Mycorrhizal Fungi from Protocorms, Seedlings and Mature Plants of the Eastern Prairie Fringed Orchid, Platanthera leucophaea (Nutt.) Lindley: A Comprehensive List to Augment Conservation

Author(s) :Lawrence W. Zettler and Kurt A. Piskin
Source: The American Midland Naturalist, 166(1):29-39. 2011.
Published By: University of Notre Dame
DOI:
URL: http://www.bioone.org/doi/full/10.1674/0003-0031-166.1.29

Am. Midl. Nat. (2011) 166:29–39

Mycorrhizal Fungi from Protocorms, Seedlings and Mature Plants of the Eastern Prairie Fringed Orchid, Platanthera leucophaea (Nutt.) Lindley: A Comprehensive List to Augment Conservation

LAWRENCE W. ZETTLER[1]
Department of Biology, Illinois College, 1101 West College Avenue, Jacksonville 62650

AND

KURT A. PISKIN[2]
Department of Biology, Saint Louis University, 3507 Laclede Avenue, Saint Louis, Missouri 63103

ABSTRACT.—The Federally threatened Eastern Prairie Fringed Orchid, *Platanthera leucophaea* (Nutt.) Lindley (Orchidaceae), has experienced long-term decline largely due to habitat loss and degradation. Although this species has been propagated from seed in the laboratory, achieving seedling survival *ex vitro* has been problematic, forcing conservationists to sow seeds directly into field sites in an attempt to generate seedlings. Given that the mycorrhizal fungi needed for germination *in situ* have sporadic distributions, sowing seeds of this threatened species indiscriminately is not a preferable option. Thus, locating fungal ''hotspots'' using seed baits, and amending soil with fungi may have practical merit. In anticipation of the latter possibility, we provide a comprehensive list of the 75 mycorrhizal fungi isolated from *P. leucophaea* protocorms, seedlings and mature plants during the past 10 y from sites in Illinois and Michigan, including newly acquired strains from five additional sites in Illinois. Collectively, 66 of the 75 isolates (88%) were assignable to the anamorphic form-genus *Ceratorhiza*, including all of the fungi recovered from the five additional sites. This further supports the hypothesis that *P. leucophaea* relies primarily on *Ceratorhiza* to fulfill its initial and long-term mycotrophic needs. Although *Ceratorhiza* appears to be an ubiquitous associate of *P. leucophaea*, it should not be assumed that specific strains of this genus are equally widespread. Thus, we advocate that the fungi used in conservation should be limited to strains acquired from the same or nearby populations.

INTRODUCTION

The Eastern Prairie Fringed Orchid, *Platanthera leucophaea* (Nutt.) Lindley (Orchidaceae; Fig. 1), of the tallgrass prairie has experienced long-term decline largely due to habitat loss/degradation and is currently listed as Federally threatened (U.S. Fish & Wildlife Service, 2007). Formerly widespread across the Midwest, east into Ontario and Maine, the species is limited to prairie remnants and wetlands (fens) north of the Wisconsinan glacial boundary (Bowles *et al.*, 2005). In 2007, 79 extant populations were identified, but only 28% of these sites had full legal protection (U.S. Fish & Wildlife Service, 2007). Well-coordinated efforts to recover this species are currently in place involving a network of scientists, private landowners and volunteers, yet the conservation of *P. leucophaea* remains a precarious endeavor. Propagation through seed has been a high priority, but progress has been limited by low fruit set and poor seed quality (*i.e.*, low embryo viability) probably linked to genetic

[1] Corresponding author: e-mail: lwzettle@ic.edu
[2] Current Address: Division of Natural Sciences, University of Illinois – Springfield, 1 University Plaza, Springfield 62703

