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Research

Shared ectomycorrhizal fungi between a herbaceous perennial (*Helianthemum bicknellii*) and oak (*Quercus*) seedlings

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Summary

• Ectomycorrhizal infection of *Quercus* seedlings can be low at a distance from established ectomycorrhizal vegetation. Here we investigate whether *Helianthemum bicknellii*, a herbaceous ectomycorrhizal perennial of prairies and oak savannas, creates patches of elevated ectomycorrhizal infection of *Quercus* seedlings.

• We performed two studies. First, ectomycorrhizas of *H. bicknellii* were compared with ectomycorrhizas of *Quercus* spp. Second, soil bioassays were conducted with *Quercus* macrocarpa seedlings grown in soils from near *H. bicknellii*; near established *Quercus* spp.; or distant from ectomycorrhizal vegetation.

• Eight species associated with *H. bicknellii* were identified: *Cenococcum geophilum*, *Russula* aff. *amoenolens*, an unknown Pezizalean fungus, *Laccaria laccata*, *Tomentella* sp., *Lactarius mutabilis*, *Russula* aff. *pectinatoides*, and *Cortinarius* sp. Internal transcribed spacer restriction fragment length polymorphism (ITS RFLP) patterns of all species except *Cortinarius* sp. matched to ectomycorrhiza from *Quercus*. In the bioassay, ectomycorrhizal infection was higher in near-*Helianthemum* soils than in distant soils, but lower than in near-*Quercus* soils.

• These results demonstrate that *H. bicknellii*, a common herbaceous plant of oak savannas, shares ectomycorrhizal partners with *Quercus* and provides patches of increased ectomycorrhizal infection of *Quercus* seedlings.

Key words: *Cenococcum geophilum*, ectomycorrhiza, facilitation, *Helianthemum bicknellii* (hoary frostweed), inoculum potential, *Quercus* spp. (oak), savanna, seedling establishment.

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Introduction

The majority of ectomycorrhizal plants are trees and woody shrubs. Nonetheless, a small number of herbaceous plants are known to form ectomycorrhizas, including members of the Cistaceae such as *Helianthemum bicknellii* (hoary frostweed), a herbaceous perennial to semi-woody shrub of dry oak woodlands, savannas and prairies of eastern North America (USDA NRCS, 2002). Although *H. bicknellii* can be semi-woody, at our study sites in Minnesota, USA it grows as an entirely herbaceous perennial. Herbaceous ectomycorrhizal plants may play a unique ecological role. As a herbaceous perennial, *H. bicknellii* may be able to establish or persist on sites that are inhospitable to ectomycorrhizal trees. For example, *H. bicknellii* has been shown to increase in abundance with increasing fire frequency, while woody trees and shrubs generally decline (Tester, 1996). In studies at the Cedar Creek Long-Term Ecological Research (LTER) site in Minnesota, *H. bicknellii* also re-establishes an average of 37 yr after agricultural abandonment (Gleeson & Tilman, 1990), substantially faster than other ectomycorrhizal vegetation. The ability of *H. bicknellii* to persist or re-establish on disturbed sites may permit the survival of ectomycorrhizal fungi even when woody ectomycorrhizal hosts are absent. In turn, the presence of established ectomycorrhizal fungi in soils may facilitate the establishment or re-establishment of seedlings of

ectomycorrhizal tree species following disturbance (Kropp & Trappe, 1982; Molina & Trappe, 1982; Newman, 1988; Perry *et al.*, 1989), potentially contributing to succession from prairie or old-field to savanna or oak woodland. However, such interactions between *H. bicknellii* and woody vegetation depend on whether *H. bicknellii* shares ectomycorrhizal partners with *Quercus*, and whether infection of *Quercus* seedlings is actually increased near *H. bicknellii*.

