Germination ecology of the boreal-alpine terrestrial orchid *Dactylorhiza lapponica* (Orchidaceae)

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In order to reveal some of the germination requirements of the boreal-alpine terrestrial orchid *Dactylorhiza lapponica*, several experiments were carried out on seeds collected from the Sølendet nature reserve, central Norway. Seeds were sown in seed packets made of nylon cloth and deployed *in situ* vertically in the peat in order to study the temporal pattern of seed germination and determine if the seeds became part of the soil seed bank. *In vitro* germination experiments were carried out varying the growth media, fungal partner and chilling treatment, in order to study the effect of a fungal symbiont on germination and early protocorm development, and the possibility of a physiological seed dormancy. A high rate of germination a short time after sowing in the *in vitro* experiments together with a very low survival after deployment in the *in situ* experiments (0.2% after three years) indicate that *D. lapponica* seeds are not part of a long-lived soil seed bank. *In vitro* experiments also demonstrated that a fungal symbiont was not required for germination. Seeds sown *in situ* had very low germination rate (11%–12%) and lack of available nutrients is suggested as a possible explanation. Presence of a fungal symbiont clearly enhanced the early development of protocorms *in vitro*, and is probably necessary for the seedling to grow beyond the earliest protocorm stages under natural conditions. The results indicate that recruitment is highly variable and very low relative to population size, indicating that survival of established plants is crucial to the fate of a population.

Key words: Central Norway, life strategy, orchid mycorrhiza, protocorm development, rich fen, seed bank, seedling survival

Introduction

Terrestrial orchids form mycorrhizal interactions at all life history stages but the characteristics and ecological consequences of the interactions are known for relatively few species (Rasmussen 1995). At one end of a spectrum of dependence on mycorrhizal interactions are non-photosynthetic

orchids (e.g., species of *Corallorhiza*) that are fully mycotrophic at all life history stages and the interactions are obligatory. Other species depend on mycorrhizal interactions to varying degrees. Seeds of *Liparis lilifolia*, for example, will not germinate in the field or laboratory unless an appropriate fungus is present. The same fungus occurs at all life history stages (Whigham *et al.* 2006) and it is very similar genetically across the range of distribution of the orchid (McCormick *et al.* 2004). Beyond the seeding stage, all terrestrial orchids that were examined are mycorrhizal during some part of the growing season (Rasmussen 1995). Perhaps most importantly, all terrestrial orchids form protocorms, an underground life history stage between seed germination and seedling establishment, that are fully mycotrophic and the orchid–fungus interaction is obligatory. The establishment and maintenance of populations of terrestrial orchids thus depends on the establishment of successful mycorrhizal interactions at one or more life history stages and knowledge about the characteristics of orchid mycorrhizal interactions is very important. Especially for rare and endangered species as their decline may be due, in part, to disturbances of the orchid-fungal interactions, particularly the loss of required fungi as a result of habitat changes. Thus, if we are to successfully protect, maintain or even reintroduce populations into areas where they have been extirpated, such knowledge is essential.

Following the development of methods allowing *in situ* studies of orchid seed germination (Rasmussen & Whigham 1993, 1998a, Masuhara & Katsuya 1994, Van der Kinderen 1995a, Whigham *et al.* 2002), a number of studies on seed germination and early seedling development have expanded our knowledge base on orchid–fungal interactions in terrestrial orchids. These studies reveal that seed germination is highly variable temporally and spatially, both regarding germination percentage and the time from imbibition to germination. Important knowledge is still lacking, however, for many species and information based on the few species that have been examined show enormous variability and overall patterns seem to be lacking. There is a general impression, for example, that holarctic species germinate in spring, although

