

Spatial patterns of ectomycorrhizal fungal inoculum in arbuscular mycorrhizal barrens communities: implications for controlling invasion by *Pinus virginiana*

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Abstract Invasion of globally threatened ecosystems dominated by arbuscular mycorrhizal plants, such as the alkaline prairies and serpentine barrens of eastern North America, by species of ectomycorrhizal (ECM) pine (*Pinus*) seriously threatens the persistence, conservation, and ongoing restoration of these rare plant communities. Using Maryland serpentine barrens and an Ohio alkaline prairie complex as model systems, we tested the hypothesis that the invasiveness of Virginia pine (*Pinus virginiana* L.) into such communities is regulated by the spatial pattern of ECM fungal inoculum in the soil. ECM colonization of pine seedlings can occur by (1) hyphae growing from the roots of mature ECM pines colonizing nearby seedlings (contagion model), (2) pine seedlings being infected after germinating in open areas where spores are concentrated in feces of animals that have consumed sporocarps (centers of infection model), and (3) colonization from spores that are wind-dispersed across the landscape (background model). To test these models of dispersal of ECM fungal inoculum into these barrens, we used autocorrelation and spatially explicit mapping techniques (semivariance analysis and kriging) to characterize the distribution and abundance of ECM inoculum in soil. Our results strongly suggest that

ECM fungi most often disperse into open barrens by contagion, thereby facilitating rapid pine colonization in an advancing front from mature pine forests bordering the barrens. Spatial patterns consistent with the centers of infection model were present but less common. Thus, current management techniques that rely on cutting and fire to reverse pine invasion may be ineffective because they do not kill or disrupt hyphal mats attached to mature roots of neighboring pines. Management alternatives are discussed.

Keywords Alkaline prairie · Serpentine grasslands · Ectomycorrhizae · Virginia pine · Invasion ecology

Introduction

Isolated, unique, and biodiverse oak barrens and grassland–savanna outcrop communities exist within many forested landscapes in eastern North America. The persistence of these outcrop communities has been threatened by *Pinus virginiana* invasion for the past several decades. Characterizing the mechanisms of invasion is more relevant than ever as biological invaders threaten the persistence of native ecosystems and hamper efforts to restore degraded ones. Considerable study has been directed toward understanding why some plant species are successful invaders and why some community types are particularly vulnerable to invasion (e.g., grasslands; Richardson and Bond 1991; Richardson et al. 1994; Lonsdale 1999). Nonetheless, invasion ecology focuses almost exclusively on aboveground dynamics (Rejmanek and Richardson 1996; Lonsdale 1999), while little is known about the role of the mycorrhizal fungal community in plant invasions.

Ectomycorrhizal (ECM) fungal inoculum spreads across communities and ecosystems via three potential mecha-

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nisms. The first is hyphal growth from the root mass of existing ECM plants; hyphal networks may extend up to tens of meters away from the base of ECM trees (Read et al. 1985; Read 1998), and distinct, vegetatively spread ECM genets at least 100 m long (Sawyer et al. 2001) and 300 m² in size (Bonello et al. 1998) have been documented. Seedlings of ECM-dependent species can then successfully establish near existing ECM trees, producing a pattern of colonization resembling a slowly advancing front (Francis and Read 1994). This model for species spread has been termed the contagion model (Alexander et al. 1992; Forman 1995; Read 1998).

ECM fungi also disperse by means of spores produced within sporocarps. Consumption of sporocarps by small mammals and subsequent deposition of densely packed fungal spores in feces can effectively disperse ECM inoculum over great distances (≥ 100 ha) and infect incoming and germinating seeds of ECM plant species (Maser et al. 1978; Kotter and Farentinos 1984; Alexander et al. 1992; Claridge et al. 1992; Cazares and Trappe 1994; Janos et al. 1995; review by Johnson 1996). This pattern of ECM inoculum dispersal follows the centers of infection model of epidemiology and landscape ecology, in which distinct foci for colonization appear far from the existing colonization or species concentration, and spread occurs outward from those centers of colonization (Forman 1995; Janos et al. 1995).

Spores of some ECM fungi may also be dispersed by wind after loss from sporocarps. Given the generally low wind speeds in the forest understory (Geiger 1965), the spatial pattern of inoculum produced by this dispersal mode may not be qualitatively different from that expected under the centers of infection model. However, when sporocarps occur at the edge of a forest or in more open habitats, wind dispersal may produce a generalized background level of inoculum spread broadly and evenly across the ecosystem; in this case, ECM inoculum in open areas is ubiquitous.

Communities dominated by arbuscular mycorrhizal plant species (e.g., grasses, forbs) may resist the invasion of ECM trees (e.g., pines) because of a lack of ECM inoculum. When invasion by ECM trees into open areas dominated by AM plants does occur, the spatial distribution of the trees should mirror the spatial pattern of ECM inoculum. Thus, one might expect ECM trees to occur in a contagion-like pattern, a centers of infection-like pattern, or both. Therefore, in cases where the conservation or restoration goal is to prevent invasion by ECM trees such as species of *Pinus*, the spatial pattern of ECM inoculum spread must be documented and appropriate management strategies to counter that pattern of spread implemented. Within this context, our study objectives were (1) to determine if ECM inoculum is present in two open, barrens ecosystems of high conservation value in eastern North

America whose long-term persistence is threatened by pine invasion, (2) to quantify the spatial distribution and abundance of ECM inoculum in these sites, and (3) to determine whether the abundance of ECM inoculum is more closely linked to soil chemical properties or to host distribution/abundance. We then utilize the results of this study to suggest management strategies for curtailing pine invasiveness based on an understanding of the role of ECM distribution and dispersal.