Our first hypothesis was that *H. bicknellii* shares multiple ectomycorrhizal fungal associates with *Quercus* spp. The ectomycorrhizal habit is believed to have evolved independently in the Fagales (including *Quercus*) and Cistaceae (Brundrett, 2002), which might lead to some degree of incompatibility between mycorrhizal associates. Nonetheless, host specificity of many ectomycorrhizal fungi is often low (Molina *et al.*, 1992), leading to the possibility that *Quercus* and *H. bicknellii* might share many associates. Old-world *Helianthemum* spp. mycorrhizal associates appear to be somewhat host-specific, particularly *Terfezia* spp. and *Tirmania* spp. (Díez *et al.*, 2002), but there is some evidence that new-world Cistaceae have less specific associations (Malloch & Thorn, 1985). In addition, one of the most commonly described associates of *Helianthemum* spp. is *Cenococcum geophilum* (Read *et al.*, 1977; Read & Haselwandter, 1981), a species known to infect *Quercus*.

Based on the assumption that *Helianthemum* and *Quercus* do share ectomycorrhizal fungi, our second hypothesis was that ectomycorrhizal infection of *Quercus* seedlings would be low in soil collected at a distance from any established ectomycorrhizal vegetation, and higher in soil from near *H. bicknellii* or established *Quercus* spp. trees. Established ectomycorrhizal vegetation can greatly increase the mycorrhizal infection of *Quercus* spp. seedlings, while seedlings germinating at a distance from established ectomycorrhizal vegetation may have very low ectomycorrhizal infection (Berman & Bledsoe, 1998; Dickie *et al.*, 2002; Lindahl, 2002). Similar results have also been obtained in a number of other ectomycorrhizal tree species (Perry *et al.*, 1989; Borchers & Perry, 1990; Boerner *et al.*, 1996; Terwilliger & Pastor, 1999). As *Quercus* nutrient uptake is correlated with ectomycorrhizal infection (Dickie *et al.*, 2002), increased ectomycorrhizal infection of *Quercus* seedlings near established vegetation has the potential to increase seedling nutrient uptake and growth such that established vegetation may facilitate *Quercus* seedling establishment (Newman, 1988; Dickie *et al.*, 2002).

One of the most commonly described mycorrhizal associates of *Helianthemum* spp. is *C. geophilum* (Read *et al.*, 1977; Read & Haselwandter, 1981). *Cenococcum geophilum* is a cosmopolitan fungus with a very low degree of host specificity (LoBuglio, 1999). In a previous study (Dickie *et al.*, 2002) we found that *C. geophilum* was proportionally more abundant on *Quercus* seedlings planted distant from established *Quercus* spp. trees than on seedlings planted near established *Quercus* spp. trees. Based on the common observation of *C. geophilum* on *Helianthemum* spp., the low host-specificity of *C. geophilum*, and previous observations of low *C. geophilum* infection on *Quercus* seedlings planted near established *Quercus* trees, our third hypothesis was that ectomycorrhizal infection of *Quercus* seedlings in soil from near *H. bicknellii* would be dominated by *C. geophilum*.

In order to test our three hypotheses we conducted two studies. First, we tested hypothesis 1 by comparing ectomycorrhizas collected from harvested *H. bicknellii* roots with known associates of *Quercus* spp. using restriction fragment length polymorphism (RFLP) and DNA sequence analysis. Second, we tested hypotheses 2 and 3 by growing *Quercus macrocarpa* seedlings in glasshouse bioassays of soils collected near either *H. bicknellii* or established *Quercus* spp. trees, or distant from either ectomycorrhizal host (distant treatment).

Glasshouse bioassays have been used successfully to investigate mycorrhizal infection in a number of other studies (Perry *et al.*, 1982; Borchers & Perry, 1990), but they do involve disruption of soil and unnatural growing conditions. *Ex situ* bioassays are also limited in the types of interaction that can be investigated, permitting investigation of changes in inoculum potential (as in this study), but not permitting investigation of interactions via a common mycelial network (see review by Newman, 1988). Our chosen research site, located on a scientific and natural reserve, was ideal in having isolated *Helianthemum* and areas without any other known ectomycorrhizal vegetation, but also had high conservation value as one of the highest diversity and most 'native' of prairie sites in the reserve. Establishing and protecting trees in this site was therefore discouraged by reserve policies, while removing small amounts of soil for a bioassay was viewed as having a lesser impact. As part of another study, we replicated the near-*Quercus* and distant treatments with an *in situ* bioassay in nearby old fields; data from that study are discussed for comparison.