input of dead biomass during autumn which stimulates fungal activity should provide ideal conditions for mycotrophic seedlings to begin their development shortly after the seeds are shed (Rasmussen 1995). *In situ* observations of seed germination in *Epipactis helleborine* indicates a long period of imbibition (6 months) before germination, and rupture of testa was first observed after 8–9 months (Van der Kinderen 1995a, 1995b). Seeds of *Goodyera pubescens* and *Corallorhiza odontorhiza* required about 6 months in the soil before germinating (Rasmussen & Whigham 1993). On the other hand, seeds of *Dactylorhiza maculata* germinated in large numbers after just 3.5 months, seeds of *Microtis parviflora* after 12 weeks, and seeds of the winter-green species *Spiranthes sinensis* after only eight weeks in the soil (Masuhara & Katsuya 1994, Perkins & McGee 1995, Van der Kinderen 1995a). Studies of species confined to Mediterranean conditions indicate that very few seedlings reach a stage of development allowing them to survive the subsequent dry season, and that most species are believed to have transient seed banks, lasting for less than one growing season (Batty *et al.* 2000, 2001). Recent studies, however, provide evidence that seeds of some species become part of a long-lived soil seed bank (Whigham *et al.* 2006). We are not aware of any seed bank studies that were carried out on orchid populations in a boreal-alpine climate, with an extensive period of snow cover and frost, and a very short growing season. We do not know whether the seeds remain viable for a longer or shorter time under such conditions, but most biological processes slow down in a cold climate, including decomposition and fungal activity, and the risk of being exposed to excessive drought is much smaller.

Populations of the boreal-alpine orchid *Dactylorhiza lapponica* have been followed since the late 1970s as part of a major long-term monitoring programme in the Sølendet nature reserve in central Norway. The monitoring forms a part of a larger programme in the reserve, the main aim of which is to learn more about the composition, production and dynamics of the plant communities, the ecology and population biology of a number of fen and grassland species, and how different management practices affect their popu-

lation and communities (Moen 1990, Moen *et al.* 1999). We consider vegetative reproduction to be virtually absent in *D. lapponica* (Øien & Moen 2002). Recruitment, or population input, is thus totally dependent on the survival and establishment of seedlings. In this paper we report results from ongoing investigations on the germination requirements of *D. lapponica*, in particular those related to fungal symbionts and seed dormancy. Considering the lack of vegetative reproduction and the species' life strategy that give prority to the survival of the perennial plant, we wanted to establish whether the species has a seed bank, and increase our knowledge of seedling survival prior to the photosynthetic stage. The ecological consequences of the findings are emphasized, particularly in view of population input (recruitment). In the present paper, the following issues are addressed:

- The effect of chilling on germination in relation to a possible physiological dormancy.
- The effect of the presence of a fungal symbiont on germination, early protocorm development and growth.
- Germination *in situ* and the presence of a persistent seed bank.

The study species

Dactylorhiza lapponica belongs to an orchid genus that constitutes a large group of species occurring mainly in northern and central parts of Eurasia. They occupy a wide range of biotopes such as mires, dry meadows and woodlands (Delforge 1995). For a number of *Dactylorhiza* species the seeds germinate rather readily *in vitro*, also asymbiotically and on a wide range of substrates, even water (Downie 1941, Vermeulen 1947, Harvais & Hadley 1967b, Van Waes & Debergh 1986, Rasmussen 1995). Seeds of *D. maculata* are also observed to germinate readily under natural conditions *in situ* (Van der Kinderen 1995a). Introduction of a fungus results in increased germination in both *D. majalis* (Rasmussen *et al.* 1990) and *D. purpurella*, particularly at lower temperatures (Harvais & Hadley 1967b), and association with a mycorrhizal fungus is believed to be required for fur-

ther development of the protocorm to take place in these species under natural conditions (Harvais & Hadley 1967b, Rasmussen 1990).

Dactylorhiza lapponica is largely confined to the central parts of Fennoscandia and the Alps (Øien & Moen 2002). It is found in open low vegetation on wet, calcareous or base-rich soil, such as rich fens, wet grasslands, springs and riverbanks. In Fennoscandia it is exclusively found in extremely rich fens and springs in the boreal vegetation zones (zonation after Moen 1999), where it is a characteristic species of *Caricion atrofuscae*, *Schoenion ferruginei* and *Cratoneurion commutati* communities (Moen 1990). Studies of the population biology of *D. lapponica* (Øien & Moen 2002) revealed an irregular flowering pattern influenced by the high cost of flowering and periodic extreme weather conditions. Previous studies also suggested that the persistence of populations depend more on the long-term survival of established plants and less on recruitment from seeds (Øien & Pedersen 2005). It should be noted that *D. lapponica* is a controversial taxon. It is an allotetraploid and probably formed through hybridisation between *D. fuchsii* and *D. incarnata* and recent studies conclude that *D. lapponica* should be recognised as a subspecies of *D. majalis s. lato* (Hedrén 1996, 2005, Pedersen 2004). In our study area we find a secondary hybrid zone between *D. lapponica* and *D. incarnata* ssp. *cruenta* (Aagaard *et al.* 2005).

