessment of AM colonization for each individual plant. The measurement index used for colonization was percent colonization (length of root colonized by hyphae, arbuscules, or vesicles over total length of root per plant).

Roots were cleared and stained through a method modified from Phillips and Hayman (1970). Roots were washed in running tap water and cut into segments to fit into glass vials where they were immersed in 10% KOH solution for 48 hours at room temperature. Then the KOH solution was poured off, and the roots were rinsed twice in tap water, placed in 1% HCl acid solution for 30 minutes, and then rinsed in tap water afterward. Cleared samples were immersed for 12 hours at room temperature in a staining solution of 0.5% trypan-blue (which stains fungal wall cells) in lactoglycerol, rinsed with tap water, and stored in lactoglycerol or water at 4 °C until examination. Stained roots were examined under a microscope at 10–40x magnification to determine presence or absence of mycorrhizal fungi. Roots were placed in a Petri dish with a 1-cm grid marked on the bottom. Total root length of each plant was estimated using the grid, and mycorrhizal colonization was estimated by scanning all the roots under a microscope and recording the presence of any hyphae, arbuscules, vesicles, or internal spores in each centimeter of root.

Nonparametric tests were used to detect possible differences in the extent of mycorrhizal colonization between sites and phenologic stages. Data were nonparametric, even after multiple transformations. Mann-Whitney U-tests were done to test for differences between sites (at each phenologic stage as well as overall) and between phenologic stages (within sites) for mycorrhizal colonization of *H. perforatum*. Software used for statistical analyses and figures were R version 2.9.1 (Copyright © 2009 The R Foundation for Statistical Computing) and MYSTAT version 12.02.00 (Copyright © 2007 SYSTAT Software, Inc.).

Results

Among all 89 *H. perforatum* plants collected, the root colonization varied from 0% to 63%. The mean percent of root colonization for all plants collected was 10% with a standard deviation of 13%, and most plants we observed had colonization levels below the average (Fig. 1).

There were no differences in root colonization levels between the serpentine and granite sites (Mann-Whitney U-test *P*-values all > 0.25). However, within each site, there was a significant effect of phenology on colonization level; post-flowering individuals exhibited significantly higher colonization levels compared to the other two stages, regardless of site (Mann-Whitney U-test *P* -values < 0.05; Fig. 2).

Discussion

Our results showed no significant difference in the extent of AM colonization in *Hypericum perforatum* between the serpentine and granite sites. Other studies
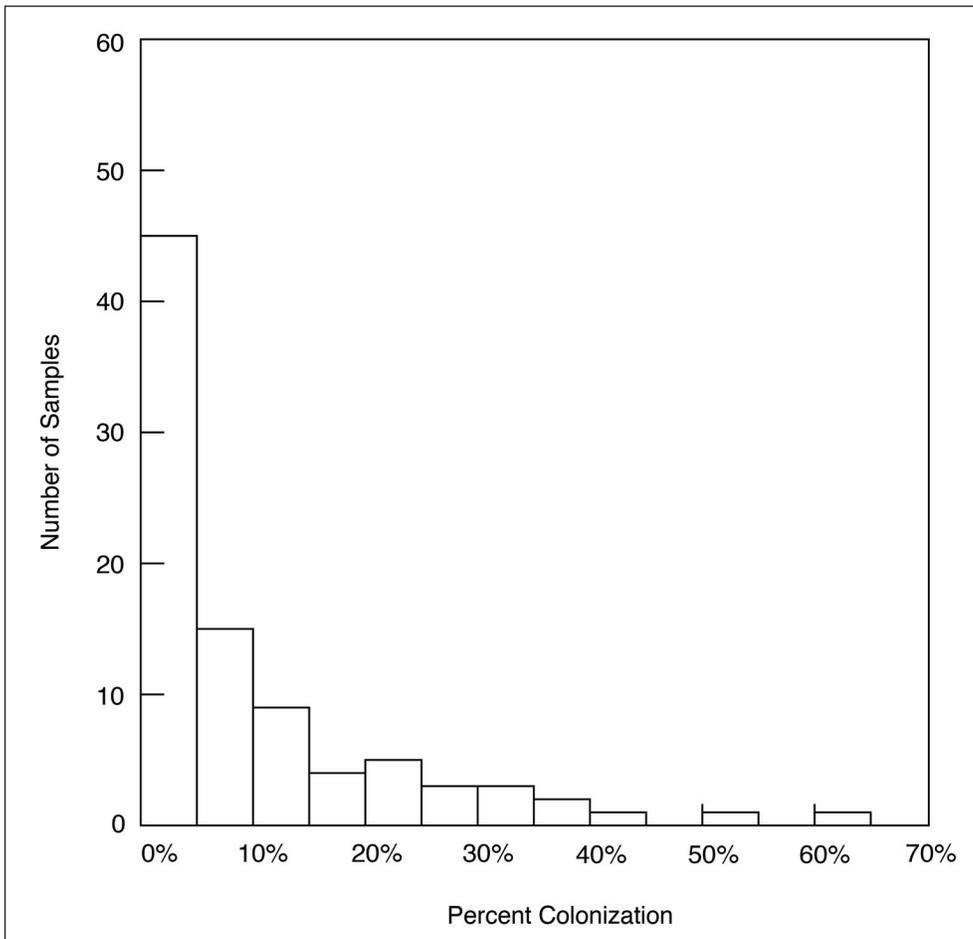