Materials and Methods

Field collection of *H. bicknellii* ectomycorrhiza

Root systems of a total of 12 *Helianthemum bicknellii* Fern. plants were collected in July 2002 from two sites (old field 57 and savanna burn unit 104) at the Cedar Creek Natural History Area (CCNHA), an LTER site in central Minnesota, USA (Latitude 45°24′45″, Longitude 93°12′45″). Roots were excavated by hand in an attempt to obtain as much of the root system as practical. Excavated root systems were stored under refrigeration for no more than 3 d. Root systems were then washed under running water and all fine roots excised for further examination.

Fine roots of *H. bicknellii* were examined for ectomycorrhizal infection under a dissecting microscope, with all ectomycorrhizal morphotypes described. The fungus *C. geophilum* was identified on the basis of characteristic morphology (LoBuglio, 1999). All other unique ectomycorrhizal morphotypes encountered on each plant were described under both

low- and high-power magnification, and two or three samples saved for DNA extraction and RFLP and sequence analysis.

DNA was extracted from root tips using a modified commercial DNA extraction kit (REDExtract-N-AmpTM Plant PCR kit, XNA-P, Sigma, Saint Louis, MO, USA), following the protocol of Avis *et al.* (2003). Genomic DNA extracts were amplified with ITS1F and ITS4 primers (Gardes & Bruns, 1993) and restriction digested with *Hin*fI and *Dpn*II enzymes (NEB, Beverley, MA, USA). Resulting RFLP patterns were compared with a database of 200 unique RFLP patterns from 69 known ectomycorrhizal sporocarps (D.J. McLaughlin and co-workers, unpublished data) and over 1000 RFLP collections from *Quercus* seedling roots (I.A.D., P.G. Avis and D.J. McLaughlin, unpublished data) using the GOOD-ENOUGH RFLP MATCHER (GERM) program (Dickie *et al.*, 2003) to match RFLP patterns.

One sample of each unique RFLP pattern was sequenced and compared with published sequences in GenBank using BLAST (Altschul *et al.*, 1997). Internal transcribed spacer region (ITS1F and ITS4 primers) PCR products were cleaned with QiaQuick PCR Purification kits (Qiagen, Mississauga, Ontario, Canada) and sequenced at the Advanced Genetic Analysis Center of the University of Minnesota.

Bioassay of soils from near *H. bicknellii*

Soils near and distant from *H. bicknellii* individuals and near *Quercus* trees were collected from an approx. 5.6 ha area of an old field (CCNHA number 81 = burn unit 102) at the CCNHA LTER site. The site was a former agricultural field, abandoned from agricultural use before 1938. Vegetation on the site includes many native prairie and savanna forbs and grasses. The site has been maintained in an open condition by burning 1 out of 3 yr since 1964. Further description of CCNHA site conditions is given in Peterson & Reich (2001).

Ten established *H. bicknellii* were located spread widely throughout the field. On 8 August 2002 we collected two intact soil monoliths (intact blocks of soil) adjacent (< 30 cm) to each of these established plants (near-*Helianthemum* treatment). Ten additional pairs of intact soil monoliths were taken at 5–10 m from each near-*Helianthemum* sample (distant treatment). Locations of distant samples were interspersed with locations of near-*Helianthemum* samples. Distant samples were taken at least 5 m from any established *H. bicknellii* and at least 20 m from any established *Quercus* spp. tree or other known ectomycorrhizal plant. These distances are probably sufficient to avoid any influence of *H. bicknellii* or *Quercus* trees on mycorrhiza, based on known distributions of *Quercus* roots (Lyford, 1980) and mycorrhizas (Dickie *et al.*, 2002; I.A.D., unpublished data), and the relatively small size of *H. bicknellii* plants at this site (typically < 40 cm tall). Nine additional pairs of samples were taken from the edge of the study site, 1–3 m from the trunk of established *Quercus ellipsoidalis* and *Quercus macrocarpa* trees at the edge of the field (near-*Quercus* treatment). Near-*Quercus* treatment soils were taken without regard to the proximity of *H. bicknellii*. The slightly lower replication of the near-*Quercus* treatment was inadvertent.