Methods

Seeds were collected at the beginning of August 2003 and 2004, approximately 3–4 weeks after the peak of flowering, from plants growing in the Sølendet nature reserve, central Norway (62°40′N, 11°50′E). Sølendet is situated 700–800 m a.s.l at the transition between the middle boreal and northern boreal vegetation zones and between the oceanic and continental vegetation sections (Moen 1999). The winter is long and cold, and the summer is short and cool, giving a short growing season, usually from the beginning of June until late August (Øien & Moen 2002). The vegetation at Sølendet is dominated by birch woodlands and sloping fens. About half

the area consists of rich fen vegetation, mostly extremely rich lawn communities belonging to *Caricion atrofuscae*; the rest is mainly wooded grassland belonging to *Lactucion alpinae* and heath vegetation belonging to *Vaccinio–Piceion*.

Seeds were obtained by cutting flower spikes containing fully matured seed capsules of a few randomly selected individuals in the nature reserve. The flower spikes were air dried and stored in room temperature for 2–4 months until further use in subsequent experiments. *In vitro* germination of seeds and growth of protocorms were carried out at the Smithsonian Environmental Research Center, Maryland, USA (SERC), and at the Plant Biocentre, Norwegian University of Science and Technology, Trondheim, Norway (NTNU).

Germination *in situ*

Seeds were sown in seed packets made of nylon cloth (mesh 50 μm) put between slide frames according to a method described by Rasmussen and Whigham (1993). The seed packets were deployed vertically in the peat with the top edge of the slide frames visible above ground, leaving most of the seeds buried 2–4 cm below the surface. The packets were deployed in early September 2003 in two different types of rich-fen lawn vegetation at the Sølendet nature reserve, rich-fen expanse and rich-fen margin. Seed packets were deployed in areas where established plants were abundant. The rich-fen lawn vegetation at Sølendet is dominated by grasses and sedges, particularly species like *Carex panicea* and *Trichophorum cespitosum*. Characteristic species of the rich-fen expanse vegetation are *Carex hostiana* and *Eriophorum latifolium*, while *Kobresia simpliciuscula* and *Molinia caerulea* along with a relatively high abundance of herbs like *Leontodon autumnalis* and *Succisa pratensis* are characteristic of the rich-fen margin vegetation (*see* Øien & Moen [2002] for more details on species composition). Approximately 100–400 seeds from one seed capsule were placed in each seed packet. The packets were retrieved about 11 months (mid August 2004), 12 months (late September 2004) and three years (late September 2006) after deployment. From both vegetation types, ten seed packets were retrieved at each sampling in 2004. After retrieval the packets were cleaned with tap water, opened and scored for percent germination. A germinated seed was defined as one that had clearly swelled, ruptured its testa and produced its first rhizoid. In 2006 three seed packets were retrieved from each vegetation type. After being cleaned with tap water, a test was performed to determine if protocorms found in these packets were alive and if ungerminated seeds were still viable. The seed packets were soaked in a 1% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) for 5 days in darkness and at room temperature. Ungerminated seeds that remained whitish to yellow were considered non-viable, while seeds and protocorms that had turned orange to pink were considered to be alive and metabolically active.

