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SYMBIOTIC GERMINATION OF A FEDERALLY ENDANGERED HAWAIIAN ENDEMIC, *PLATANTHERA HOLOCHILA* (ORCHIDACEAE), USING A MYCOBIONT FROM FLORIDA: A CONSERVATION DILEMMA

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ABSTRACT. *Platanthera holochila* (Hbd.) Krzl. (Orchidaceae), one of three orchid species endemic to Hawaii, is among the rarest orchids in the world (U.S. federal endangered species, C1; global rank, G1) with ca. 18 surviving individuals. We provide a protocol for cultivating *P. holochila* to the leaf-bearing stage in-vitro, using a mycorrhizal fungus originally isolated from Florida (*Epulorhiza repens,* UAMH 9824). This study describes two separate experiments aimed at inducing leaf formation: (1) a low agar pH, and (2) an agar medium containing nutrients (e.g., $MgSO_4 \cdot 7H_2O$). Seeds sown on acidified (pH 4.3) modified oats medium (MOM), containing nutrients, resulted in the highest percent germination recorded for the study (16.8%), and leaf formation (Stage 4) in up to 2.3% of the viable seed total. Also the ethics of releasing *P. holochila* seedlings harboring the Florida fungus into Hawaii is discussed. We advocate that such seedlings not be introduced into natural habitats in Hawaii, given the state's high number of vulnerable taxa and the potential for the fungus to further upset the ecological balance there.

Key words: *Platanthera holochila,* conservation, Hawaii, seed germination, mycorrhizal fungi

RESUMEN. *Platanthera holochila* (Hbd.) Krzl. (Orchidaceae), es una de las tres especies de orquídeas endémicas de Hawaii y una de las orquídeas más escasa en el mundo (rango C1 de las especies federales en peligro de extincción E.E.U.U., y G1 rango global) con aproximados 18 sobrevivientes. En este artículo, proveemos un método para cultivar *P. holochila* hasta la etapa de produción de hojas in-vitro utilizando un micobionte aislado originalmente desde Florida (*Epulorhiza repens,* UAMH 9824). Este proyecto describe dos experimentos distintos, ambos enfocados en inducir formación de hojas: 1) agar de pH baja, y 2) un medio de agar nutritivo (e.g., $MgSO_4 \cdot 7H_2O$). Las semillas sembradas en un medio modificado de avena (MOM) ácido (pH 4.3) y nutritivo resultó en el porcentaje de germinación más alto del proyecto (16.8%), y para la formación de hojas (Stage 4) en hasta 2.3% del total viable de semillas. Nosotros abogamos para que las plantas que contienen el micobionte de Florida (UAMH 9824) no sean introducidas en áreas naturales en Hawaii, dado el número alto de taxa vulnerable en el estado, y el potencial que existe para que el hongo interfiera con el balance ecológico local.

Palabras claves: *Platanthera holochila,* conservación, Hawaii, germinacion simbiotica, micobionte

INTRODUCTION

Known locally as "puahala a kane," *Platanthera holochila* (Hbd.) Krzl. (Orchidaceae) is one of three orchid species endemic to the Hawaiian Islands and among the rarest orchids in the world. Once assumed to be extinct, fewer than two-dozen individuals remain in four scattered populations on the islands of Kauai, Maui, and Molokai. Three additional plants (seedlings)

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were observed in August 2003 on Molokai (L. Zettler & S. Perlman pers. obs.), raising the total number of plants thought to exist to ca. 18. The species has not been reported on Oahu since the early 1940s and has never been seen on "the big island" of Hawaii (Walker 1994), which is the youngest (<0.5 million years old) and largest (10,433 sq km) island of the archipelago (Sakai et al. 2002). Currently, *P. holochila* is listed as a C1 U.S. Federal Endangered species, and has achieved a global G1 ranking (1–5 sites or 1000 individuals known). Despite receiving legal protection, the species remains vulnerable to competition from alien plants, illicit collecting, and trampling by humans near established trails.