For each treatment, an intact monolith of soil was cut with a shovel, lifted by hand, and immediately placed into a 12.5 cm diameter, 12.5 cm tall round plastic pot. A second paired sample was taken within 0.5 m of the first soil monolith. Pairs were treated similarly and averaged before statistical analyses. The shovel used to collect intact soil monoliths was washed in 10% household bleach with a water rinse between collection of monoliths. Any plants growing within the soil sample were pulled by hand or clipped at the soil surface, minimizing soil disturbance. Pots with soil were wrapped in plastic to prevent cross-contamination and immediately transported to the glasshouse. Pot locations were randomized in the glasshouse. Within 6 h of sample collection (also on 8 August 2002), two pregerminated (with emerged root radicle 0–3 cm long), surface-sterilized *Q. macrocarpa* acorns were planted into each intact soil monolith. Pots were maintained in the glasshouse, watering as needed, until 2 December 2002. Sterile controls were not included. Airborne contamination of glasshouse experiments with *Quercus* appears to be rare as compared with conifers, and sterile controls from previous (P.G. Avis and co-workers, unpublished data) and concurrent (I.A.D. and P.G. Avis, unpublished data) studies in the same glasshouse room found no airborne contamination on sterile controls. Any airborne contamination of bioassays would only decrease, not increase, differences between the distant and other treatments.

In all pots, at least one seedling successfully established and grew. In pots with two established seedlings, one seedling was chosen at random for measurement of mycorrhizal infection. Roots of seedlings were cleaned by shaking off excess soil, then washing roots over a screen with running water. All fine roots were excised from seedlings and stored in water under refrigeration for up to 2 wk before measurement of ectomycorrhizal infection. No visible degradation of roots occurred during storage. Fine roots were chopped into fragments 1–2 cm long, and a random subsample of these root fragments was collected for visual assessment of ectomycorrhizal infection under ×40 magnification with a dissecting microscope. Our goal was to obtain a minimum of 100 root tips per sample; we actually observed an average of 462 root tips per sample, with a minimum of 192. Any root tips of uncertain infection status were examined under compound microscope for the presence of a Hartig net and mantle. All mycorrhizal infection was measured by a single researcher (I.A.D.). *Cenococcum geophilum* was distinguished based on morphological characters. No other ectomycorrhizal fungi were identified to species for this part of the study. The decision not to identify fungi in the bioassay was, in part, a result of poor morphological differentiation of mycorrhiza, as appears to be typical for non-*C. geophilum* ectomycorrhizas on young *Quercus* seedlings (Dickie *et al.*, 2002).

Table 1 Identification of field-collected *Helianthemum bicknellii* ectomycorrhiza with number of collections, morphology and best RFLP match to *Quercus* ectomycorrhizal RFLP database

Putative ID	*N**	Morphology†	RFLP match
Cenococcum geophilum	6	Black to dark brown, with characteristic stellate mantle pattern; wide, unbranched hyphae	NA‡
Laccaria laccata	2	White to light gold, few hyphae	*Laccaria laccata*
Russula aff. *amoenolens*	2	Light gold, abundant bottle-shaped cystidia	*Russula* aff. *amoenolens*
Pezizalean I	2	Dull white, smooth, no clamp connections, septate, light gold smooth hyphae	Pezizalean I
Russula aff. *pectinatoides*	1	Light gold, bottle-shaped cystidia	*Russula* aff. *pectinatoides*
Tomentella sp.	1	Tan to gold, many coarse hyphae, septate	*Tomentella* sp.
Lactarius cf. *mutabilis*	1	White, no hyphae observed	*Lactarius mutabilis*
Cortinarius sp.	1	White, 'fuzzy' with abundant tan to white hyphae, clamps present	No match in database

*Number of collections.
†All morphotypes were monopoid (unbranched) root tips.
‡*Cenococcum* was identified based on morphology only.

We did not measure vesicular arbuscular mycorrhizal (VAM) infection in the present study, although VAM infection does occur in *Quercus* seedlings (Dickie *et al.*, 2001; Egerton-Warburton & Allen, 2001). In other studies at Cedar Creek we have observed VAM on *Q. macrocarpa* seedlings at very low frequency and abundance, even where ectomycorrhizal infection was low (I.A.D., personal observation), suggesting that VAM infection is a relatively unimportant factor at this site.