Figure 1. Frequency histogram of AM colonization from 89 *H. perforatum* plants collected in 2009.

have noted neutral, negative, and positive effects of various toxic elements (such as those naturally occurring in serpentine areas) on different AM fungal species (Bartolome-Esteban and Schenck 1994, Pawlowska and Charvat 2004, Smith and Read 2008). Schechter and Bruns (2008) examined AM assemblages in ecotypes of *Collinsia sparsiflora* Fischer and C. Meyer (Spinster's Blue-Eyed Mary; Scrophulariaceae) on and off serpentine substrates in California and found that there were no significant differences in colonization between samples. These workers, however, found that each ecotype was associated with a distinct AM fungal assemblage, and they suggested that it is necessary for serpentine-adapted plants to associate with serpentine-tolerant mycorrhizal fungal taxa in ultramafic soils, whether the plants are "choosing specific" fungi or "tapping nonspecifically" into an edaphically influenced AM community. In contrast, in a study on the serpentine-tolerant grass *Avenula sulcata* (J. Gay) Dumort, Fitzsimons and Miller
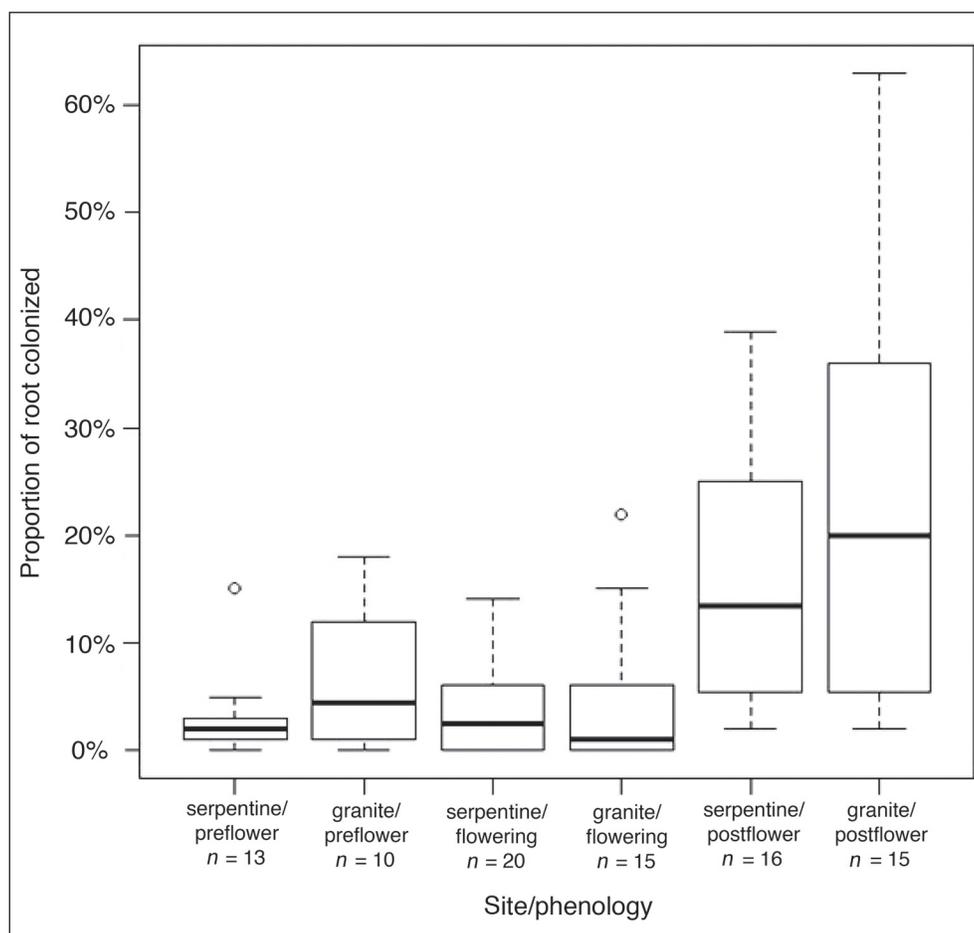