As a terrestrial orchid, *Platanthera holochila* is assumed to require mycorrhizal fungi to prompt seedling development to an autotrophic stage and relies on mycotrophy to supplement photosynthesis during adulthood. Evidence for the latter possibility is exemplified by the presence of numerous, intact pelotons observed in the root-like organs of mature plants (L. Zettler pers. obs.). Subsequent efforts to isolate mycobionts from pelotons in *P. holochila* have, thus far, been unsuccessful. This species has also resisted efforts aimed at its propagation from seed using asymbiotic techniques (Walker 1994). Taken together, the immediate recovery of *P. holochila* largely hinges upon the development of a protocol that implements symbiotic seed germination.

Given that symbiotically derived seedlings harbor active mycobionts, the release of orchid seedlings into native habitats also results in the release of the fungus into the surrounding substrata. Unless that fungus originated from the same habitat or site, it is conceivable that the fungus could cause irreversible ecological harm by altering the existing microflora, or by perhaps triggering plant diseases in non-orchids. This possibility seems more likely for orchid mycobionts because of their close taxonomic affinities to the form-genus *Rhizoctonia* s.l., which is well known worldwide for its plant pathogens (e.g., *Rhizoctonia solani*). In Hawaii, the release of a fungus antagonistic toward the endemic flora could be catastrophic, considering that 52.5% of the 1159 taxa already are at risk of extinction (Sakai et al. 2002). Consequently, symbiotic techniques aimed at cultivating *Platanthera holochila* should employ endemic fungi, but this will not be possible until such fungi are successfully recovered. The precarious status of *P. holochila* (18 individuals), coupled with the fact that some terrestrial orchids can be cultured with fungi originating from unrelated taxa (e.g., Masuhara & Katsuya 1989, Smreciu & Currah 1989, Stewart & Zettler 2002), justifies the immediate cultivation of this species, at least in-vitro. Should *P. holochila* suddenly become extinct in the wild, seedlings cultured in the laboratory, albeit with "alien" mycobionts, would then serve as the sole source of living plant material (e.g., seeds) utilized in the recovery of the species. Furthermore, this protocol could then be immediately adopted if ongoing attempts to recover natural mycobionts are successful.

We provide a protocol for cultivating *Platanthera holochila* to the leaf-bearing stage in-vitro, using a mycobiont, *Epulorhiza repens* (Bernard) Moore, originally isolated from Florida (Stewart et al. 2003). In a pilot study (L. Zettler & S. Perlman unpubl. data), symbiotic germination of *P. holochila* was achieved using a standard oats/agar-based medium (see Dixon 1987), but protocorm development was arrested at an early growth stage. This study describes two subsequent, separate experiments aimed at inducing leaf formation using: (1) a lower agar pH, and (2) an agar medium containing nutrients (e.g., $MgSO_4 \cdot 7H_2O$). The ethics of releasing the resulting mycorrhizal *P. holochila* seedlings into Hawaii is also discussed.

Materials and Methods

Description of the Orchid and Habitat

Platanthera holochila (Hbd.) Krzl. is perennial and found in wet, acidic, mountainous bogs at 600–1500 m elevation. It is identified in flower by its tall (50 cm) inflorescence, containing 5–60 densely clustered, inconspicuous flowers (Figure 1) from May through November. No known pollinators have been observed, and it is assumed to be self-pollinating. The species produces numerous long (>10 cm) and brittle branch roots heavily infected with peloton-forming, mycorrhizal fungi along yellow-orange colored regions, typical of the genus in North America (Zettler 1997). These branch roots often infiltrate the surrounding substrate that consists of a thick (0.5 m), spongy, moist layer of organic debris derived from leaf litter and moss. On the island of Molokai, *P. holochila* inhabits a wet forest of *Metrosideros polymorpha* dominated by *Cheirodendron trigynum* and *Cibotium glaucum.* Other associated angiosperms include *Machaerina angustifolia, Vaccinium calycinum, Myrsine lessertiana, Astelia menziesiana, Viola chamissoniana* subsp. *robusta, Labordia hirtella, Coprosma* spp., *Styphelia tameiameiae, Ilex anomala, Melicope mauiensis, Broussaisia arguta, Cyrtandra procera,* and *Liparis hawaiiensis.* Fern associates include *Dicranopteris linearis, Elaphoglossum* spp., *Schizea robusta,*

FIGURE 1. Two specimens of *Platanthera holochila* in flower within the Kamakou Preserve, Molokai, photographed by L.W. Zettler in August 2003.