We compared infection rates among near-*Helianthemum*, distant, and near-*Quercus* treatments using the mean infection level of the two paired samples from each sample point as our observations ($n = 29$). As infection data violated the assumptions of parametric tests, means were compared using the nonparametric Kruskal–Wallis test, with Nemenyi test for means separation (Zar, 1999). Statistical tests were performed using MINITAB ver. 10.5 (Minitab Inc., State College, PA, USA).

Results

Comparison of *H. bicknellii* and *Quercus* ectomycorrhizas

The root architecture of *H. bicknellii* was such that finding ectomycorrhizal root tips was difficult. All the samples collected were characterized by a small number of long, unbranched woody roots approx. 3–5 mm in diameter with extremely limited numbers of fine roots (where ectomycorrhizal fungi would be found). Of 12 plants harvested, we obtained ectomycorrhizal root tips from 10, although very few (< 20) root tips were obtained from any one plant. Four plants had one unique morphotype; three had two morphotypes; and the remaining three plants had three morphotypes each.

Of the 19 total morphotype observations (Table 1), six were *C. geophilum*. Three samples failed to amplify in PCR and remained unidentified. Of the 10 remaining morphotypes, three RFLP patterns were found twice each, matching *R.* aff. *amoenolens*, *L. laccata*, and a Pezizalean fungus (Pezizalean I) in comparisons of RFLP patterns. Previous phylogenetic analysis of large subunit sequences placed Pezizalean I in clade VI of Hansen *et al.* (2001) with *Ruhlandiella*, several *Peziza* and *Tirmania*; this clade also includes *Terfezia* (K. Hansen, personal communication). Four RFLP patterns were found once each, these were *R.* aff. *pectinatoides*, *Tomentella* sp., *L. mutabilis* and an unknown fungus. The unknown fungus was later identified as a *Cortinarius* sp., based on sequence data. In general, sequence analysis supported the RFLP matches (Table 2). We failed to obtain a sequence from one sample (RFLP matching to *L. mutabilis*), despite repeated efforts and an attempt to clone the PCR products generated from this sample. The ITS sequence of Pezizalean I matched to a *Terfezia* sequence, but given the relative paucity of ITS sequences for North American Pezizalean fungi in GenBank, this identity is probably valid only to family level. The ITS sequence of *Tomentella* was short (281 bp) because of poor sequence quality, but the BLAST results were identical to RFLP-based identification. Six of the seven identified species RFLPs matched to previous collections of *Quercus* mycorrhiza from Cedar Creek, the one *Cortinarius* sp. collection being the exception.

Bioassays

Ectomycorrhizal infection of *Quercus* seedlings was greatest in the near-*Quercus* treatment (68%), lowest in the distant treatment (9%), and intermediate in the near-*Helianthemum* treatment (44%, Fig. 1; $P < 0.001$). The differences were pronounced, and all treatments were significantly different from one another at $P < 0.05$.

Table 2 Sequence-matching data with putative ID, GenBank accession number, sequence length, best match in GenBank, number of identities and *E* values

Putative ID	Accession number	Length (bp)	GenBank match	Identities	*E* value
Laccaria laccata	AY640408	700	AF204814: *Laccaria laccata*	623/669	0.0
Russula aff. *amoenolens*	AY640409	389	AF418615: *Russula amoenolens*	374/381	0.0
Pezizalean I	AY640410	461	AF501260: *Terfezia spinosa*	186/195	2e-82
Russula aff. *pectinatoides*	AY640411	637	AY061732: *Russula pectinatoides*	614/634	0.0
Tomentella sp.	AY640412	281	AF272904: *Tomentella atramentaria*	276/280	e-146
Cortinarius sp.	AY640413	550	AF495455: *Cortinarius* sp.	501/506	0.0

All sequences are the ITS region, using primers ITS1F and ITS4.