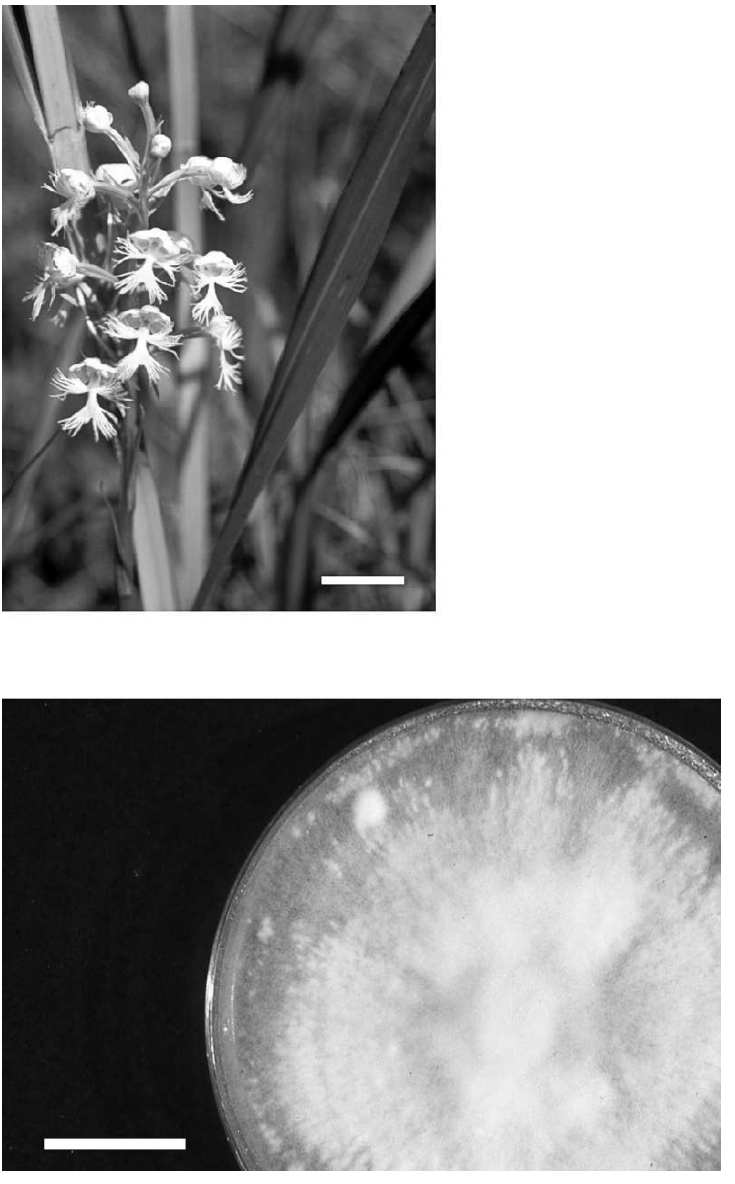

FIG. 1.—*Platanthera leucophaea* in flower (A) and its mycorrhizal associate, *Ceratorhiza* sp., on potato dextrose agar within a 9 cm diameter Petri dish (B). The cottony texture of the culture's aerial mycelium—typical of *Ceratorhiza* strains—is clearly evident. Scale bars = 2 cm

inbreeding among closely-related individuals in smaller populations (Bowles *et al.*, 2002). These problems are compounded by the species' reliance on mycorrhizal fungi necessary to propel the orchid life cycle to completion *in situ*—a requirement of all members of the Orchidaceae (Rasmussen, 1995).

To address the latter, the technique of symbiotic seed germination (Clements *et al.*, 1986; Dixon, 1987) was applied as a means to acquire laboratory-grown seedlings for reintroduction into suitable habitats. Although *Platanthera leucophaea* has since been propagated in this manner to a leaf-bearing stage under laboratory conditions (Zettler *et al.*, 2001, 2005), achieving seedling survival *ex vitro* has been problematic for reasons that remain unclear. Until this dilemma can be resolved, the propagation of this orchid hinges solely on directed seed sowing *in situ*. This practice, coupled with artificial hand pollination, has been employed for almost a decade and may explain an increase in 20 populations (59 to 79) since 1999 (U.S. Fish & Wildlife Service, 2007). The appearance of spontaneous seedlings suggests that the mycorrhizal fungi specific to the germination and maturation process have persisted in these sites. Unfortunately, the fungi utilized by *P. leucophaea* have sporadic distributions (Zettler *et al.*, 2005; Piskin *et al.*, 2003), and targeting their whereabouts remains a hit or miss affair. Considering that viable *P. leucophaea* seeds are sometimes difficult to acquire in sufficient quantity, sowing seeds indiscriminately is not a preferable option.

To improve seed germination *in situ*, techniques are needed to locate fungal ''hotspots'' and to artificially amend soil with fungal inoculum. In Australia, several such protocols have surfaced (*e.g.,* Batty *et al.*, 2006a, b; Swarts, 2007) that may have practical merit for *Platanthera leucophaea* here in North America. In anticipation of the latter possibility, we provide a comprehensive list of the mycorrhizal fungi isolated from *P. leucophaea* protocorms, seedlings and mature plants during the last 10 y, from sites in Illinois and Michigan. The strains that have prompted seed germination and development *in vitro* are highlighted, and their accession numbers at the University of Alberta Microfungus Collection and Herbarium in Canada (UAMH) are provided. We also report mycorrhizal fungi from roots of established plants from three additional sites in Illinois (Nachusa, Ogle Co.; Grant Creek, Will Co.; Somme Prairie Grove, Cook Co.), and from protocorms acquired from two prairie remnants that currently lack the species (Anderson Prairie, Christian Co.; Denby Prairie, Macoupin Co.).