Materials and methods

Study areas

The Soldiers Delight Natural Environment Area is a 770-ha serpentine grassland/savanna located 15 km west of Baltimore in Owings Mills, MD (Fig. 1). Soldiers Delight is one of the largest intact tracts of serpentine barrens remaining along the extensive serpentine deposits that span the eastern Piedmont of the Appalachian Mountains from Alabama to New Jersey. The barrens outcrops at this site



Fig. 1 Locations of the Edge of Appalachia Prairie Preserve system in Adams County, OH and Soldiers Delight Natural Environment Area in Owings Mills, MD

are dominated by prairie grasses, particularly little bluestem (*Schizachyrium scoparius* Michx. Nash; Knox 1984).

These open barrens were present at the time of European colonization and may have been maintained before that time through a combination of edaphic factors, frequent fire, and grazing by native animals (Marye 1955a, b). After European colonization, ca. 1750, fire and livestock grazing continued to prevent woody species from successfully establishing in these open barrens (Marye 1955a, b). According to both early land surveys and aerial photographs of Soldiers Delight, Virginia pine (*P. virginiana*) and eastern red cedar (*Juniperus virginiana*) were absent from this area until the mid-1930s (Shreve 1910). After the site was released from grazing in the 1930s, pine spread to the site from nearby commercial pine plantations and, since then, aggressive invasion by Virginia pine has reduced the area of open serpentine grasslands at Soldiers Delight by approximately 50% (Tyndall 1992). The speed with which Virginia pine has formed closed canopy forests at Soldiers Delight is remarkable; since 1970, even-aged stands of pine have become dominant in many sites that were barrens openings just a few decades earlier (Knox 1984). Thus, rare serpentine plant populations are threatened with permanent loss from Soldiers Delight due to consequences of pine invasion such as shading, pine-mediated changes in soil properties, and loss of serpentine habitat.

The Lynx Prairie complex is part of the Edge of Appalachia Preserve System (EOA), an assemblage of >6000 ha of mixed oak forests, oak savannas, and alkaline hill prairies in southern Ohio (Fig. 1). The alkaline prairies establish and persist only on the shallow, droughty, and highly alkaline soils formed in Silurian dolomites (Braun 1928). Within the larger EOA Preserve, we focused on the Lynx Prairies, an area first described by and brought under protection through the efforts of the noted ecologist E. Lucy Braun.

The prairie plant association at Lynx Prairie is dominated by big bluestem (*Andropogon gerardii* Vitman), little bluestem (*S. scoparius* Michx. Nash), and side-oats grama (*Bouteloua curtipendula* (Michx.) Torr.). Indian grass (*Sorghastrum nutans* (L.) Nash) and plains Muhlenbergia (*Muhlenbergia cuspidata* (Torr.) Rydb.) are also common, and more than a dozen rare plant species grow in the barrens (Braun 1928; Rankin and O'Bryan 1982).

The alkaline prairie outcrops of Lynx Prairie exist within a forest matrix of Virginia pine, eastern red cedar, and a mixture of oaks (*Quercus* spp.). In contrast to the serpentine barrens in Maryland, it is not known whether these prairie areas were present before European settlement. Following settlement in the early 1800s, edaphic factors, fire, and livestock grazing would have helped prevent the establishment of woody species in these prairie barrens until release from grazing in the early 1900s. In the absence

of grazing and the effective suppression of wildfire in the region, invasion by woody species (Virginia pine in particular) has threatened the persistence and integrity of these prairies. Prairie coverage in the Lynx Prairie complex decreased from 47 to 16% of the region between 1938 and 1971 (Kapustka and Annala 1982; Annala and Kapustka 1983).

Experimental design

We sought to determine whether the three models of ECM inoculum dispersal (contagion, centers of infection, and/or background) were operating in these communities by quantifying the spatial pattern of ECM fungal inoculum in the Soldiers Delight serpentine barrens (areas F and G) and in the Lynx Prairies (Lynx 3 and 5). In June 1999, in each of the four study sites, two to three belt transects 3 m wide and 6–9 m long were randomly established, each of which ran from the forested area through the transitional ecotone into open barrens/prairie vegetation. In addition, belt transects were established in open barrens/prairie areas at least 30 m from any forest vegetation, except in Lynx 5, which was too small to sample an open barren transect that was at least 30 m from forests.