Figure 2. Box plot showing AM colonization for 89 *H. perforatum* plants. Y-axis displays percent colonization. X-axis displays each unique pair of site and phenologic stage. The bottom and top of a box show the first and third quartiles, respectively. Bold lines show medians. Bottom and top bars show minimum and maximum values, respectively, except outliers, which are represented as open circles.

(2010) found no strong evidence of association with distinct AM communities and concluded that plant adaptation to serpentine soils does not involve adapting to unique microbial assemblages.

It is known that exposing arbuscular mycorrhizae to increasing metal concentrations, regardless of whether the fungal strains involved are sensitive or resistant to toxic metals, usually reduces the percentage of root colonization, though populations of fungi isolated from metal-laden environments are often more metal-resistant (Meharg and Cairney 2000). In a review of the available literature, Meharg and Cairney (2000) concluded that AM fungi provide little to no enhanced metal resistance to their hosts. Furthermore, these workers suggested that AM associations in metal-laden environments are not maintained because they confer metal resistance to the plant host but rather for the same reasons they are maintained in other environments, namely improved nutrient acquisition and water relations. They further suggest that AM fungi and their hosts have co-evolved to survive in metal-enriched environments and thus can exploit extremely metalliferous ecological niches. This co-evolution, coupled with evidence that the evolution of metal resistance in AM fungi can be rapid (Meharg and Cairney 2000), could possibly explain why our investigation found no difference in colonization between the serpentine and granite sites despite the fact that increased metal concentrations are known to decrease colonization levels. Similarly, Gonçalves et al. (2001), finding that Ni concentration at a serpentine site in Portugal did not affect the extent of AM colonization in *Festuca brigantina* (Markgr.-Dann.) Markgr.-Dann., suggested that the fungi are adapted to serpentine soil. In contrast to the conclusion reached by Meharg and Cairney (2000), at least one study has shown that AM fungi can reduce the toxic effects of metals on their host plants (Joner and Leyval 1997).

Throughout our study, the values we observed for percent root colonization were fairly low, as two-thirds of our samples exhibited between 0–10% mycorrhizal colonization (Fig. 1). By contrast, Hopkins (1987), studying 27 species in a serpentine grassland in California in May, found high levels of colonization: 91.6% of the herbaceous cover sampled exhibited over 75% mycorrhizal colonization, and 97.7% of the herbaceous cover exhibited over 50% colonization. In 24 samples of *Collinsia sparsiflora* taken in March from serpentine and nonserpentine sites in California, Schechter and Bruns (2008) found all samples to be highly colonized (44–57%), whereas our 89 *H. perforatum* plants had a mean of 10%, with only 3 plants exhibiting colonization levels equal to or greater than 40%. *Hypericum perforatum* is native to Europe, North Africa, and Asia (Maron et al. 2004) but not to North America (Haines and Vining 1998). There is evidence that the species has been introduced into North America multiple times (Maron et al. 2004). Populations of the plant in North America invest less in root biomass and more in first-year reproduction than populations in the native range, suggesting a shift towards a more annual, weedy life history compared to conspecifics in the native range—a pattern observed in many introduced species (Seifert et al. 2009). Furthermore, there is strong evidence that North American

populations of *H. perforatum* have evolved reduced dependence on mycorrhizal fungi (Seifert et al. 2009). Several introduced species have been found to be less dependent on AM fungi than native species with which they co-occur (Seifert et al. 2009, Vogelsang and Bever 2009). Seifert et al. (2009) speculated on the selective pressures involved in causing this phenomenon in *H. perforatum* and non-native species in general, and suggested that anthropogenic disturbance that disrupts and degrades natural AM fungal communities (e.g., intensive agriculture) could select for plants with reduced mycorrhizal dependence. *Hypericum perforatum*'s reduced mycorrhizal dependence in North America could explain the low root colonization values we observed.