Germination *in vitro*

Separate experiments were carried out on the two seed batches, varying the growth media, fungal partner and chilling treatment (Table 1). In all experiments seeds from several individuals were mixed and pretreated for 2 hours in a saturated solution of calcium hypochlorite $Ca(ClO)_2$ with 0.05% detergent (Tween 80). The seeds were then rinsed in sterile water before sowing onto agar media plates (in 60 mm sterilized polystyrene Petri dishes). Asymbiotic germination was carried out on three different media: LIRIO = water mixed with 5% dried and finely ground partly decomposed wood of *Liriodendron tulipifera*, a medium poor in readily accessible carbon and other nutrients that has been shown to support germination and protocorm growth (i.e. Whigham *et al.* 2006); OAT = 2.5 g finely ground oat in one liter of water gives a medium slightly richer in readily accessible carbon; and P0931 = a commercial medium (Sigma-Aldrich) containing large amounts of readily accessible carbon (sucrose) and all necessary macro- and micronutrients for plant growth, including large amounts of inorganic nitrogen (ammonium nitrate). As OAT gave the best result in the asymbiotic germination of seeds from the 2003 batch, this was the medium mainly used in the subsequent symbiotic germination experiments. However, SALIX,

a lean medium made from water mixed with 5% dried and finely ground partly decomposed grasses, sedges and wood of *Salix* spp. from Sølendet, was used in symbiotic experiments with seeds from the 2004 batch. Unchilled plates were incubated at room temperature immediately after sowing. The others were chilled for three months at 3–4 °C before incubation at room temperature. No plates were exposed to light, either during chilling or incubation. Inoculation with an endophyte was done prior to chilling or 1 to 5 days after incubation started in unchilled plates. The fungi used in this study had been isolated from roots or protocorms of orchid species (Table 1) using a method described in Whigham *et al*. (2002), and kept in culture on a basal salt media (Caldwell *et al*. 1991) or a medium similar to Clements *et al*. (1986), prior to inoculation. A vitality test (*see* above) was performed on the seeds from the 2004 batch at the end of the germination experiment, to check the viability of ungerminated seeds. Approximately 10 ml TTC was added to petri dishes, and the seeds were soaked in TTC for 5–10 days in darkness and at room temperature.

Early development and growth of protocorms *in vitro*

Well developed protocorms were transferred from the Petri dishes used in the *in vitro* germination experiments to new agar media plates about 2–4 weeks after incubation, three protocorms on each plate. The plates were kept at room temperature and those originating from experiments on the 2003 seed batch (carried out at SERC) were exposed to light during daytime. The diameter of the protocorms were measured in a dissection microscope at intervals up to three months after incubation.

Statistical analyses

The effect of various fungi and media (including chilling) on germination in the *in vitro* experiments were tested separately, using the Tukey multiple comparison test in a one-way ANOVA. Similarly, differences in protocorm growth were analysed testing differences in protocorm size after 10–12 weeks of incubation. The effect of

Table 1. Overview of the *in vitro* germination experiments carried out on seeds of *Dactylorhiza lapponica* collected at the Sølendet nature reserve, central Norway.

Seed batch	Fungal symbiont[a] (isolated from roots of orchid)	Growth medium	Chilling[b]	N
2003	asymbiotic	OAT	no	8
2003	asymbiotic	OAT	yes	5
2003	asymbiotic	LIRIO	no	5
2003	asymbiotic	P0931	no	5
2003	*Tulasnella* sp. 159 (*Goodyera pubescens*)[c]	OAT	yes	5
2003	*Ceratobasidium* sp. 468-2 (*Goodyera tesselata*)	OAT	no	5
2003	*Ceratobasidium* sp. 530-1 (*Coeloglossum viride*)	OAT	no	5
2003	*Tulasnella calospora* D47.8 (*Dactylorhiza majalis*)/ B326.47 (*Orchis morio*)	OAT	no	5
2003	*Tulasnella* sp. 120-e (*Liparis lilifolia*)	OAT	no	5
2003	*Tulasnella* sp. 144-1/149-1 (*Goodyera pubescens*)[c]	OAT	no	5
2003	*Tulasnella* sp. M109-1 (*Goodyera pubescens*)	OAT	no	3
2004	asymbiotic	OAT	yes	5
2004	asymbiotic	SALIX	yes	5
2004	*Tulasnella* sp. D12 (*Dactylorhiza lapponica*)	OAT	yes	9
2004	*Tulasnella* sp. D12 (*Dactylorhiza lapponica*)	SALIX	yes	5
2004	*Sebacinaceae* D13 (*Dactylorhiza lapponica*)[d]	OAT	yes	5
2004	*Sebacinaceae* D13 (*Dactylorhiza lapponica*)[d]	SALIX	yes	5