Nephrolepis cordifolia, and *Asplenium lobulatum.*

Seed Collection and Storage

Seeds of *Platanthera holochila* (Hbd.) Krzl. were collected from 12 capsules on four separate plants within the Kamakou Preserve (1165 m elevation) on the island of Molokai on 23 September 2002 by S. Perlman (#18,271). The upper portion of each inflorescence containing ripening (pale green) capsules was detached, placed in an envelope, and immediately sent to Illinois College via airmail. Upon arriving 3–4 days after collection, capsules were promptly dried over Drierite® desiccant ($CaSO_4$) at 22°C for 1 week. Dried seeds (ca. 0–5% RH) were then removed from capsules using a sterile scalpel, placed in sealed vials containing $CaSO_4$, and stored at −7°C in darkness (L:D 0:24 hours), according to Zettler (1997). Considerable care was exercised to exclude capsule debris from the seed samples to minimize the introduction of surface contaminants.

TABLE 1. Soil analysis from the *Platanthera holochila* population at Kamakou Preserve, Molokai, Hawaii, August 2003. The sample consisted of a mixture of 10 separate samples (= grab sample) acquired at the same depth supporting root-like organs of adult plants nearby.

pH	Organic matter %	Nutrients kg/ha							
		K	P	S	Zn	Fe	Mn	Cu	B
4.3	3.8	211.2	11.2	22.5	1.9	98.8	75.2	3.6	2.8

Seed Sowing, Inoculation, and Incubation In-Vitro

Seeds were sown according to techniques outlined in Clements et al. (1986), Dixon (1987), and Zettler (1997). Seeds were removed from cold storage, allowed to warm at ambient (22°C) temperature, and added to a vial containing a surface sterilization solution of 5 ml ethanol, 5 ml 5.25% NaOCl (Clorox® bleach), and 90 ml DI water. Seeds were rinsed in this solution for 1 min. by vigorous shaking, then rinsed in sterile DI water twice, with each rinse lasting 1 min. Using a sterile inoculation loop, seeds were removed from the vial and spread across the surface of a 1 × 4 cm filter paper strip (Whatman No. 4) within a Petri plate containing ca. 20 ml agar. In the first experiment, seeds were sown on standard oats/agar medium (2.5 g rolled oats, 7.0 g agar, 1 L DI water; see Dixon 1987) and exposed to one of four pH treatments (4.0, 5.0, 6.0, 7.0) to determine the optimal pH range for the species. Agar pH was adjusted prior to autoclaving (121°C for 20 min.) and differed only slightly (pH +0.1) thereafter. In the second but concurrent experiment, seeds were sown on a medium containing oats and added nutrients (modified oats medium, MOM; see Clements et al. 1986) adjusted to a pH of 4.3. This pH, low compared to related studies that employ the symbiotic technique, was chosen to parallel the pH of the soil at the existing *Platanthera holochila* population on Molokai (TABLE 1). Seeds were subjected to one of five $MgSO_4 \cdot 7H_2O$ treatments: 0.00, 0.02, 0.05, 0.1, 0.2 g/L. Standard MOM utilizes 0.1 g/L $MgSO_4 \cdot 7H_2O$, and the second experiment attempted to determine the effects of high (0.2 g/L) and low (<0.1 g/L) concentrations on germination and growth. $MgSO_4 \cdot 7H_2O$ was chosen because it contained an available form of sulfur in solution, an element often associated with soils of volcanic origin and present on Molokai (TABLE 1). After adding 6–50 seeds to the filter paper strip in each plate, at least 10 replicate plates were prepared for each treatment in both experiments. Each plate was inoculated with a 1-cm^3 agar block containing mycelium from a fungus identified as *Epulorhiza repens* (Bernard) Moore, which is a widespread and common anamorph in the *Rhizoctonia* s.l. complex. Originally isolated from the root-like organs of *Spiranthes brevilabris* Lindley (Orchidaceae) in Florida (Stewart et al. 2003), the strain was chosen for its effective promotion of seedling development in a range of taxa: *Certopodium punctatum* (L.) Lindley (S. Stewart unpubl. data), *Epidendrum nocturnum* Jacquin (S. Stewart, S. Hopkins unpubl. data), *Habenaria repens* Nuttall (Stewart & Zettler 2002), *Platanthera ciliaris* (L.) Lindley (L. Zettler unpubl. data), and *S. brevilabris* (Stewart et al. 2003). This fungus was deposited in the University of Alberta (Canada) Microfungus Collection and Herbarium as UAMH 9824 for safekeeping and future use. Following inoculation, plates were sealed with Parafilm "M" (American National Can, Greenwich, Connecticut), wrapped in aluminum foil to exclude light, and incubated in darkness (L:D 0:24 hours) at ambient temperature.

