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Seed longevity in terrestrial orchids – Potential for persistent in situ seed banks

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ABSTRACT

Terrestrial orchids typically produce numerous small seeds that contain very small nutrient reserves. The seeds are structurally adapted for wind dispersal but little is known about their fate after dispersal. Some studies of seed viability in situ indicate survival for up to two years in temperate orchid species. Seeds stored in the laboratory may last much longer. We investigated seed viability of seven North American orchid species with seed packets buried in a range of soil and wood substrates within their natural habitats. In *Goodyera pubescens* most seeds germinated within one year. Four other species continued to germinate sparsely during the observation period, but after almost seven years many seeds were still viable. In one species, *Liparis liliifolia*, seeds that had been in situ for four years had germination rates as high as 68% when sown in vitro with a compatible fungus. The remaining two species did not germinate during the observation period but the seeds were judged to be intact and tested positively for viability after four years in the ground. These observations are interpreted as different species-specific strategies for in situ germination and their seed bank potential is discussed.

1. Introduction

Orchid seeds are minute, contain very small nutrient reserves, and the seedlings require a fungal symbiont to become established. When the seeds are shed, the embryos are morphologically immature and morphophysiological dormancy is likely to be common in orchids (Baskin and Baskin, 1998). Little is known about the fate of orchid seeds after dispersal. Do they become part of a seed bank, a common feature in many plant communities (Leck et al., 1989)? Does germination follow a predictable pattern over time (e.g., Grime, 1981; Baskin and Baskin, 1998)? Because of their small size, many seeds could be dispersed to sites unsuitable for germination or destroyed by soil processing animals or parasitic fungi (Rasmussen, 1995). On the other hand, orchid seeds stored under low humidity and low temperatures ex situ have remained viable for decades (Seaton and Pritchard, 2003; Ramsey and Dixon, 2003) and they are known to be highly resistant to chemical surface treatments (sulfuric acid and hypochlorites) used for sterile in vitro germination (Malmgren, 1996; Hicks, 1999).

The introduction of a method for sowing orchid seeds in situ and retrieving them later for germination assessment (Rasmussen and Whigham, 1993) has provided information on seed longevity in a few species. With the seed packet technique, the seeds are subjected to the physical and chemical conditions of the substrate, and contact with small soil organisms is possible but the sseeds are protected from larger

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animals such as millipedes and earthworms. In a study of the North American species *Spiranthes lacera*, Zelmer and Currah (1997) found that most seeds had germinated within a year. Also in the European species *Dactylorhiza maculata* and *Epipactis helleborine*, the seeds proved to be relatively short-lived (van der Kinderen, 1995). Seeds of *Caladenia arenicola* and *Pterostylis sanguinea* had either germinated or decomposed within a year in the dry Mediterranean climate of Western Australia (Batty et al., 2000). Many seeds of *Corallorhiza trifida* germinated within the first year but some seeds remained intact and appeared viable even after 31 months of burial (McKendrick et al., 2000b).

Whigham et al. (2002) reported on in situ studies of seeds of five orchid species that occur in deciduous forests in eastern North America. After one year in the ground, only one species (*Goodyera pubescens*) showed a high germination percentage and few viable seeds of this species remained. All seed packets of the other four species contained viable seeds but the small number of protocorms observed suggested a high degree of spatial variation in germination within and among the study plots. In the present paper we present additional results from that experiment and from a second field sowing experiment. Our objective is to provide evidence that seeds of some orchids persist well beyond one or two years, thus having the potential to form a persistent seed bank. We discuss the types of seed banks that were formed and their implications for conservation and restoration of threatened and endangered terrestrial orchids. The results of these studies are also interpreted in the context of orchid–fungal interactions.