In each belt transect, 2.5-cm diameter soil cores were collected to 7.5-cm depth at 0.5- to 1.0-m intervals in a regular 0.5-m grid pattern and returned to the laboratory under refrigeration. We also performed bioassays on soil taken from the base of 14 individual, isolated trees located in barrens areas >30 m from the border of neighboring forested areas: six Virginia pine saplings at Soldiers Delight, plus five post oak trees (*Quercus stellata*), and three redbuds (*Cercis canadensis*, an AM mycorrhizal understory tree species) at Lynx Prairie. The soil probe was sterilized with 80% ethanol between each sample. Within 48 h, each soil sample was transferred to a 2.5-cm diameter × 5-cm long Conetainer[®] pot (Stuewe and Sons, Corvallis, OR) and was planted with three seeds of Virginia pine (Lawyer Nursery, Plains, MT). Seedlings were grown under typical Ohio summer day/night length in a glass-house and thinned to one seedling/pot after 2 weeks. Pots containing only autoclave-sterilized soil were seeded to serve as controls. Pots were randomized and watered with deionized water as needed, and care was taken to prevent splashing of water and soil among pots. Pine seedlings were harvested after 8–9 weeks, rinsed carefully in a tap water bath, and refrigerated in a formalin/acetic acid/ethyl alcohol solution until analysis. Analysis of ECM colonization of root tips took place over a period of three consecutive months in 2000.

Quantification of ECM colonization was done using a dissecting microscope (×10.5–60) by counting the number of root tips infected with ECM fungi on the basis of the

following criteria: presence of a conspicuous, well-developed mantle; hyphae emanating from lateral roots; ramification, swelling, and/or clumping of lateral roots; and absence of root hairs (Ingleby et al. 1990; Visser 1995; Gehring et al. 1998; Baar et al. 1999). Initially, squash preparations were prepared after roots were cleared and stained with trypan blue (Phillips and Hayman 1970; Boerner et al. 1996) to more precisely distinguish among similar morphotypes. Clearing and staining destroyed many of our initial root bioassays; thus, we used squash preparations and staining only initially to distinguish among ECM morphotypes, so we could accurately identify them later using only a dissecting scope. The number of different ECM morphotypes on each seedling root system and at each study site was recorded. No ECM colonization was observed on control bioassays.

We identified ECM morphotypes instead of conducting DNA analyses because characterizing the specific ECM species present at the site was not germane to our study objectives; rather, we wished to use ECM morphotypes as markers to detect and test ECM dispersal mechanisms across these sites. Further, the morphological characteristics of particular morphotypes can be used to infer their ecological functions, such as dispersal potential, e.g., the presence of ramifying hyphae implies good dispersal via hyphae (Agerer 2001).

Because primary productivity in these barrens outcrops is relatively low, and pH is high at the Lynx Prairie outcrops, we suspected that pH and total C would be most limiting of ECM colonization potential at these sites. Thus, the pH of each soil sample was analyzed in 0.01 M CaCl₂ (Hendershot et al. 1993) and soil organic C determined using Dumas combustion in a Carlo Erba 2100 NCS analyzer (serpentine soils) or Walkley–Black oxidation (calcareous soils; Allison 1965).

Statistical analyses

Differences in colonization intensity among vegetation zones (open barrens, forests, transitional areas) were analyzed by nonparametric rank analyses of variance [ANOVA (PROC RANK, PROC GLM, SAS Institute 1996)], as the large proportion of bioassay seedlings that had no detectable colonization, the large range of colonization within individual transects, and the strongly non-normal distribution of colonization within a site precluded traditional ANOVA.

In any system structured by nonrandom processes, samples taken closer together are more likely to be positively correlated than samples taken farther apart. The degree to which ECM inoculum density exhibited non-random patterning was determined at the transect level using semivariance analysis (GS⁺ Version 2.0, Gamma Design

Software, Plainwell, MI, 1992). Semivariance analysis is based on the premise that if sample points show spatial patterning at the scale measured, then samples will be autocorrelated (Isaaks and Srivastava 1989; Morris 1999). Semivariance analysis allowed us to quantify the degree of spatial autocorrelation in ECM colonization that existed among our bioassay samples and to produce semivariograms that were used to convert the ECM colonization data to spatially explicit contour maps using kriging. Put simply, our spatially explicit experimental data for ECM colonization were used to determine the degree of spatial dependency in ECM inoculum potential among samples (spatial autocorrelation), and maps were then generated in GS⁺ by extrapolating inoculum potential to points between our actual sample points, based upon the degree of spatial dependency of samples and the strength (r^2) of the model (kriging). These maps were then used to infer which dispersal model is dominant in the system for which it was generated. This technique has been used previously to quantify the spatial distribution of soil microbial and chemical properties in other systems in this region (Boerner et al. 1996; Morris 1999; Choesin and Boerner 2000). Nonparametric Spearman's rank-order correlation coefficient analyses (PROC CORR OUTS, SAS Institute 1996) were used to assess whether ECM colonization intensity was correlated with soil chemical properties at $p \leq 0.05$.

Results

Mycorrhizal colonization potential

Five ECM morphotypes were identified in samples from Soldiers Delight, and ECM species composition was strongly dominated by two of them (Table 1). Seven ECM morphotypes were identified at the two Lynx Prairie sites sampled, and ECM species composition at both sites was strongly dominated by three morphotypes (Table 1). Five ECM morphotypes were found in Lynx 3, and despite the low colonization potential of soils from Lynx 5, that site contained five ECM morphotypes.