Assessment of Seed Viability, Germination, and Growth

After 21 days of initial incubation, plates were examined every other week to assess germination and development, exposing the seeds to brief (<30 min.) episodes of illumination. After inspection, plates were returned to experimental conditions (darkness). Seed viability and germination were recorded with the aid of a dissection microscope. Viable seeds were counted as those containing robust, rounded creamy-white embryos. Seed viability was based on two seed sources: Kamakou Preserve on Molokai and Alakai Swamp on Kauai (collected from 10 capsules, 5 September 2003 by S. Perlman). Seed germination percentages were based solely on viable seeds. Germination and development were scored on a 0–5 scale, where Stage 0 = no germination (intact testa), 1 = initiation of rhizoids, 2 = rupture of testa by enlarged embryo, 3 = appearance of the shoot, 4 = emergence of leaf from shoot region, and 5 = elongation of leaf. Statistical tests were conducted using SPSS 12.0 for Windows software (SPSS 2003). To assess optimal pH levels for germination (Stage 1) and rupture of the testa

TABLE 2. Experiment 1: Effect of pH on in-vitro germination and development of *Platanthera holochila* 118 days after sowing on standard oats/agar medium (2.5 g rolled oats, 7.0 g agar, 1 L DI water; see Dixon 1987). Seeds were inoculated with *Epulorhiza repens* (UAMH 9824) and incubated in darkness at 22°C. No significant differences in percent germination were detected among the four pH treatments.

pH	Replicates* no.	Seeds** no.	Stage†			Germination	
			0	1	2	%	±SE
4.0	11	182	171	3	8	5.7	1.9
5.0	11	188	167	1	20	10.6	2.8
6.0	9	130	121	1	8	7.0	2.7
7.0	11	187	171	3	13	7.7	2.1

* Number of replicate Petri plates for a given treatment. Unequal subsample sizes resulted after contaminated plates were discarded.

** Viable seeds.

† Stage 0 = no germination (intact testa), 1 = initiation of rhizoids, and 2 = rupture of testa by enlarged embryo.

(Stage 2), an analysis of variance (ANOVA) was conducted. To assess optimal levels of $MgSO_4 \cdot 7H_2O$, an ANOVA was conducted to compare germination and development to Stage 4 across the conditions.

RESULTS AND DISCUSSION

Seed Viability

Seeds of *Platanthera holochila* from Kamakou Preserve on Molokai and Alakali Swamp on Kauai were monoembryonic. Seed viability was generally higher for *P. holochila* compared to other U.S. Federally listed *Platanthera* species: *P. holochila,* Molokai = >85%, *P. holochila,* Kauai = 89%, *P. integrilabia* (Correll) Luer = 75–85% (L. Zettler unpubl. data), *P. leucophaea* (Nutt.) Lindley = 40–85% (for outcrossed seeds; Bowles et al. 2002, Wallace 2003), and *P. praeclara* Sheviak & Bowles = 9–37% (Sharma et al. 2003). High seed viability in *P. holochila* is perplexing, considering that the species, assumed to be self-pollinating, conceivably would suffer from inbreeding depression resulting in low seed viability. One possible explanation is that *P. holochila* experienced a "genetic bottleneck" in the recent past (see Falk & Holsinger 1991) that resulted from inbreeding brought about by extreme geographic isolation. In this scenario, the colonization of the archipelago by few individuals (=founder effect), followed by a population enlargement shortly thereafter, would have forced inbreeding and survivorship of individuals with low genetic diversity, purging the deleterious recessive alleles in the process (Lande & Schemske 1985, Barrett & Charlesworth 1991). A lack of pollinators on Hawaii initially would have selected for self-pollination and an immediate shift towards homozygosity, eventually leading to high seed viability.