2. Study area

The two field sowing experiments described below were conducted at the Smithsonian Environmental Research Center (SERC) in Maryland, USA. Both were established in the natural habitats of the study species. The sites for the first field sowing were all located in mature hardwood forests where the dominant trees were species of *Quercus* and *Carya* as well as *Liriodendron tulipifera* L., *Liquidambar styraciflua* L., *Fagus grandifolia* Ehrh., and *Acer rubrum* L. (Parker and Tibbs, 2004). Dominant understory trees were *Cornus florida* L. and *Carpinus caroliniana* Walter. Orchid species included in the first field sowing were: *Aplectrum hyemale* (Muhl ex Willd.) Nutt, *Corallorhiza odontorhiza* (Willd.) Nutt., *Goodyera pubescens* (Willd.) R. Br., *Liparis liliifolia* (L.) Rich ex Lindl. and *Tipularia discolor* (Pursh) Nutt. In conjunction with this first field sowing we carried out an in vitro germination study of *Liparis* seeds that had been buried for four years. The second field sowing involved *Galearis spectabilis* (L.) Raf. and *Platanthera lacera* (Michx) G. Don. Nomenclature for non-orchids follows Radford et al. (1968), and for orchids we used the World Checklist of Monocots (2004) as listed by The Board of Trustees of the Royal Botanic Gardens, Kew. Published on the Internet; http://www.kew.org/monocotChecklist/home.do.

3. Methods

3.1. *First field sowing: Aplectrum, Corallorhiza, Goodyera, Liparis and Tipularia*

In autumn 1997, locally collected seeds of these species were briefly dried in the laboratory and seeds from numerous capsules mixed. A sample containing approximately 50–300 seeds was put into each seed packet (270 seed packets per species) constructed of 50 μm pore size plankton netting fitted in plastic frames (Gepe glassless slide mounts). The packets were then placed vertically into linear plastic trays that are designed for slide projectors (Fig. 1). The trays were buried in eight different types of substrates at each of the three forest sites (Whigham et al., 2002). The schedule for examination of seed packets is shown in Table 1. Further details of the experiment and results after the first year for *Goodyera* can be found in Whigham et al. (2002). Because most seeds of *Goodyera* had germinated or deteriorated after one year, no further observations of that species were made.

Seed packets were periodically returned to the laboratory for examination and viability testing (Table 1). Seed packets not processed immediately were stored at 6 C until recorded. A visual inspection of each seed packet included examination for the presence of germinating seeds and protocorms. Germination was indicated if the embryo had emerged from the testa. Intact seeds were evaluated for viability based on visual conditions of the seed coats and embryos. After four years (Table 1) we continued to make visual observations of seed packets but we also conducted viability tests on subsets of seed packets (Table 1) using the Triphenyltetrazolium chloride (TTC) method (Rasmussen, 1995; Baskin and Baskin, 1998; Hicks, 1999; Ramsey and Dixon, 2003).

We also determined the germination rates of *Liparis* seeds after they had been in the field for four years (Table 1). The

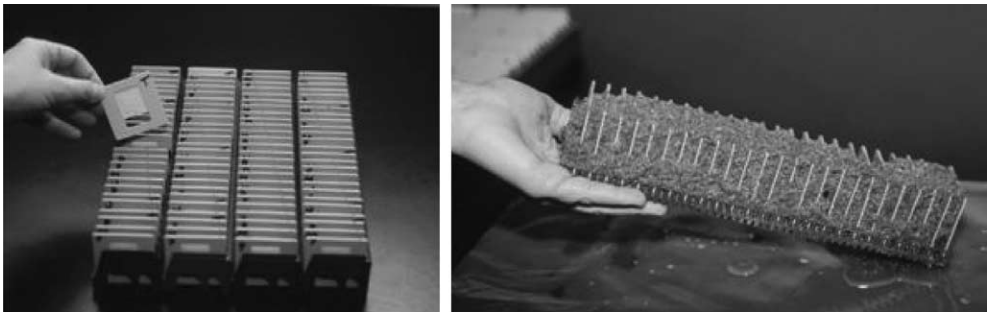

Fig. 1 – Photos of slide trays containing seed packets (left) and a slide tray that has been filled with ground wood and seed packets (right).