[a] code referring to the fungus collection at SERC (McCormick *et al*. 2004) or NTNU.
[b] chilling period: 10–12 weeks.
[c] isolated from protocorms.
[d] no close match found, but it is closest to *Efibulbobasidium* sp. and *Sebacina allantoidea*.
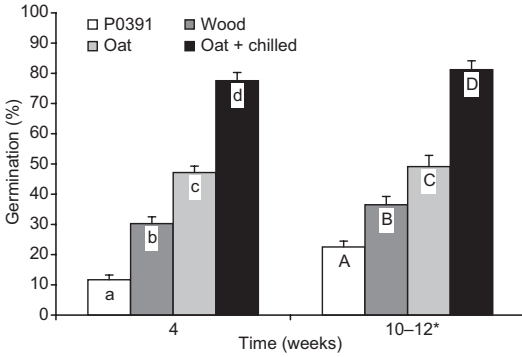
Fig. 1. Germination *in vitro* of seeds of *Dactylorhiza lapponica* collected in 2003 and sown on various growth media. Letters indicate significant differences (*p* < 0.05) in germination percentage between treatments. * Some oat plates were scored after 9 weeks.
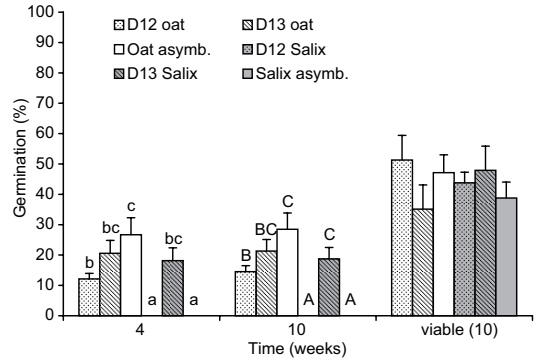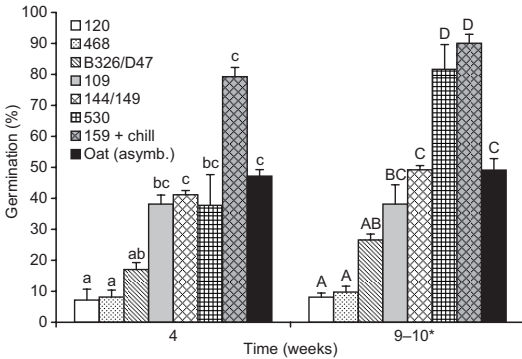

Fig. 2. Germination *in vitro* of seeds of *Dactylorhiza lapponica* collected in 2003, sown on oat and with or without various fungal symbionts (*see* Table 1). Letters indicate significant differences (*p* < 0.05) in germination percentage between treatments. * Plates with fungus 159 were scored after 12 weeks.

vegetation type in the *in situ* sowing experiment was analysed by comparing germination percentages in a *t*-test. The statistical analyses were performed by use of the program package S-PLUS ver. 6.2 (Insightful Corp., Seattle, USA).

Results

Germination *in vitro*

Sowing of seeds from the 2003 batch on various growth media resulted in the highest germination percentage on OAT (Fig. 1). After only four weeks about 47% of the seeds had germinated

Fig. 3. Germination *in vitro* and viability of seeds of *Dactylorhiza lapponica* collected in 2004, sown on various growth media and with or without fungal symbionts found at the Sølendet nature reserve (*see* Table 1). Letters indicate significant differences (*p* < 0.05) in germination percentage between treatments.