MATERIALS AND METHODS

Fungal isolation from orchid tissues.—All mycorrhizal fungus strains were isolated using standard protocols (Currah *et al.*, 1987, 1990; Zettler *et al.*, 2003, 2005). Living orchid tissues from leafless, mycotrophic seedlings (protocorms) and lateral roots from mature plants and seedlings were promptly transported to the laboratory within 24 h of collection. Protocorms were obtained using the seed baiting technique described by Rasmussen and Whigham (1993). Briefly, seeds of *Platanthera leucophaea* were placed between two sheets of nylon mesh suspended within 35 mm plastic slide mounts (Polaroid Corp., Cambridge, MA) which were then subsequently buried in sod for 1–2 y. The small pore size of the mesh (95 μm) allowed fungal hyphae present in the sod to infiltrate the mesh, infect the seeds and prompt germination to the protocorm stage. Protocorms trapped between the mesh are then recovered, and their associated mycorrhizal fungi are subsequently isolated in the laboratory. Orchid tissues (roots, protocorms) were initially rinsed under tap water to remove surface debris, followed by surface sterilization using a solution of 5% absolute ethanol (EtOH), 5% Clorox® bleach (5.25% NaOCl; Clorox Company, Oakland, CA) and 90% sterile DI water. Tissues were immersed in this solution for 1 min under agitation. After this time, the solution was decanted off, and tissues were then rinsed twice in sterile DI water. Protocorms were added to a drop of sterile DI water within empty 9 cm diameter Petri plates. The epidermis of roots from mature plants and seedlings was scraped off by scalpel,

and clumps of cortical tissues were removed and placed in a drop of sterile DI water within separate Petri dishes. These tissues were then macerated by scalpel, releasing individual cortical cells into the water droplet that were visible to the naked eye. Molten modified Melin-Norkran's agar (MMN; Marx, 1969) or fungal isolation medium (FIM; Hollick, 2004) was poured over the droplet, gently swirled and allowed to cool and solidify. Plates were incubated at ambient temperature (ca. 22 C) for up to 1 wk until individual hyphae could be observed emanating from cortical cells/pelotons suspended in the agar. Using a dissection microscope, pure cultures were obtained by excising hyphal tips with a sterile scalpel, followed by transfer to the surface of potato dextrose agar (PDA; Difco™, Becton, Dickinson and Co., Sparks, MD).

Fungal identification and storage.—Genus-level taxonomic treatment of the mycorrhizal fungi reported herein follows Moore (1987). Orchid mycorrhizal strains were distinguished from common molds facilitated by published descriptions, namely Currah *et al.* (1987, 1990, 1997), Richardson *et al.* (1993) and Zettler *et al.* (2003). All strains recovered from *Platanthera leucophaea* that were assignable to known mycorrhizal genera were retained for further study. Strains that originated from the same tissue source were carefully screened for subtle differences in pure culture. Those that exhibited visual differences (*e.g.,* growth rate, colony coloration, presence/absence of monilioid cells) were retained and assigned an accession number. These cultures were then stored at Illinois College under refrigeration (4 C) in darkness on PDA slants in screw-cap tubes and/or over modified oats medium (Dixon, 1987). Every attempt was made to retain mycorrhizal fungi that appeared unique in culture at the time of isolation. Cultures were sub-cultured to fresh agar slants yearly. To retain culture viability in storage >1 y between sub-culturing, sterile heavy mineral/paraffin oil (Fisher Scientific, Fair Lawn, NJ) was added over the surface of the culture on agar slants and stored at 4 C. Cultures that either prompted germination and development to the protocorm stage, or were deemed potentially important (*e.g.,* acquired from different protocorms *in situ*) were deposited into the University of Alberta (Canada) Microfungus Collection and Herbarium (UAMH) for safekeeping and future use through cryopreservation.