The proportion of root tips of bioassay pines infected by ECM fungi was highly variable within and among transects (Table 2). There was no detectable colonization in pine bioassays of soils from two of the three open barrens only (O) transects and <2% mean colonization in the third (Table 2). Mean and maximum colonization intensity was also low in the barrens–oak forest (OB) transects at Lynx Prairie (Table 2). The five barrens–pine forest (PB) transects varied more in the percent of root tips infected in bioassays than did the O or OB transects. Mean colonization in the PB transects ranged from 2.4 to 12.2%, and

Table 1 Ectomycorrhizal morphotypes observed in the serpentine barrens of Soldiers Delight Natural Environment Area in Owings Mills, MD and the alkaline prairie barrens at the Lynx Prairies at the Edge of Appalachia, OH

Morphological type	Distinguishing morphological characters	Soldiers Delight F	Soldiers Delight G	Lynx Prairie 3	Lynx Prairie 5
1	Black; club-shaped; surface hairy; lateral roots ramified and unramified; emanating hyphae wiry and abundant; probably <i>Cenococcum geophilum</i> (Fr.)	Not present	+ (PB)	++++ (PB)	++++ (PB, OB)
2	Dark brown-black; surface smooth except at root base; lateral roots unramified; emanating hyphae silky and abundant at base of root tip only	+ (O)	Not present	++ (PB)	Not present
3	White; surface smooth, shiny; lateral roots dichotomously ramified; emanating hyphae absent	++++ (PB, O)	++++ (PB, O)	++ (PB)	++ (OB)
4	Translucent, white; surface smooth, shiny; lateral roots strongly, frequently ramified; emanating hyphae absent	++++ (PB)	++++ (PB, O)	Not present	+ (OB, PB)
5	White; surface smooth, shiny; lateral roots strongly, frequently ramified; emanating hyphae yellow, cottony, abundant on root tips only	+ (PB)	Not present	Not present	++ (PB)
6	Rusty brown; surface smooth; lateral roots ramified; emanating hyphae absent	Not present	Not present	++++ (PB, OB)	++++ (OB, PB)
7	Black; surface smooth; lateral roots unramified; emanating hyphae abundant, cottony, cream-colored on root tips only	Not present	Not present	+ (PB)	Not present
8	White; surface cottony; lateral roots strongly, frequently ramified into tuberculate clumps; emanating hyphae abundant, cottony, golden	Not present	Not present	Not present	++++ (PB)

(*Single plus sign*) rare; (*double plus sign*) common; (*quadruple plus sign*) abundant. Transect types denoted in parentheses are: *PB* barrens–pine forest, *OB* barrens–oak forest, and *O* open barrens only.

maximum observed colonization ranged from 22.8 to 85.0% (Table 2).

Bioassays performed on soil taken from the base of the 14 isolated trees showed that rhizosphere soils of 9 of the 11 pines and oaks lacked sufficient inoculum to colonize even a single root tip of our bioassay pines, as did the soils from under the three redbud trees. One of six rhizosphere soils collected below established but isolated pines at Soldiers Delight contained sufficient inoculum to colonize the bioassay pine but only enough to colonize 4.5% of its root tips. One

of five rhizosphere soils from established post oaks at Lynx Prairie contained enough inoculum to colonize the bioassay pine, infecting 12.7% of its root tips.

Nonparametric ANOVA indicated no significant difference in colonization intensity among seedlings grown in soils from open barrens, pine transition, and pine forest areas at Soldiers Delight ($F=1.83$, $p<0.163$), despite the pine forest seedlings having mean colonization intensity 2.7-fold greater than seedlings grown in barrens–pine forest transition zones and 2.9-fold greater than seedlings grown in open barrens soil

Table 2 Mean and maximum ectomycorrhizal (ECM) colonization (percent of root tips colonized) of *Pinus virginiana* bioassays of soils from Soldiers Delight, MD and the Lynx Prairies, OH

Transect	Transect type	Mean (\pm SE) % colonization	Maximum % colonization	<i>N</i>
Lynx Prairie 3, Transect O	Barrens only	0.0 (0.0)	0.0	35
Soldiers Delight Area G, Transect O	Barrens only	0.0 (0.0)	0.0	28
Soldiers Delight Area F, Transect O	Barrens only	1.9 (0.9)	22.8	26
Lynx Prairie 3, Transect OB	Barrens–oak forest	0.4 (0.3)	10.6	50
Lynx Prairie 5, Transect OB	Barrens–oak forest	1.5 (0.6)	14.0	39
Lynx Prairie 3, Transect PB	Barrens–pine forest	12.2 (3.2)	85.0	41
Lynx Prairie 5, Transect PB	Barrens–pine forest	2.4 (1.2)	36.0	33
Soldiers Delight Area F, Transect PB1	Barrens–pine forest	2.5 (1.2)	40.6	45
Soldiers Delight Area F, Transect PB2	Barrens–pine forest	4.1 (1.0)	22.8	44
Soldiers Delight Area G, Transect PB	Barrens–pine forest	4.2 (1.7)	46.7	37

Minimum percent of root tips colonized in each transect was zero.