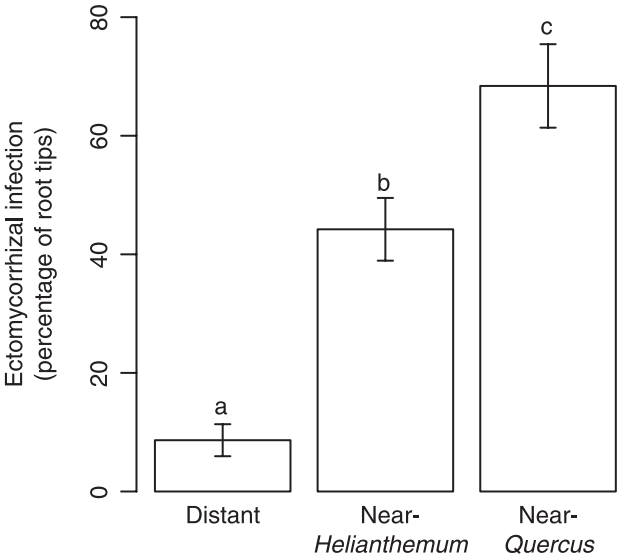

Fig. 1 Ectomycorrhizal infection of bioassay seedlings in experiment 2. Error bars are 1 SEM. Letters indicate significant differences between treatments at $P < 0.05$ (Nemenyi test).

Cenococcum geophilum was present on 79% of *Quercus* seedlings overall. Absolute infection rates (percentage of root tips) of *C. geophilum* were significantly higher in the near-*Quercus* treatment than in the near-*Helianthemum* or distant treatments (Fig. 2a; $P < 0.001$). In contrast to our hypothesis, relative *C. geophilum* infection rates (as a percentage of all mycorrhizal root tips) were high in both the distant and near-*Quercus* treatments, and significantly lower in the near-*Helianthemum* treatment (Fig. 2b; $P = 0.017$).

Discussion

Cenococcum geophilum and six of the other seven RFLP types found on *H. bicknellii* were known associates of *Quercus* spp., providing strong support for our first hypothesis, that *H. bicknellii* shares multiple ectomycorrhizal fungal associates with *Quercus* spp. Four of the six fungal genera identified, *Cenococcum*, *Cortinarius*, *Laccaria* and *Russula*, were previously described as producing sporocarps near *Helianthemum* spp. or other Cistaceae (Malloch & Thorn, 1985; Fellner & Biber, 1989). We are unaware of any previous report of *Tomentella* or *Lactarius* ectomycorrhiza in the Cistaceae; however there have been few previous reports on the mycorrhizal associates of new-world Cistaceae. Pezizalean I was not identified to genus, but is consistent with observations of Pezizalean fungi on old-world Cistaceae (Díez *et al.*, 2002). The frequency of host-generalist, epigeous fungi observed in our study is consistent with the observation of Malloch & Thorn (1985) that new-world Cistaceae appear to be somewhat distinct in mycorrhizal associates from old-world Cistaceae, where more host-specific fungal associates are frequently described (Díez *et al.*, 2002). This may, however, be more indicative of a reporting bias than a true biological pattern (Malloch & Thorn, 1985). More work on mycorrhizal associates of *Helianthemum* across its entire range is needed to test this hypothesis.

The one sample that failed to RFLP match to a previous collection from *Quercus* was a *Cortinarius* spp. ectomycorrhiza. *Cortinarius* spp. are a highly diverse component of the mycorrhizal community at Cedar Creek (Avis *et al.*, 2003), and the failure to match RFLP types could simply reflect an inadequacy of the database, rather than host specificity. At present our RFLP database includes 200 unique RFLP patterns, including eight RFLP patterns from *Cortinarius* spp. sporocarps, while we estimate total fungal diversity at Cedar Creek at around 260 species based on species area curve extrapolation (I.A.D. and co-workers, unpublished data). Alternatively, the high diversity of *Cortinarius* species in these sites may suggest greater specialization than other fungi, potentially including greater host specificity as some *Cortinarius* species are apparently quite host specific (Arnolds & Kuyper, 1995).

Quercus macrocarpa seedlings growing in soils collected near *H. bicknellii* showed much higher ectomycorrhizal infection than those growing in soils collected at a distance from established ectomycorrhizal vegetation. This supports our second hypothesis, that ectomycorrhizal infection of *Quercus* seedlings would be low in soil distant from any established