RESULTS AND DISCUSSION

To date, 75 mycorrhizal fungus strains have been recovered from *Platanthera leucophaea* protocorms, seedlings and mature plants spanning nine populations in Illinois (Fig. 2) and Michigan (Table 1). The majority (66/75 or 88%) of the fungi were identified as members of the anamorphic genus *Ceratorhiza* (Fig. 1) and the remaining number were assignable to *Epulorhiza* (Table 1). A third anamorphic genus associated with orchids worldwide – *Moniliopsis* (teleomorphs = *Thanatephorus/Waitea*; Currah *et al.*, 1997) – has yet to be isolated from *P. leucophaea*. Nearly half (35/75) of these strains have been deposited into the UAMH collection for safekeeping and future use (Table 1). This total (75) expands upon the previous number (41) reported by Zettler *et al.* (2005), in part, because of subsequent isolations that ensued during 2007–2008 involving three new sites in Illinois (Ogle, Will and Cook Cos.; Table 1). Two additional prairie sites (Christian, Macoupin Cos., IL) that lacked *P. leucophaea* but appeared suitable for the species were also sampled for fungi though the use of seed baits. This resulted in 15 additional strains, 14 of which originated from Denby Prairie in Macoupin Co. (Table 1). Of special significance is that these 15 strains were isolated from protocorms – the earliest growth stage of the orchid (Fig. 3). The acquisition of protocorms from sites lacking *P. leucophaea* suggests that these sites harbor suitable fungi for germination, but seedling recruitment might be restricted by limited seed dispersal.

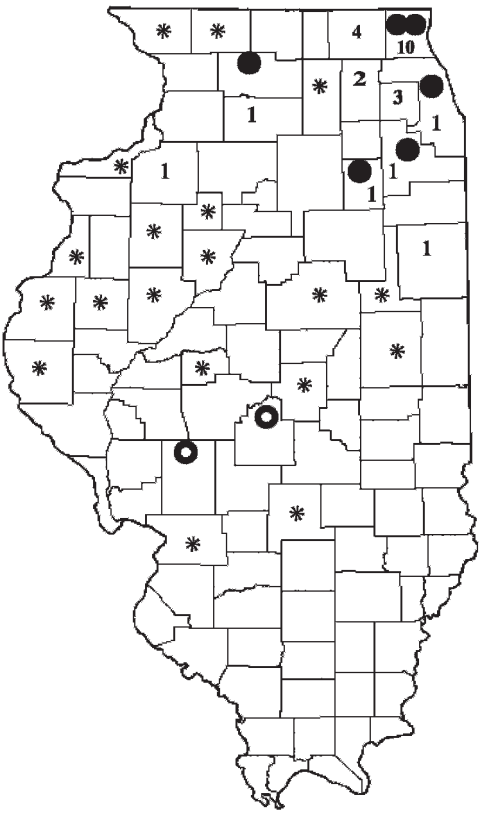

FIG. 2.—Illinois distribution map of *Platanthera leucophaea* populations that yielded mycorrhizal fungi to date (solid dark circles) and the number of extant orchid populations per county. Open circles denote the two remnant sites in Christian Co. (Anderson Prairie) and Macoupin Co. (Denby Prairie) that lacked *P. leucophaea* but yielded mycorrhizal fungi from protocorms acquired from seed baits. Counties marked by an asterisk represent the orchid's former distribution based on Sheviak (1974) as cited in Bowles *et al.* (2005)

Protocorms of terrestrial orchids are subterranean and colorless, deriving all of their carbon from fungi during this initial growth stage (Rasmussen, 1995). In a natural setting, orchids like *P. leucophaea* must make physical contact with this fungus host via seed dissemination, and failure to do so results in an abrupt end to the orchid's life cycle. For *ex situ* conservation protocols aimed at terrestrial orchids (*e.g.,* Swarts and Dixon, 2009a, b), acquiring fungal strains that prompt germination is of paramount importance and is best achieved by their extraction from orchid tissues (Rasmussen, 1995), preferably protocorms (Zettler *et al.*, 2003). The 15 protocorm-derived strains reported herein, combined with the seven reported previously (Zettler *et al.*, 2005) brings the total number of protocorm isolates to 22 spanning three different prairie sites (Hildy, Anderson, Denby). Of these 22, six have been secured in Canada (UAMH 10217, 10218, 10457, 19498, 10499, 10500). The total deposition of 35 mycorrhizal strains from *P. leucophaea* into UAMH represents a start, but only six orchid populations have been surveyed for fungi so far. In Illinois alone, >30 additional populations await study (Fig. 1). Studies that utilize molecular techniques (*e.g.,*

Table 1.—Summary of mycorrhizal fungi isolated from *Platanthera leucophaea* in Illinois and Michigan prairies (1998–2008). Cultures marked by an asterisk (*) were acquired from protocorms. UAMH accession numbers listed in **bold** represent cultures that prompted seed germination *in vitro* (Zettler *et al.*, 2001, 2005). All UAMH cultures listed may be obtained at: www.devonian2.ualberta.ca/uamh/