Our results show significantly higher colonization in the postflowering stage (October) than in the preflowering and flowering stages (April and July). Phenology appears to have a strong effect on patterns of AM colonization. A study of mycorrhizae in prairie tallgrasses found colonization to be greatest in late summer or fall, and it was suggested that the mycorrhizal fungi involved might be parasitic at that time (Bentivenga and Hetrick 1992, Kennedy et al. 2002). This possibility could be true for our observations, especially in the context of reduced mycorrhizal dependence of *H. perforatum* in North America (Seifert et al. 2009). Thus, the greater colonization we observed in postflowering collections could represent fungal symbionts opportunistically parasitizing their plant hosts as the plants age.

It appears that mycorrhizal colonization in *H. perforatum* is lower on Pine Hill (serpentine) than on Settlement Quarry (granite), because mean colonization is higher on Settlement Quarry in the preflowering and postflowering stages. Our results, however, indicate that the effect of substrate is minor, while the effect of phenology is significant. It is possible that in this case plant phenology is a proxy for other seasonal variables, such as temperature and/or soil moisture. If plant phenology is the "actual" effect, however, it implies that investigators comparing mycorrhizae from different substrates should take particular care in noting whether phenology is aligned between samples. Otherwise, differences that are due to variations in plant phenology among sites could be mistakenly interpreted as being due to edaphic effects.

Literature Cited

Alexander, E.B. 2009. Serpentine geoecology of the eastern and southeastern margins of North America. Northeastern Naturalist 16 (Special Issue 5):223–252.

Bartolome-Esteban, H., and N.C. Schenck. 1994. Spore germination and hyphal growth of arbuscular mycorrhizal fungi in relation to soil aluminum saturation. Mycologia 86:217–226.

Bentivenga, S.P., and B.A.D. Hetrick. 1992. Seasonal and temperature effects on mycorrhizal activity and dependence of cool- and warm-season tallgrass prairie grasses. Canadian Journal of Botany 70:1596–1602.

Boyd, R.S., A.R. Kruckeberg, and N. Rajakaruna. 2009. Biology of ultramafic rocks and soils: Research goals for the future. Northeastern Naturalist 16 (Special Issue 5):422–440.

Brady, K.U., A.R. Kruckeberg, and H.D. Bradshaw, Jr. 2005. Evolutionary ecology of plant adaptation to serpentine soils. Annual Review of Ecology, Evolution, and Systematics 36:243–266.

Branco, S., and R.H. Ree. 2010. Serpentine soils do not limit mycorrhizal fungal diversity. PloS ONE 5:e11757.

Briscoe, L.R.E., T.B. Harris, E. Dannenberg, W. Broussard, F.C. Olday, and N. Rajakaruna. 2009. Bryophytes of adjacent serpentine and granite outcrops on the Deer Isles, Maine, USA. Rhodora 111:1–20.

Casper, B.B., and J.P. Castelli. 2007. Evaluating plant-soil feedback together with competition in a serpentine grassland. Ecology Letters 10:394–400.

Doherty, J.H., B.M. Ji, and B.B. Casper. 2008. Testing nickel tolerance of *Sorghastrum nutans* and its associated soil microbial community from serpentine and prairie soils. Environmental Pollution 151:593–598.

Doubková, P, J. Sudaa, and R. Sudováa. 2012. The symbiosis with arbuscular mycorrhizal fungi contributes to plant tolerance to serpentine edaphic stress. Soil Biology and Biochemistry 44: 56–64.

Fitzsimons, M.S., and R.M. Miller. 2010. Serpentine soil has little influence on the root-associated microbial community composition of the serpentine-tolerant grass species *Avenula sulcata*. Plant and Soil 330:395–405.

Gleason, H.A., and A. Cronquist. 1991. Manual of Vascular Plants of Northeastern United States and Adjacent Canada. 2nd Edition. The New York Botanical Garden, Bronx, NY.

Gonçalves, S.C., M. A. Martins-Loução, and H. Freitas. 2001. Arbuscular mycorrhizas (AM) of *Festuca brigantina*, an endemic serpentinophyte from Portugal. South African Journal of Science 97:571–572.

Haines, A., and T.F. Vining. 1998. Flora of Maine: A Manual for Identification of Native and Naturalized Vascular Plants of Maine. V.F. Thomas Company, Bar Harbor, ME.

Harris, T.B., F.C. Olday, and N. Rajakaruna. 2007. Lichens of Pine Hill, a peridotite outcrop in Eastern North America. Rhodora 109:430–447.