asymbiotically on this medium, with an insignificant increase to 49% after 10 weeks. Percentage germination on LIRIO and P0931 was 23% and 36% respectively after 10 weeks. Chilling had a positive effect on germination and resulted in more than 80% germination after 10 weeks incubation on oat medium. Two fungal symbionts, both isolated from American orchids, had a positive effect on germination (Fig. 2). Ten weeks after incubation, 82% of the seeds had germinated symbiotically with fungus 530 (*Ceratobasidium* sp. from roots of *Coeloglossum viride*), and 90% of the seeds had germinated symbiotically with fungus 159 (*Tulasnella* spp. from protocorms of *Goodyera pubescens*). The latter had been chilled for three months, but still had a significantly higher germination percentage than the chilled seeds grown asymbiotically (Fig. 1). Neither of the fungi isolated from Norwegian *D. lapponica* had a positive effect on germination of the seeds collected in 2004 (Fig. 3), but fungus D13 greatly enhanced germination when seeds were grown on the lean SALIX medium. After 10 weeks 18.7% of the seed grown symbiotically had germinated, while no germination was detected in the seeds grown asymbiotically. Several of the fungi had a negative effect on germination, particularly fungus 120 (*Tulasnella* sp. from roots of *Liparis lilifolia*) and fungus 468 (*Ceratobasidium* sp. from roots of *Goodyera tesselata*). After 10 weeks less than 10% of the seeds had germinated with one of these fungi present. The results also

show much higher germination percentage in seeds from the 2003 batch (Fig. 2) as compared with that in seeds from the 2004 batch (Fig. 3), and all seeds from the latter had been chilled for three months. The viability test indicates that less than 50% of the seeds from the 2004 batch were viable (Fig. 3), while seeds from the 2003 batch reached above 80% germination for several of the treatments both chilled and unchilled (Fig. 2).

Germination *in situ*

The germination in seed packets was very low (Fig. 4). After 11 months only about 11% of the seeds had germinated, but germinated seeds were found in 90% of the packets, including all packets in the fen expanse vegetation. After 12 months, germination had only increased to about 12%, but percent germination was significantly higher in the fen expanse compared to the fen margin (Fig. 4). No seeds had developed beyond the early stages of germination with rupture of testa and production of a few rhizoids.

After 11 months a number of seeds had disintegrated in nine (45%) of the seed packets that were retrieved, four from the fen expanse and five from the fen margin. In most of these seed packets the nylon mesh was partly covered with green algae. After 12 months this was the situation in 14 seed packets (70%), six from the fen expanse and eight from the fen margin. In the seed packets retrieved after three years almost all the seeds had disintegrated, and a vitality test using TTC showed that very few (about 0.2%) of the remaining seeds were alive. No protocorms were observed to have survived the earliest stages of development. Throughout the whole experiment we also observed a number of seeds that seemed to be empty. After some imbibition they had collapsed without producing rhizoids or any sign of germination. Most of the viable seeds were found in the seed packets deployed in the fen margin and retrieved after 12 months.

Early development and growth of protocorms *in vitro*

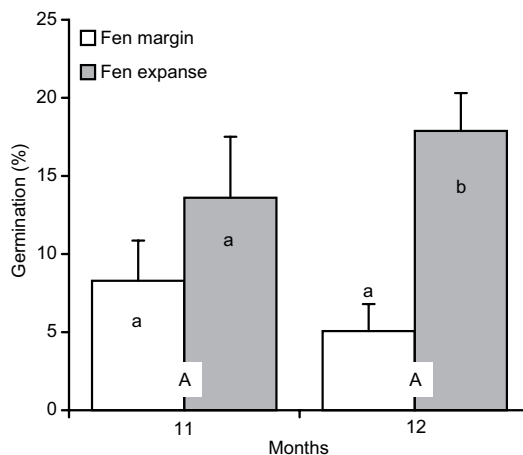Three different endophytes clearly enhanced

Fig. 4. Germination *in situ* of seeds of *Dactylorhiza lapponica* collected in 2003 and deployed in seed packets in September 2003 at the Sølendet nature reserve. Letters indicate significant differences ($p < 0.05$) in germination percentage, lower case letters between vegetation types and upper case letters between sampling times.

the early development of protocorms. Those grown with D12, 159 and 144 were on average about 1.4–1.5 mm in diameter 10–12 weeks after sowing (Fig. 5), significantly larger than those grown asymbiotically which were typically 0.4–0.6 mm in diameter. Protocorms inoculated with D12, a fungus isolated from roots of *D. lapponica*, reached a size of 1 mm already after 4 weeks. Furthermore, symbiotically grown protocorms grew more rapidly on OAT than on a wood media and asymbiotically grown protocorms grew better on P0931 than on oat (Fig. 5). After 10–12 weeks most of the largest protocorms had developed a clearly visible shoot tip with leaf primordium, but no root initial.