State	Year	Population	# *Epulorhiza* strains (mature plant)	# *Ceratorhiza* strains			UAMH accession number
				Protocorm	Seedling	Mature	
MI	1998	Monroe Co.[1]	—	—	—	4	**9611**
IL	1998	Lake Co. Wrigley/ Abbott				4	**9610**
IL	1999	Lake Co. Wadsworth	5	–	4	14	9855, 9856, 9857, 9858, 9859, 9860, **9861**
IL	2002	Grundy Co. Hildy	4	7	—	—	10219, 10220, 10217*, 10218*
IL	2004	Christian Co. Anderson	—	1	—	—	10457*
IL	2004	Macoupin Co. Denby	—	14	—	—	10498*, 10499*, 10500*
IL	2007	Ogle Co. Nachusa	—	—	—	6	10974, 10975, 10976, 10977, 10978, 11011
IL	2007	Will Co. Grant Creek	—	—	—	2	10979, 10980
IL	2008	Cook Co. Somme Prairie Grove	—	—	—	10	10981, 10982, 10983, 10984, 10985, 10986, 10987, 10988, 10989, 10990
Total		**9 populations** (8 Illinois, 1 Michigan)	**9**	**22**	**4**	**40**	**35 deposited into UAMH**

[1] The identity of the Michigan population has been withheld at the request of Michigan DNR

FIG. 3.—Opened seed packet revealing a cluster of eight *Platanthera leucophaea* protocorms from Denby Prairie (Macoupin Co., IL). The packet was recovered on 21 Jul. 2004 after remaining in sod for ca. 25 mo. Mycorrhizal fungi (*Ceratorhiza* sp.) isolated from these protocorms were deposited into the University of Alberta Microfungus Collection and Herbarium as UAMH 10498, 10499, 10450. Scale bar = 3 cm

DNA sequencing of ITS genes) to ascertain genetic variation among these (and newer) strains are also needed.

Compared to other terrestrial orchids in North America, the prairie fringed orchids (*Platanthera leucophaea*, *P. praeclara*, *P. lacera*) have received modest attention with respect to their mycorrhizal associations (*e.g.,* Bowles *et al.*, 2005; Sharma *et al.*, 2003a, b; Zelmer and Currah, 1995; Zelmer *et al.*, 1996; Zettler *et al.*, 2001, 2005; L.W. Zettler, unpub. data). As a group, fungi assignable to *Ceratorhiza* have been recovered from protocorms, seedlings and mature plants of both *P. leucophaea* and *P. praeclara* alike, and with regularity (Zettler *et al.*, 2001, 2005; Sharma *et al.*, 2003a, b). Strains of *Epulorhiza* - one of the most common mycorrhizal associates of terrestrial orchids worldwide (Currah *et al.*, 1997) – have also been isolated from these orchids but to a lesser extent. While *Ceratorhiza* isolates have prompted *in vitro* seed germination of *P. leucophaea* (Zettler *et al.*, 2001, 2005), *Epulorhiza* strains have not been effective. Taken together, the prevalence of *Ceratorhiza* spanning all three growth stages (protocorms, seedlings, mature plants) combined with *in vitro* germination results suggests that *P. leucophaea* relies primarily on *Ceratorhiza* to fulfill its mycotrophic needs (Zettler *et al.*, 2005). Our recovery of additional *Ceratorhiza* strains from *P. leucophaea* at five new sites in Illinois lends further support for this hypothesis. In SW Australia, the abundance and composition of orchid mycorrhizal fungi is influenced by fire (Ramsay *et al.*, 1986), and the same could be true for *P. leucophaea* of the tallgrass prairie. Thus, additional types of mycorrhizal fungi from frequently-burned areas may await discovery. Curtis (1939)

predicted that orchid species restricted to particular habitats would harbor fewer fungal associates. Similarly, Swarts and Dixon (2009a) proposed that rare orchids (''specialists'') may be closely tied to specific biotic agents (pollinators, mycorrhizal fungi) that themselves have narrow distributions, whereas common orchids (''generalists'') are less discriminate. Whether or not *P. leucophaea* should be regarded as a ''specialist'' or a ''generalist'' remains unclear; however, the degree that *P. leucophaea* relies on *Ceratorhiza*, combined with its requirement for moth pollinators (*e.g., Sphinx erremitus*; Pollack, 2009), suggests the balance to be tilted in favor of the former.