N Number of bioassays evaluated

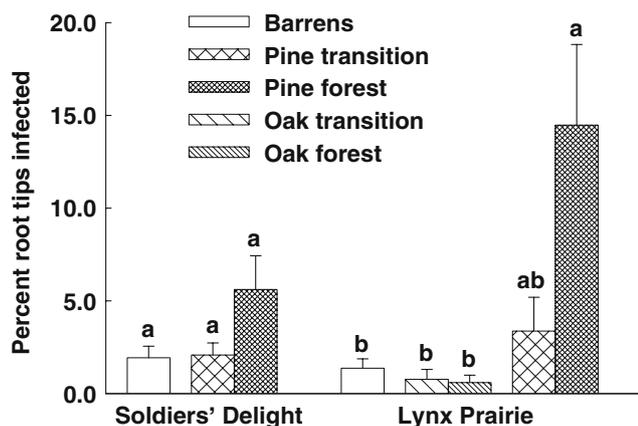


Fig. 2 ECM colonization of *P. virginiana* seedlings grown in soils from alkaline prairie barrens in the Lynx Prairie complex at the Edge of Appalachia, OH and from serpentine barrens at Soldiers Delight, MD. Histogram bars represent means with standard errors shown. Within a site, histogram bars indicated by the same lower case letter were not significantly different at $p \leq 0.05$ following nonparametric ANOVA

(Fig. 2). At Lynx Prairie, seedlings grown in pine forest soil had significantly greater colonization ($F=8.32, p < 0.001$) than did seedlings grown in soil from the open barrens, oak forests, or barrens–oak forest transition zones (Fig. 2).

Spatial pattern of inoculum

Semivariance analysis produced statistically significant models of spatial autocorrelation in ECM inoculum potential for all transects except the open barrens transect at Lynx 3, where no ECM inoculum was detected in any of the samples we bioassayed (Table 3). Among the transects that ran from forest (either pine or oak) to open barrens, the best fit

semivariance models accounted for 68–99% of the total variance among samples within a transect. In contrast, in open barrens transects, the proportion of variance that could be attributed to spatial structure averaged 64% (Table 3).

At Soldiers Delight, the maximum distance at which samples were significantly autocorrelated was <1.4 m in barrens-to-forest transects and >4.4 m in open barrens transects. This suggests greater fine scale structure to ECM inoculum in the barrens forest ecotones than in the open barrens themselves. There was no such relationship among the Lynx Prairie samples, although this is in part due to the complete lack of ECM inoculum potential in soils from the open barrens transect at that site.

The interpolated (kriged) maps of inoculum potential of the open barrens transects indicated little heterogeneity, as two of the three barrens transects lacked inoculum entirely (Fig. 3). The open barrens transect in Soldiers Delight Area F had four small, concentrated areas of inoculum (Fig. 3) and presented a spatial pattern consistent with the centers of infection model.

The spatial patterns of the sparse ECM inoculum present in the two barrens-to-oak forest transects in Lynx Prairie differed somewhat (Fig. 3). At Lynx 3, inoculum decreased, as one moved from the oak forest to the open barrens, thus, suggesting a contagion pattern. In contrast, at Lynx 5, there were two small clusters of inoculum and a pattern more consistent with the centers of infection model (Fig. 3).

Two distinct spatial patterns were apparent among the five barrens-to-pine forest transects (Fig. 4). In the two Lynx Prairie transects and the Soldiers Delight Area F Transect PB1, there was a consistent and gradual decrease in inoculum from the pine forest through the transition zone and into the

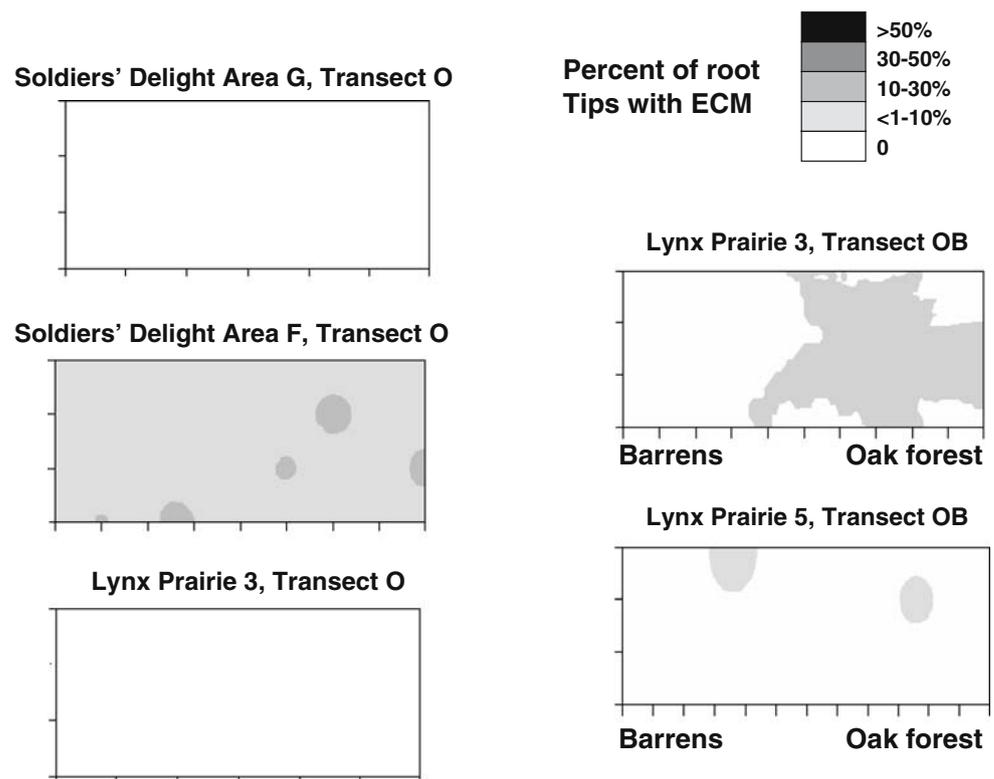
Table 3 Semivariance analysis of spatial structure of proportional colonization of Virginia pine (*Pinus virginiana* L.) roots by ectomycorrhizal (ECM) fungi