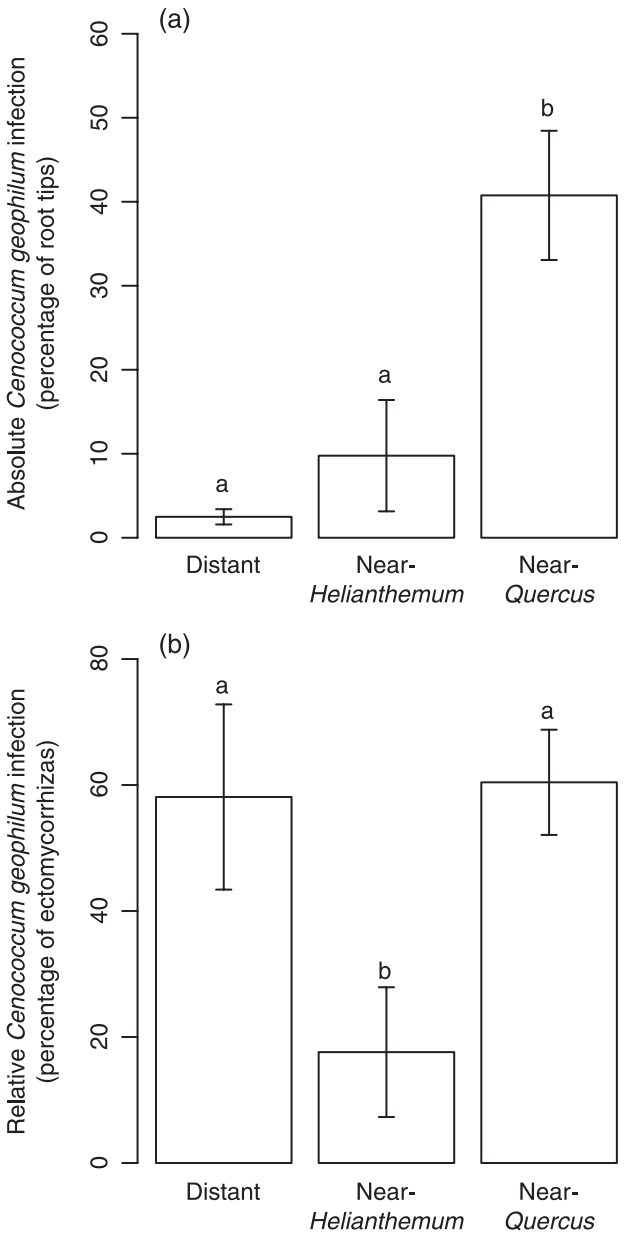

Fig. 2 *Cenococcum geophilum* infection of bioassay seedlings in experiment 2 measured as percentage of total root tips (a) and percentage of ectomycorrhizal root tips (b). Error bars are 1 SEM. Letters indicate significant differences between treatments at $P < 0.05$ (Nemenyi test).

ectomycorrhizal vegetation, and high in soil from near *H. bicknellii* or established *Quercus* spp. trees. The low levels of ectomycorrhizal infection of *Q. macrocarpa* seedlings in the distant treatment is consistent with previous observations of low ectomycorrhizal infection of *Quercus* spp. planted distant from established ectomycorrhizal trees (Berman & Bledsoe, 1998; Dickie *et al.*, 2002; Lindahl, 2002). By creating patches of elevated ectomycorrhizal infection potential, *H. bicknellii* may facilitate the establishment of *Quercus* spp. seedlings in old fields and savanna openings. Our results are similar to reports of facilitation of *Pseudotsuga menziesii* seedling ectomycorrhizal infection by pioneering hardwoods (Borchers & Perry, 1990) or *Arctostaphylos* (Horton *et al.*, 1999).

Interestingly, ectomycorrhizal infection of the near-*Helianthemum* treatment, while higher than the distant treatment, was significantly lower than in the near-*Quercus* treatment. Given the lack of any evidence of strict host specificity in experiment 1, we do not think that host specificity played a role in the lower levels of *Q. macrocarpa* infection in near-*Helianthemum* as compared with near-*Quercus* soil. *Helianthemum bicknellii* is a relatively small plant with a low density of ectomycorrhizal root tips on its long, widely spreading roots. Populations of ectomycorrhizal fungi near *H. bicknellii* may therefore be less dense than near established *Quercus* spp. trees, potentially leading to lower levels of inoculum in the soil. This is consistent with the very low numbers of ectomycorrhizal root tips that we were able to collect from any individual *H. bicknellii* in experiment 1.