New approaches to conservation with mycorrhizal fungi.—In Australia, several protocols within the framework of integrated conservation (Falk, 1990; Ramsay and Dixon, 2003; Stewart, 2007; Swarts, 2007) have already been advanced that may have an application for rare species in North America (*e.g., Platanthera leucophaea*). Of particular interest are *in situ* and *ex situ* methods used to detect the presence of mycorrhizal fungi in suitable habitats and methods that involve inoculation of soil. Quay *et al.* (1995) acquired seedlings of two *Caladenia* species from seed in 10 wk on pre-inoculated potting mix (*ex vitro* germination), and noted that seed germination was absent in containers lacking mycorrhizal fungi and in potting mix that was not previously sterilized. Batty *et al.* (2006a) inoculated potting media with mycorrhizal fungi to promote the survival of symbiotically-grown seedlings in a greenhouse setting but concluded that the additional dose of inoculum was unnecessary. Brundrett *et al.* (2003) devised an innovative fungal baiting technique that could detect the presence of mycorrhizal fungi *ex situ*. They concluded that fungal inoculum was most prevalent in the layer of coarse organic matter (>2 mm litter) and topsoil. The same study also yielded a novel *in situ* method for detecting mycorrhizal fungi using multi-chambered seed packets that permitted simultaneous assessment of seed germination spanning multiple orchid genera. Swarts (2007) used *ex situ* baiting to locate ''fungal hotspots'' in field sites and proceeded to reintroduce seedlings of critically rare *Caladenia huegelii* into these areas. Our recovery of mycorrhizal fungi from *P. leucophaea* protocorms using Rasmussen and Whigham's (1993) initial seed baiting protocol can also be used to locate ''fungal hotspots,'' but these newer Australian techniques may prove more effective.

The availability of the 35 *Platanthera leucophaea* isolates combined with protocols applied in Australia should provide conservationists with an opportunity to amend soil with mycorrhizal fungi *in situ*. Doing so could conceivably lead to the establishment of additional fungus ''hotspots'' for seed germination and the establishment of ''safe sites'' that improve survival rates of re-introduced seedlings (Swarts, 2007). Developing a ''safe site'' protocol for *P. leucophaea* seems warranted given the difficulties associated with the establishment of laboratory-grown seedlings to date. To our knowledge, amending soil *in situ* has yet to be attempted in North America. In Australia, Swarts (2007) utilized sterilized millet seed colonized by mycorrhizal fungi to amend soil prior to reintroducing *Caladenia huegelii* seedlings. However, he reported that mice seeking the millet seed as a food source destroyed the out-planted seedlings by digging under wire cage barriers. Given the likelihood of small mammals in the prairie ecosystem, a more distasteful substitution for millet seed might be considered. Batty *et al.* (2006b) inoculated field sites with fungi using small (3 mm) cubes of oat meal agar placed adjacent to translocated tubers of two species grown symbiotically (*Thelymitra manginiorum*, *Diuris micrantha*) but concluded that there was no benefit to this practice on orchid survival rates possibly because fungi were already present at the site. Additional studies are needed to determine if amending soil imparts a benefit on seedling survival in sites that lack suitable mycorrhizal fungi determined through seed baiting (Batty *et al.*, 2006b). We also urge caution when selecting specific fungal strains

for this purpose, at least until more is known about fungal invasion ecology. Desprez-Loustau *et al.* (2007) warned that the introduction and spread of exotic fungi is one of the most important problems facing conservation biology exacerbated by limited baseline data on fungal communities. Although *Ceratorhiza* appears to be a ubiquitous associate of *P. leucophaea*, it should not be assumed that specific strains of that genus are equally widespread. Wright *et al.* (2010) determined that mycorrhizal associates differ between populations of *Caladenia tentaculata* and that local fungus diversity exists within individual orchid populations. Thus, we advocate that the fungi used in amending soil should be limited to strains acquired from the same or nearby populations until more information is gathered.

Acknowledgments.—We express gratitude to Dr. Lynne Sigler for deposition of mycorrhizal fungi into UAMH and to Marlin L. Bowles (The Morton Arboretum) for the photograph of *P. leucophaea* depicted in Fig. 1. Special thanks are extended to Kelly Neal (Illinois Nature Preserves Commission) and to Dave Nance (Jacksonville, IL) for permission to sow seed baits into Denby and Anderson Prairies, respectively. We also thank Stephen Packard and Bill Kleiman for access to material at Somme Prairie and Nachusa Grasslands, respectively. Valuable feedback from Dr. Nevin Aspinwall (Saint Louis University), Dr. Scott L. Stewart (Kankakee Community College, IL), and Cathy Pollack (U.S. Fish & Wildlife Service) is also appreciated. The technical support of the following Illinois College students is graciously acknowledged: Jenifer Fortney, Jared Hartsock, Erin Wood.