Transect	Best-fit model	Structural variance (%)	Model fit (r^2)	Range (m)
Soldiers Delight Area F				
Transect PB1	Isotropic, spherical	99.9	0.949	0.75
Transect PB2	Isotropic, spherical	76.1	0.807	0.30
Transect O	Anisotropic, spherical	63.7	0.733	>4.40
Soldiers Delight Area G				
Transect PB	Isotropic, exponential	99.9	0.734	1.38
Transect O	Anisotropic, linear	65.2	0.441	>4.80
Lynx Prairie 3				
Transect OB	Anisotropic, linear	88.5	0.418	>8.30
Transect PB	Anisotropic, exponential	90.5	0.758	>8.50
Transect O	No colonization, therefore, no spatial model applies			
Lynx Prairie 5				
Transect OB	Isotropic, spherical	99.9	0.459	0.81
Transect PB	Anisotropic, exponential	68.4	0.362	8.14

Structural variance is the proportion of total variance attributable to spatial autocorrelation, i.e., it signifies the degree of dependency among samples at the range given. The range represents the distance between samples at which spatial autocorrelation is no longer significant.

PB Pine forest to open barrens, OB oak–pine forest to open barrens, O open barrens

Fig. 3 Spatial patterns of ECM colonization potential of soils from open barrens (*O*) and oak forest-to-barrens (*OB*) transects at Soldiers Delight, MD and Lynx Prairie, OH. Each division on the axes represents 1.0 m. See Table 3 for details of the semivariogram models used to construct each plot map



open barrens. In contrast, in the remaining two Soldiers Delight transects, the inoculum appeared to diffuse outward from small, distinct clusters of concentrated inoculum (Fig. 4).

Relationships between ECM colonization and soil chemical properties

At Soldiers Delight, there was no significant correlation between colonization potential and soil pH, either overall or among only those samples with >10% ECM root colonization. Samples from the open barrens tended to fall at the higher end of the pH range, whereas those from the pine forests were somewhat lower in pH.

In contrast, there was a significant negative relationship between soil pH and ECM colonization potential in soils from the Lynx Prairies: overall ($r=-0.56$, $p<0.001$), in samples from barrens-to-pine forest transects ($r=-0.53$, $p<0.001$), and samples from barrens-to-oak forest transects ($r=-0.217$, $p<0.041$), but not among samples from open barrens transects. Pine forest samples that produced the greatest ECM colonization were generally arrayed at the lower end of the pH gradient, with those from barrens and oak forests falling higher on the pH gradient.

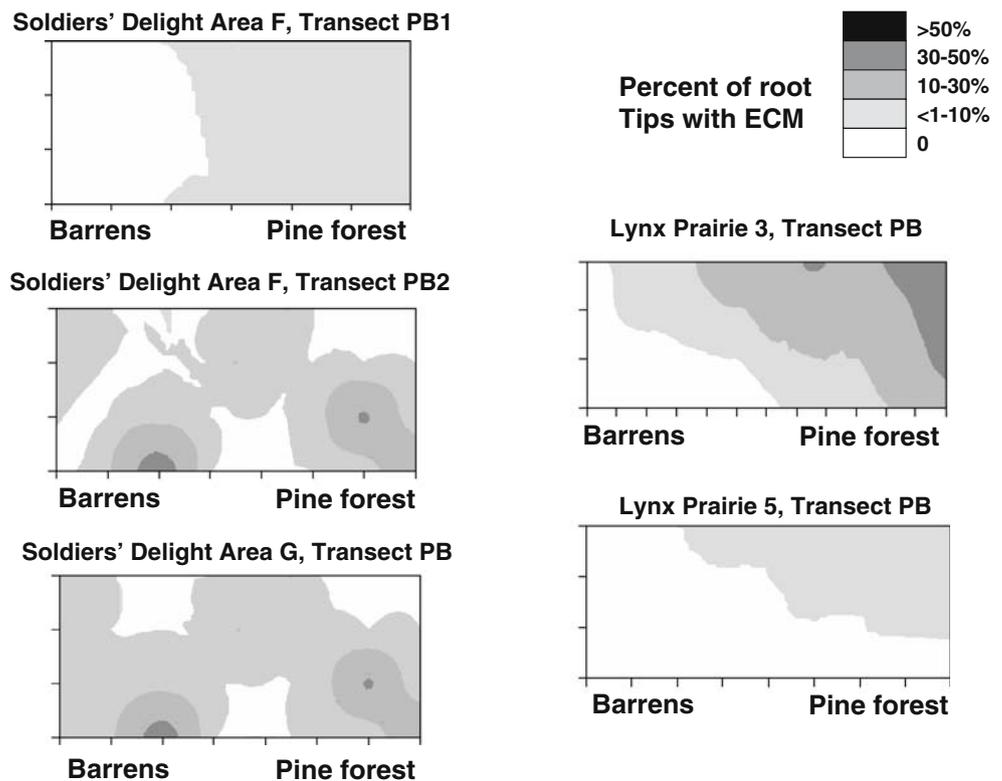
There was no linear relationship between ECM colonization and soil organic C content in either site, either overall or when the samples were stratified by transect type. ECM colonization potential seemed to be greatest in samples in the middle of the range of soil organic C content in both sites.