Harrison, S.P., and N. Rajakaruna. 2011. What have we learned from serpentine about evolution, ecology, and other sciences? Pp. 417–428, *In* S.P. Harrison and N. Rajakaruna (Eds.). Serpentine: The Evolution and Ecology of a Model System. University of California Press, Berkeley, CA.

Hopkins, N.A. 1987. Mycorrhizae in a California serpentine grassland community. Canadian Journal of Botany 65:484–487.

Joner, E.J., and C. Leyval. 1997. Uptake of ^{109}Cd by roots and hyphae of a *Glomus mosseae/Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium. New Phytologist 135:353–360.

Kennedy, L.J., R.L. Tiller, and J.C. Stutz. 2002. Associations between arbuscular mycorrhizal fungi and *Sporobolus wrightii* in riparian habitats in arid southwestern North America. Journal of Arid Environments 50:459–475.

Kruckeberg, A.R. 2002. Geology and Plant Life: The Effects of Landforms and Rock Type on Plants. University of Washington Press, Seattle, WA.

Maron, J.L., M. Vilà, R. Bommarco, S. Elmendorf, and P. Beardsley. 2004. Rapid evolution of an invasive plant. Ecological Monographs 74:261–280.

Meharg, A.A., and J.W.G. Cairney. 2000. Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. Pp. 69–112, *In* A.H. Fitter and D.G. Raffaelli (Eds.). Advances in Ecological Research. 30. Academic Press, London.,UK

O'Dell, R.E., and V.P. Claassen. 2009. Serpentine revegetation: A review. Northeastern Naturalist 16 (Special Issue 5):253–271.

Pawlowska, T.E., and I. Charvat. 2004. Heavy metal stress and developmental patterns in arbuscular mycorrhizal fungi. Applied and Environmental Microbiology 70:6643–6649.

Phillips, J.M., and D.S. Hayman. 1970. Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55:158–161.

Pilon-Smits, E.A.H., and J.L. Freeman. 2006. Environmental cleanup using plants: Biotechnological advances and ecological considerations. Frontiers in Ecology and the Environment 4:203–210.

Pope, N., T.B. Harris, and N. Rajakaruna. 2010. Vascular plants of adjacent serpentine and granite outcrops on the Deer Isles, Maine, USA. Rhodora 112:105–141.

Rajakaruna, N., T.B. Harris, and E.B. Alexander. 2009. Serpentine geoecology of eastern North America: A review. Rhodora 111:21–108.

Read, D.J., and J. Perez-Moreno. 2003. Mycorrhizas and nutrient cycling in ecosystems: A journey towards relevance? New Phytologist 157:475–492.

Redecker, D. 2008. Glomeromycota. Arbuscular mycorrhizal fungi and their relative(s). Version 14 January 2008. Available online at http://tolweb.org/Glomeromycota/28715/2008.01.14. Accessed 20 January 2008.

Safford, H.D., J.H. Viers, and S.P. Harrison. 2005. Serpentine endemism in the California flora: A database of serpentine affinity. Madroño 52:222–257.

Schechter, S.P., and T.D. Bruns. 2008. Serpentine and non-serpentine ecotypes of *Collinsia sparsiflora* associate with distinct arbuscular mycorrhizal fungal assemblages. Molecular Ecology 17:3198–3210.

Seifert, E.K., J.D. Bever, and J.L. Maron. 2009. Evidence for the evolution of reduced mycorrhizal dependence during plant invasion. Ecology 90:1055–1062.

Smith, S.E., and D.J. Read. 2008. Mycorrhizal Symbiosis. 3rd Edition. Academic Press, London, UK.

Strauss, S.Y., and R.S. Boyd. 2011. Herbivory and other cross-kingdom interactions on harsh soils. Pp. 181–200, *In* S.P. Harrison, and N. Rajakaruna (Eds.). Serpentine: The Evolution and Ecology of a Model System. University of California Press, Berkeley, CA.

Tyndall, R.W., and J.C. Hull. 1999. Vegetation, flora, and plant physiological ecology of serpentine barrens of eastern North America. *In* R.C. Anderson, J.S. Fralish, and J.M. Baskin (Eds.). Savannas, Barrens, and Rock Outcrop Plant Communities of North America. Cambridge University Press, New York, NY.

Vogelsang, K.M., and J.D. Bever. 2009. Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. Ecology 90:399–407.

Whittaker, R.H. 1954. The ecology of serpentine soils. IV. The vegetation response to serpentine soils. Ecology 35:275–288.

Wilkinson, D.M., and N.M. Dickinson. 1995. Metal resistance in trees: The role of mycorrhizae. Oikos 72:298–300.