LITERATURE CITED

BATTY, A. L., M. C. BRUNDRETT, K. W. DIXON AND K. SIVASITHAMPARAM. 2006a. New methods to improve symbiotic propagation of temperate terrestrial orchid seedlings from axenic culture to soil. *Aust. J. Bot.*, **54**:367–374.

———, ———, ——— AND ———. 2006b. *In situ* symbiotic seed germination and propagation of terrestrial orchid seedlings for establishment at field sites. *Aust. J. Bot.*, **54**:375–381.

BOWLES, M. L., K. A. JACOBS, L. W. ZETTLER AND T. WILSON DELANEY. 2002. Crossing effects on seed viability and experimental germination of the Federal threatened *Platanthera leucophaea* (Orchidaceae). *Rhodora*, **104**:14–30.

———, L. W. ZETTLER, T. BELL AND P. KELSEY. 2005. Relationships between soil characteristics, distribution and restoration potential of the Federal threatened Eastern Prairie Fringed Orchid, *Platanthera leucophaea* (Nutt.) Lindl. *Am. Midl. Nat.*, **154**:273–285.

BRUNDRETT, M. C., A. SCADE, A. L. BATTY, K. W. DIXON AND K. SIVASITHAMPARAM. 2003. Development of *in situ* and *ex situ* seed baiting techniques to detect mycorrhizal fungi from terrestrial orchid habitats. *Mycol. Res.*, **107**:1210–1220.

CLEMENTS, M. A., H. MUIR AND P. J. CRIBB. 1986. A preliminary report on the symbiotic germination of European terrestrial orchids. *Kew Bull.*, **41**:437–445.

CURRAH, R. S., L. SIGLER AND S. HAMBLETON. 1987. New records and new taxa of fungi from the mycorrhizae of terrestrial orchids of Alberta. *Can. J. Bot.*, **65**:2473–2482.

———, E. A. SMRECIU AND ———. 1990. Mycorrhizae and mycorrhizal fungi of boreal species of *Platanthera* and *Coeloglossum* (Orchidaceae). *Can. J. Bot.*, **68**:1171–1181.

———, C. D. ZELMER, ——— AND K. A. RICHARDSON. 1997. Fungi from orchid mycorrhizas, p. 117–170. *In:* J. Arditti and A. M. Pridgeon (eds.). Orchid Biology: reviews and perspectives, VII. Kluwer Academic Publishers, UK.

CURTIS, J. T. 1939. The relation of specificity of orchid mycorrhizal fungi to the problem of symbiosis. *Am. J. Bot.*, **26**:390–399.

DESPREZ-LOUSTAU, M. L., C. ROBIN, M. BUÉE, R. COURTECUISSE, J. GARBAYE, F. SUFFERT, I. SACHE AND D. M. RIZZO. 2007. The fungal dimension of biological invasions. *Trends Ecol. Evo.*, **22**(9):473–480.

DIXON, K. 1987. Raising terrestrial orchids from seed, p. 47–100. *In:* W. K. Harris (ed.). Modern orchid growing for pleasure and profit. Orchid Club of S. Australia, Inc. Adelaide, S. Australia.

FALK, D. A. 1990. Integrated strategies for conserving plant genetic diversity. *Ann. Missouri Bot. Gard.*, **77**:38–47.

HOLLICK, P. 2004. Mycorrhizal specificity in endemic Western Australian terrestrial orchids (tribe Diuridae): implications for conservation. Ph.D. Thesis, Murdoch University, Perth.

PISKIN, K., J. J. HARTSOCK, L. W. ZETTLER AND M. L. BOWLES. 2003. Seed germination requirements of a Federally threatened orchid (*Platanthera leucophaea*) in nature, and a technique to establish leaf-bearing seedlings onto soil. *Southeastern Bio.*, **50**(2):170 (abstract).

POLLACK, C. 2009. Restoration of the Eastern Prairie Fringed Orchid (*Platanthera leucophaea*): natural pollinators and abundance of larval host plants. Master's Thesis. Northeastern Illinois University.

MARX, D. H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. Part I. Antagonisms of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology*, **59**:153–163.

MOORE, R. T. 1987. The genera of *Rhizoctonia*-like fungi: *Ascorhizoctonia*, *Ceratorhiza* gen nov., *Epulorhiza* gen nov., *Moniliopsis*, and *Rhizoctonia*. *Mycotaxon*, **29**:91–99.