Discussion

Intensive, aboveground-focused management efforts to curtail the invasion of *P. virginiana* into globally threatened barrens communities in eastern North America have been only marginally effective; thus, we sought more effective answers belowground. Our first objective was to determine if there was sufficient ECM inoculum present in these barrens communities to effectively colonize newly established seedlings of pine. In only two of the ten transects did we find a complete absence of ECM inoculum potential, and those were in open barrens areas >30 m from the nearest ECM tree. At the same time, in only one transect did we observe ECM colonization in >10% of the pine bioassay points, and that was one which ran from an established oak forest to the open barrens and was lacking significant pine. Based on the limited scope of this study, it appears that ECM inoculum is present in low but highly variable amounts in the surface soils of these barrens and of the ecotones between the barrens and neighboring woody plant communities.

Our second objective was to determine the spatial pattern of the ECM inoculum that was present and to determine which of the three dispersal models might be supported by that pattern. In no transects was ECM inoculum ubiquitous, which would have suggested a background dispersal pattern. Gradual, gradient-like changes in inoculum potential consistent with a contagion model were present in three of the Lynx Prairie transects and one at Soldiers Delight. At

Fig. 4 Spatial patterns of ECM of soils from pine forest-to-barrens (PB) transects at Soldiers Delight, MD and Lynx Prairie, OH. Each division on the axes represents 1.0 m. See Table 3 for details of the semivariogram models used to construct each plot map



the same time, distinct, relatively concentrated patches of inoculum embedded in a larger matrix of non-infective soil were present in three of the transects at Soldiers Delight and one at the Lynx Prairies. The final two transects had no inoculum at all. Thus, spatial patterns consistent with a centers of infection model were as common as those consistent with the contagion model.

Documented field patterns of pine colonization at Soldiers Delight (Knox 1984; Tyndall 1992), in other serpentine outcrops in southeastern PA (Wallenstein 1996), and at the Lynx Prairies (Thiet 2002) are consistent primarily with the contagion model of ECM dispersion. Colonization of open barrens by pines is most pronounced at pine forest edges at both study areas, and barrens at Soldiers Delight are punctuated by even-aged clumps of small pines that grow clustered under established mature pine trees. Five of the eight ECM morphotypes found at these sites have abundant emanating hyphae, evidence of the presence of short-, medium-, or long-distance “exploration types” (Agerer 2001) of ECM that could facilitate a contagion dispersal model.

Studies have consistently documented contagion of ECM fungal colonization through common mycorrhizal networks (CMN) linking plant root systems (Molina and Trappe 1982; Read et al. 1985; Read 1988; Perry et al. 1989; review by Simard and Durall 2004). Hyphae on mature root systems can aid in the establishment of neighboring linked seedlings by providing them with

nutrients before their photosynthetic output can support the energy cost of an ECM association. Recent research documents mycorrhizal-mediated C transfer between plants of the same or different species directly through intact CMN (Wu et al. 2001), via hyphae comminuted by soil organisms (Tuffen et al. 2002), and/or through mycorrhizal–soil pathways in which C compounds move from one mycorrhizal root system to another through the soil (Newman et al. 1989; also see Reid and Woods 1969; Finlay and Read 1986; Simard et al. 1997; review by Simard and Durall 2004). CMN transfer of N and P has also been documented among plants (review by Simard et al. 2002).

However, it should also be noted that it is not uncommon at either site to see single, isolated oaks and pines in these two barrens communities, at distances from other ECM plants that exceed the distances to which hyphal spread has been documented. Either these isolated individuals persist without ECM colonization or they are colonized by inoculum that spread by a mechanism other than hyphal spread. Examination of the roots of isolated oaks from Lynx Prairie and bioassays of inoculum potential of soils from the rhizospheres of isolated Soldiers Delight pines and Lynx Prairie oaks all suggest that most of these isolated seedlings are persisting without ECM colonization. In fact, several of the post oaks we examined from the Edge of Appalachia had fungal structures in their roots that were more consistent with AM fungi than ECM fungi. Prior studies indeed suggest that some species of *Quercus* can

harbor both AM and ECM (Molina et al. 1992). Predominantly ECM tree species may use AM in the seedling stage until they encounter ECM or until they can support the energy demands of an ECM association. Egerton-Warburton and Allen (2001) suggest that early use of AM may be important to the initial establishment of plants in low nutrient or calcareous soils (also see Lapeyrie and Chilvers 1985) such as those present at the Edge of Appalachia. The mycorrhizal dynamics in these barrens outcrops are more intricate than we anticipated; future studies of plant–mycorrhizal relationships should carefully evaluate the relative importance of AM, EM, and ectendomycorrhizae in these systems.