Seed Germination, Development, and pH

Symbiotic seed germination of *Platanthera holochila* was achieved in-vitro for all treatments in both experiments (TABLES 2, 3) within 21 days of sowing and fungal inoculation. In the first experiment, which utilized standard oats/agar medium and four pH treatments, seed germination percentages were comparable (<15%) to those in the pilot study, which utilized the same medium adjusted to a neutral (6.9) pH (L. Zettler, S. Perlman unpubl. data). Seedling development was also comparable in terms of the percentage of viable seeds that developed to Stage 2 (<10%), and in terms of the maximum growth stage achieved (Stage 2). No significant differences in percent germination ($F_{(3,38)} = 1.11$, $P > 0.05$) or the percentage of viable seeds that developed to Stage 2 ($F_{(3,38)} = 0.77$, $P > 0.05$) were detected among the different pH treatments. While significant differences did not emerge, it appears that agar adjusted to a lower (5.0) pH resulted in the highest percent germination (10.6%) for the study, and the highest number of seeds that developed to Stage 2 (TABLE 2).

Given that all treatments utilized a single fungus (*Epulorhiza repens*), it is noteworthy that this strain was able to tolerate the different pH extremes, especially those in the acidic ranges. In a previous study (Stewart et al. 2003), the same fungus was successfully used to culture *Spiranthes brevilabris* on standard oats/agar media adjusted to an alkaline pH (8.0) to parallel the soil pH of limestone-based soils in Florida. The tolerance of *E. repens* to these pH extremes may, in part, explain the "ubiquitous" distribution of the fungus worldwide (Zelmer 2001, Zettler et al. 2003). The fact that *Platanthera holochila* was able to utilize *E. repens* in our study may reflect the current presence of this fungal species in Hawaii.

TABLE 3. Experiment 2: In-vitro germination and development of *Platanthera holochila* 194 days after sowing on acidified (pH 4.3) modified oats medium (MOM), containing different amounts of $MgSO_4 \cdot 7H_2O$. Seeds were inoculated with *Epulorhiza repens* (UAMH 9824) and incubated in darkness at 22°C[a]. No significant differences in percent germination were detected among the five $MgSO_4 \cdot 7H_2O$ treatments.

$MgSO_4 \cdot 7H_2O$ g/L	Replicates* no.	Seeds** no.	Stage†					Germination	
			0	1	2	3	4‡	%	±SE
0.00	9	195	162	8	16	6	3 (1.1)	16.8	2.0
0.02	10	188	160	3	16	4	5 (2.3)	14.2	2.8
0.05	5	113	94	7	9	2	1 (1.2)	16.4	1.8
0.1	10	225	188	6	24	6	1 (0.6)	16.8	3.0
0.2	9	181	159	3	19	0	0 (0.0)	12.5	2.6

* Number of replicate Petri plates for a given treatment. Unequal subsample sizes resulted after contaminated plates were discarded.
** Viable seeds.
† Stages: 0 = no germination (intact testa), 1 = initiation of rhizoids, 2 = rupture of testa by enlarged embryo, 3 = appearance of the shoot, and 4 = emergence of leaf from shoot region.
‡ Values in parentheses reflect percentage of seeds yielding advanced (Stage 4) seedlings.