Increased mycorrhizal infection of *Quercus* seedlings near *H. bicknellii*, and any consequent increase in seedling nutrient uptake and growth potential, are not the only possible implications of interspecific sharing of mycorrhizal associates. Sharing mycorrhizal associates may permit *Quercus* and *H. bicknellii* to form links into a common mycelial network, potentially lowering initial costs of establishing mycorrhizal infection, giving seedlings more rapid access to a potentially extensive established mycelial network, shifting the community composition of fungal infection, or shifting the costs and benefits of mycorrhizal infection (Newman, 1988). It is also possible that nutrients or carbon may be transferred among plants via mycorrhizal linkages (Newman, 1988; Simard *et al.*, 1997), although unequivocal evidence of ecologically significant net transfers of carbon among photosynthetic plants remains elusive (Robinson & Fitter, 1999; Simard *et al.*, 2002). Studying interactions via a common mycelial network was not possible using the *ex situ* bioassay technique of the present study, but may be a fruitful area for future research.

Our third hypothesis, that ectomycorrhizal infection of *Quercus macrocarpa* seedlings in soil from near *H. bicknellii* would be dominated by *C. geophilum*, was contradicted by the results of the study. Absolute levels of *C. geophilum* infection were similar on near-*Helianthemum* and distant seedlings, and relative levels of *C. geophilum* infection were significantly lower on near-*Helianthemum* seedlings than on seedlings of the other treatments, even though *C. geophilum* was the most common fungal associate of *H. bicknellii* in this study and of other *Helianthemum* species in past studies (Read *et al.*, 1977; Read & Haselwandter, 1981). This result may reflect other environmental factors. In a concurrent study using *in situ* bioassay seedlings planted at different distances from established trees, *C. geophilum* was found at high relative abundances on seedlings closest to trees, and at 20 m from

trees, with a low relative abundance at intermediate distances (I.A.D. and co-workers, unpublished data). In this ecosystem, inoculum of *C. geophilum* appears therefore to be ubiquitous at low absolute abundances (giving it a high relative abundance when other fungi are absent) and highly abundant (both absolutely and relatively) immediately adjacent to *Quercus* trees.

This study utilized a glasshouse bioassay technique to determine the level of mycorrhizal inoculum in soils from near to, and at a distance from, ectomycorrhizal hosts. While intact soil bioassays provide a quick, relatively low cost measurement of relative infectivity of different soils, they may be unrepresentative of field conditions. In particular, disruption of mycelium and severing of hyphal linkages to plants may reduce the abundance of fungi without resistant propagules (Taylor & Bruns, 1999). Minimizing soil disruption and rapid planting of seedlings, as in the present study, may partially mitigate these effects. Intact soil bioassays do have several advantages in that they permit environmental factors such as light to be standardized, and minimize disruption to the research site. In the present study, using intact bioassays was preferred because the research site is a unique habitat for native prairie vegetation, and minimizing disruption was an important constraint to the experimental design. We are able to corroborate our results by comparing infection levels of two of our three treatments with those observed in first-year results from an ongoing study with *Quercus* spp. seedlings planted in the field in similar near-*Quercus* and distant-from-*Quercus* treatments. These treatments were in three other old fields at CCNHA, no more than 2 km from the present study. In that study, near-*Quercus* seedlings had 63.2 ± 4.0% of root tips infected (mean ± SE), while distant-from-*Quercus* seedlings had 12.8 ± 3.7% infection (I.A.D. and co-workers, unpublished data). These numbers are remarkably similar to the infection levels observed in the near-*Quercus* (68.4 ± 7.0%) and distant (8.7 ± 2.7%) treatments in the present study. This suggests that, at this site, intact soil monoliths give reasonable estimates of the extent of mycorrhizal inoculum in different microsites.

This study provides evidence that *H. bicknellii* can facilitate ectomycorrhizal infection of *Quercus* seedlings. Whether this will lead to *H. bicknellii* facilitating the establishment, survival or growth of *Quercus* seedlings is a more complex question. *Quercus* seedling establishment in these sites is limited by multiple abiotic and biotic factors, including limited seed dispersal, low nutrient availability, droughty soils, herbivory and periodic fires. Obtaining ectomycorrhizal infection may therefore be a necessary, but not sufficient, prerequisite for *Quercus* seedling establishment.

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