Spatial patterns of spore abundance consistent with a centers of infection model, such as those we observed in four of our Soldiers Delight transects, do not alone ensure strong inoculation potential in the field. Colonization of seedlings by isolated clumps of ECM inoculum requires either that pine seeds land on areas of high ECM basidiospore density within one growing season of their deposition in feces or that ECM fungi subsist saprophytically for some time without a host plant. The number of spores required to infect one seedling in the field may be $>10^6$ (Castellano et al. 1985). Kotter (1981) estimated that tassel-eared squirrels disseminated as many as 10^9 fungal spores/g of fecal material, but Kotter and Farentinos (1984) found that only 33% of pine seedlings inoculated with squirrel feces developed an ECM colonization. Although synchronous and sufficient temporal and spatial deposition of seeds and dense spore patches may occur, our data do not support the view that this mechanism is sufficient to account for the heavy pine recruitment we observed in these two communities, as we noted no pine seedlings at or near the points of greatest inoculum density.

We were surprised by the relative lack of ECM inoculum at Lynx 5—so much so that we sampled the same transects at Lynx 5 a second year and ran the bioassay experiment again; both years showed identical results. In addition, both our field observations and those of site managers at Lynx Prairie (Minney, personal communication) indicate that Lynx 5 is unique at EOA in lacking pine regeneration below mature pines, along pine forest edges, or in open barrens areas. These findings may be the result of fire; from 1985–2000, Lynx 5 was burned five times, while Lynx 3 was burned only twice, and fires at Lynx 5 were inadvertently more intense (Minney, personal communication). Thus, more frequent, intense fires at Lynx 5 may reduce ECM inoculum (including fire-resistant propagules, see Baar et al. 1999) and suppress ECM—and pine—recolonization of this outcrop.

Our final objective was to determine whether ECM inoculum potential was correlated with soil properties. ECM colonization was consistently negatively correlated with pH in the alkaline Lynx Prairies. Although pine litter

can acidify soil over a span of decades (e.g., Alway et al. 1933), most of the more acidic and highly ECM infective microsites we sampled in the Lynx Prairies were associated not with areas of pine dominance but instead with native oak forests on soils developed on sandstone outcrops bordering the dolomites that support the prairie soils and plants.

In contrast, ECM colonization in the Soldiers Delight serpentine barrens soil was not correlated with pH, although there was some suggestion that soils occupied by pines might be more acidic. Testing this hypothesis requires a sampling design very different from the one we designed for the purposes of geostatistical analysis. In neither site was there any significant correlation between soil organic matter and ECM colonization. Because nitrogen may also limit ECM mycorrhizal colonization, future studies of mycorrhizal dynamics at these sites should explore the relationship between mycorrhizae and soil N.

The current approach to curtailing the invasion of pine into these two threatened barrens communities is a combination of low-intensity prescribed fire and hand cutting of individual pine stems (high-intensity fires at Lynx 5 are atypical and unintended). Removal of the litter layer by low-intensity burning in *Pinus* stands may actually increase ECM fungal diversity and sporocarp production (Jansen and van Dobben 1987). Thus, infrequent, low-intensity burns that temporarily curtail pine encroachment may inadvertently improve the conditions for long-term pine recruitment.

Our research strongly suggests that Virginia pine colonization of open barrens at Soldiers Delight and the Edge of Appalachia's Lynx Prairies would not be effective without the ECM mycelial mat that extends from mature root systems of established pines. Thus, management strategies that specifically address the spread of ECM fungal inoculum into areas currently devoid of pines may be more effective and less labor intensive than current, vegetation-centered management techniques. For example, trenching around established pine forests may more effectively curtail pine recruitment into open serpentine outcrops by breaking up the mycelial network that supports and infects encroaching seedlings.

Prior field experiments suggest that this technique may be effective. Fleming (1983) found that mycelial strands severed from the roots of their host plants were not capable of infecting seedlings planted around established hosts. Tuomey and Kienholz (1931) and Lutz (1945) describe a series of trenching experiments in which the growth and survivorship of eastern white pine (*Pinus strobus* L.) stand was reduced dramatically by repeated trenching. Scientists and managers may wish to experiment with trenching as a possible technique to mitigate pine invasion at these and similar sites.

We conclude that the colonization of alkaline prairies and serpentine grasslands by pine may be regulated in large

part by movement into these systems of ECM propagules in the form of hyphal inoculum networks resembling an advancing front. Unless management efforts in these ecosystems address the underlying issue of ECM fungal dispersal, efforts to eliminate pine and to conserve and restore habitat for unique prairie and barrens plant associations will require, at the least, repeated interventions involving considerable time and effort and may, in the longer term, be in vain.

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