Influence of $MgSO_4 \cdot 7H_2O$ on Seed Germination/Development

Use of acidified (pH 4.3) modified oats medium (MOM) resulted in the highest percent seed germination recorded in the study (>12%; TABLE 3), demonstrating it to be a superior medium to standard/oats agar utilized in the first experiment. While no significant effects were found in seed germination ($F_{(4,38)} = 0.89$, $P > 0.05$) or percentage that developed to Stage 4 ($F_{(4,38)} = 0.53$, $P > 0.05$), the highlight of the study was our ability to cultivate seedlings to the leaf-bearing stage on MOM (Stage 4) in four of the five treatments. These seedlings (=protocorms) appeared healthy evidenced by their opaque appearance, creamy white color, and numerous rhizoids encircling the posterior end. Maximum length for the largest seedlings was 0.5 cm. Based on similar studies aimed at the cultivation of other *Platanthera* species in North America (Anderson 1996, Hartsock et al. 2003, Zettler & McInnis 1992, Zettler & Hofer 1998, Sharma et al. 2003), these seedlings were of sufficient size to warrant exposure to light as a means to promote leaf elongation (Stage 5) and the onset of a photosynthetic capability. This would be followed by transferring seedlings to humid conditions within a culture vessel containing sterile white silica sand over an oats medium (Batty et al. in press) to prompt additional growth. Of special interest is the fact that 2.3% of seeds developed to Stage 4 using 0.02 g/L $MgSO_4 \cdot 7H_2O$ (TABLE 3). Although this percentage seems small, it is comparable to percentages observed in other *Platanthera* species cultivated with fungi in a similar manner: *P. ciliaris* (L.) Lindley = 1% (L. Zettler unpubl. data), *P. clavellata* (Mich.) Luer = 0.3% (Zettler & Hofer 1998), *P. leucophaea* = 3% (Zettler et al. 2001), and *P. praeclara* = 13% (Sharma et al. 2003). This observation is even more surprising considering that *P. holochila* seed germination percentages were generally lower relative to the other taxa, even on MOM. Consequently, this study suggests that relatively few *P. holochila* seeds germinate, but a modest number of the germinated seeds are capable of developing to an advanced (Stage 4) growth stage on MOM. Additional studies are planned to determine what component(s) of MOM play a key role in initiating leaf formation.

Taken together, our results reaffirm the potential for this species to be cultivated with fungi leading to its eventual reintroduction in the wild. More studies directed at improving seed germination and development currently are underway to render the symbiotic technique more effective. These laboratory-based efforts also are be-

ing supplemented by intensive field studies to isolate, identify, and preserve the mycobionts of *Platanthera holochila* from the natural habitat. This is being carried out by the use of seed baits (Rasmussen & Whigham 1993) deposited on Molokai in August 2003 and 2004. Until such fungi are recovered, however, our use of the Florida mycobiont (*Epulorhiza repens* UAMH 9824), combined with MOM set at a low pH (4.3–5.0), remains the only means to cultivate the species from seed to a leaf-bearing stage.

Ethical Considerations

If efforts fail to recover natural mycobiont(s) for *Platanthera holochila,* if the species should suddenly become extinct in the near future, and if a protocol is developed to successfully establish leaf-bearing seedlings on soil ex-vitro, an ethical question arises: should these seedlings be re-introduced into Hawaii, if they harbor the Florida mycobiont (*Epulorhiza repens* UAMH 9824)? Such an act conceivably could perpetuate the species, but at what cost? Is the act of saving one species (*P. holochila*) worth the risk of potentially endangering others, especially since so many alien species have been established in Hawaii already? As symbiotic techniques improve and are adopted worldwide by conservation programs, these questions will likely resurface for a growing number of critically rare taxa that are propagated with "alien" mycobionts. With more than half (52.5%) of Hawaii's flora already at risk of extinction (Sakai et al. 2002), coupled with the close taxonomic affinities of *Epulorhiza* to established plant pathogens in the *Rhizoctonia* complex, we advocate that *P. holochila* seedlings cultured with the Florida mycobiont not be released into the natural habitat. Although it may be argued that *E. repens* is a "ubiquitous" species, different strains within the species complex may be capable of causing widespread and permanent ecological harm, particularly in isolated geographical areas (e.g., island ecosystems). Since Hawaii is currently viewed as a model for global species conservation, because the archipelago is analogous to larger areas fragmented by human activities, our study may have additional meaning for similar programs faced with propagating endangered species with "alien" symbionts. At best, and assuming that legal permission is granted to allow UAMH 9824 into Hawaii, it is conceivable that *P. holochila* may remain extant only in captivity (e.g., botanical gardens), until native mycobionts are found and utilized, if ever. This scenario seems paradoxical considering that the success of the Orchidaceae may be largely attributed to the family's ability to utilize fungi as a carbon source (=mycotrophy). In essence, it seems that this special process (mycotrophy) that helped *P. holochila* colonize the Hawaiian archipelago may also have doomed it to a life of captivity. Clearly, the conservation dilemma facing *P. holochila* should send a powerful message to the global community—orchid mycobionts should be recovered promptly from remaining habitats worldwide and preserved for safekeeping and future use.