QUAY, L., J. A. MCCOMB AND K. W. DIXON. 1995. Methods for *ex vitro* germination of Australian terrestrial orchids. *HortScience*, **30**(7):1445–1446.

RAMSAY, R. R., K. W. DIXON AND K. SIVASITHAMPARAM. 1986. Patterns of infection and endophytes associated with Western Australian orchids. *Lindleyana*, **1**(3):203–214.

RASMUSSEN, H. N. 1995. Terrestrial orchids from seed to mycotrophic plant. Cambridge Univ. Press.

——— AND D. F. WHIGHAM. 1993. Seed ecology of dust seeds *in situ*: a new study technique and its application to terrestrial orchids. *Am. J. Bot.*, **80**:1374–1378.

RICHARDSON, K. A. AND R. S. CURRAH. 1993. Basidiomycetous endophytes from roots of Neotropical epiphytic Orchidaceae. *Lindleyana*, **8**:127–137.

SHARMA, J., L. W. ZETTLER AND C. J. STARBUCK. 2003a. A survey of mycobionts of Federally-threatened *Platanthera praeclara* (Orchidaceae). *Symbiosis*, **34**:145–155.

———, ———, J. W. VAN SAMBEEK, M. ELLERSECK AND C. J. STARBUCK. 2003b. Symbiotic seed germination and mycorrhizae of Federally-threatened *Platanthera praeclara* (Orchidaceae). *Am. Midl. Nat.*, **149**:79–95.

SHEVIAK, C. J. 1974. An introduction to the ecology of Illinois Orchidaceae. Scientific Paper XIV. Illinois State Museum, Springfield.

STEWART, S. L. 2007. Integrated conservation of Florida Orchidaceae in the genera *Habenaria* and *Spiranthes*: model orchid conservation systems for the Americas. Ph.D. Dissertation. University of Florida, Gainesville.

SWARTS, N. 2007. Integrated conservation of the rare and endangered terrestrial orchid *Caladenia huegelii* H.G. Reichb. Ph.D. Thesis, University of Western Australia, Perth.

SWARTS, N. D. AND K. W. DIXON. 2009. Terrestrial orchid conservation in the age of extinction. *Ann. Bot.*, **104**:543–556.

——— AND ———. 2009. Perspectives on orchid conservation in botanic gardens. *Trends in Plant Sci.*, **14**:590–598.

U.S. FISH & WILDLIFE SERVICE. 2007. Eastern Prairie Fringed Orchid (*Platanthera leucophaea*) 5-Year Review: Summary and Evaluation. Federal Register (72 FR 41348), 27 Jul. 2007.

WRIGHT, M. M., R. CROSS, R. D. COUSENS, T. W. MAY AND C. B. MCLEAN. 2010. Taxonomic and functional characterization of fungi from *Sebacina vermifera* complex from common and rare orchids in the genus *Caladenia*. *Mycorrhiza*, **20**:375–390.

ZELMER, C. D. AND R. S. CURRAH. 1995. *Ceratorhiza pernacatena* and *Epulorhiza calendulina* spp. nov.: mycorrhizal fungi of terrestrial orchids. *Can. J. Bot.*, **73**:1981–1985.

———, L. CUTHBERTSON AND R. S. CURRAH. 1996. Fungi associated with terrestrial orchid mycorrhizas, seeds and protocorms. *Mycoscience*, **37**:439–448.

ZETTLER, L. W., J. SHARMA AND F. RASMUSSEN. 2003. Mycorrhizal Diversity, Chapter 11, p. 185–203. *In:* K. Dixon, S. P. Kell, R. L. Barrett and P. J. Cribb (eds.). Orchid Conservation. Natural History Publications, Kota Kinabalu, Sabah.

———, K. A. PISKIN, S. L. STEWART, J. J. HARTSOCK, M. L. BOWLES AND T. J. BELL. 2005. Protocorm mycobionts of the Federally threatened Eastern Prairie Fringed Orchid, *Platanthera leucophaea* (Nutt.) Lindley, and a technique to prompt leaf elongation in seedlings. *Studies Mycology*, **53**:163–171.

———, S. L. STEWART, M. L. BOWLES AND K. A. JACOBS. 2001. Mycorrhizal fungi and cold-assisted symbiotic germination of the Federally threatened Eastern Prairie Fringed Orchid, *Platanthera leucophaea* (Nuttall) Lindley. *Am. Midl. Nat.*, **145**:168–175.

SUBMITTED 2 SEPTEMBER 2010 ACCEPTED 13 JANUARY 2011