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Literature Cited

Anderson, A.B. 1996. The reintroduction of *Platanthera ciliaris* in Canada. Pp. 72–76 *in* C. Allen, ed. Proceedings of the North American Native Orchid Propagation and Production Conference. National Arboretum, Washington, D.C.

Barrett, S.C.H. and D. Charlesworth. 1991. Effects of a change in the level of inbreeding on the genetic load. Nature 352: 522–524.

Batty, A.L., M.C. Brundrett, K.W. Dixon, and K. Sivasithamparam. In press. Symbiotic propagation for the conservation of temperate terrestrial orchids. Australian J. Bot.

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.

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.

Dixon, K.W. 1987. Raising terrestrial orchids from seed. Pp. 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. and K.E. Holsinger. 1991. Genetics and Conservation of Rare Plants. Oxford University Press, Oxford, UK.

Hartsock, J.J., K.A. Piskin, and L.W. Zettler. 2003. Symbiotic seed germination of two showy terrestrial orchids (*Platanthera ciliaris, P. cristata*; Orchidaceae) using mycorrhizal fungi. SE Biol. 50(2): 149.

Lande, R. and D.W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. Evolution 39: 24–40.

Masuhara, G. and K. Katsua. 1989. Effects of mycorrhizal fungi on seed germination and early growth of three Japanese terrestrial orchids. Sci. Hort. 37: 331–337.

Rasmussen, H.N. and D.F. Whigham. 1993. Seed ecology of dust seeds in situ: a new study technique and its application in terrestrial orchids. Am. J. Bot. 80(12): 1374–1378.

Sakai, A.K., W.L. Wagner, and L.A. Mehrhoff. 2002. Patterns of endangerment in the Hawaiian flora. Syst. Biol. 51(2): 276–302.

Sharma, J., L.W. Zettler, J.W. Van Sambeek, M.R. Ellersieck, and C.J. Starbuck. 2003. Symbiotic seed germination and mycorrhizae of federally threatened *Platanthera praeclara* (Orchidaceae). Amer. Midl. Nat. 149: 104–120.

Smreciu, E.A. and R.S. Currah. 1989. Symbiotic germination of seeds of terrestrial orchids of North America and Europe. Lindleyana 1: 6–15.

SPSS for Windows, Rel. 11.0.1. 2003. Chicago: SPSS Inc.

Stewart, S.L. and L.W. Zettler. 2002. Symbiotic germination of three semi-aquatic rein orchids (*Habenaria repens, H. quinqueseta, H. macroceratitis*) from Florida. Aquatic Bot. 72: 25–35.

Stewart, S.L., L.W. Zettler, J. Minso, and P.M. Brown. 2003. Symbiotic germination and reintroduction of *Spiranthes brevilabris* Lindley, an endangered orchid native to Florida. Selbyana 24: 64–70.

Walker, J.H. 1994. Intrepid trio: three orchid species arrived in Hawaii unassisted. Now one of them is in trouble. Amer. Hort. December 1994: 38–41.

Wallace, L.E. 2003. The cost of inbreeding in *Platanthera leucophaea* (Orchidaceae). Am. J. Bot. 90(2): 235–242.

Zelmer, C.D. "Root-Associated Organisms of the Cypripedioideae (Orchidaceae)." Ph.D. thesis, University of Guelph, Ontario, Canada, 2001.

Zettler, L.W. 1997. Terrestrial orchid conservation by symbiotic seed germination: techniques and perspectives. Selbyana 18: 188–194.

Zettler, L.W. and C.J. Hofer. 1998. Propagation of the little club-spur orchid (*Platanthera clavellata*) by symbiotic seed germination, and its ecological implications. Env. Exper. Bot. 39: 189–195.

Zettler, L.W. and T.M. McInnis, Jr. 1992. Propagation of *Platanthera integrilabia* (Correll) Luer, an endangered terrestrial orchid, through symbiotic seed germination. Lindleyana 7: 154–161.

Zettler, L.W., 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. Amer. Midl. Nat. 145: 168–175.

Zettler, L.W., J. Sharma, and F. Rasmussen. 2003. Mycorrhizal diversity. Pp. 205–226 *in* K. Dixon, P. Cribb, S. Kell and R. Barrett, eds. Orchid Conservation. Natural History Publications, Kota Kinabalu, Sabah.