Discussion

Dormancy and seed bank

Immature seeds of several temperate terrestrial orchids, like *Orchis* spp., *Dactylorhiza majalis*, *D. maculata*, *D. incarnata*, and *Gymnadenia conopsea* germinate more readily *in vitro* than mature seeds (Borris 1969, Lindén 1992), and soaking seeds in hypochlorite increases germination in many species, e.g. *D. maculata* (Lindén
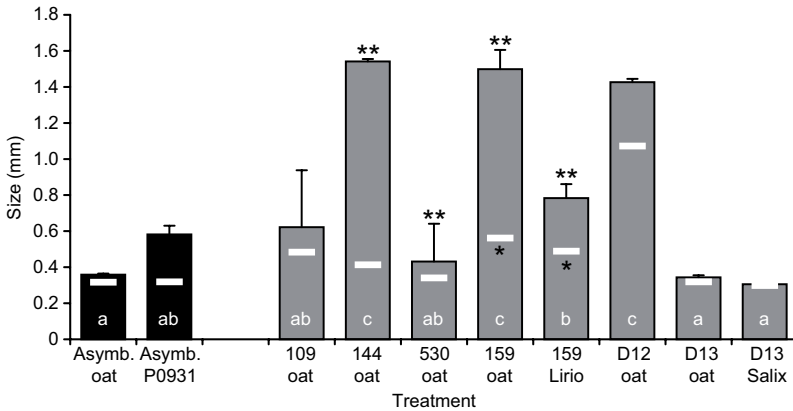
Fig. 5. Protocorm size 4 (or 6, indicated with *) weeks (white rectangles), and 10 (or 12, indicated with **) weeks (bars) after sowing of seeds from *Dactylorhiza lapponica*. The seeds were grown *in vitro* on various growth media and with or without various fungal symbionts. Letters indicate significant difference ($p < 0.05$) in protocorm size between treatments after 10–12 weeks.

1980). These are indications that a physiological or physical type of dormancy occurs in the seeds of these species. This dormancy can simply be related to the low water content of the orchid seed, which prevents further development (Rasmussen 1995). A weakening of the water-repellent and impermeable testa to allow rehydration is all that is needed for germination to begin a short time after the seeds are shed. High rates of germination *in vitro* found in our experiments indicate that this is the case for *D. lapponica*. Results of the seed sowing study further demonstrate that the species does not have seeds that are persistent in the soil and thus does not form a long-lived seed bank. The large proportion of disintegrated seeds in the seed packets one year after deployment also supports this finding.

Seeds from terrestrial orchids are known to germinate readily on a variety of media, from pure water agar to media rich in sugar, micro- and macronutrients, growth regulators, vitamins, etc. (Rasmussen 1995). In our *in vitro* experiments the lean media, with little accessible sugar and other nutrients (LIRIO and OAT) resulted in a higher germination percentage compared to a medium rich in readily accessible carbon and inorganic nitrogen (P0931) (Fig. 1). This corresponds to earlier findings. In experiments with *Dactylorhiza maculata*, Van Waes and Debergh (1986) obtained germination percentages approaching 100% of viable seeds using a medium rich in micro- and macronutrients and sugar, but without inorganic nitrogen. A rich medium for germination may not always be necessary, however, as Downie (1941) observed that seeds of species like *Coeloglossum viride*, *Gym-*

nadenia conopsea and *Dactylorhiza maculata* germinated freely on pure water agar. Rasmussen (1995) found a negative correlation between concentration of nitrate and the germination percentage of *D. majalis*. She also found that adding sugar to water solution did not increase the germination percentage in the same species, but had inhibiting effects at very high concentrations. The increased germination *in vitro* following chilling in our experiments (Fig. 1) could indicate that seeds that do not germinate after being shed can enter a dormancy that can be overcome with a period of cold stratification. However, a more probable explanation is that the pretreatment of seeds in the *in vitro* experiments did not sufficiently break down the testa to initiate germination in some of the seeds. Freezing and thawing during chilling brought about a further softening of the testa and increased the proportion of germinating seeds.

The effect of the presence of a fungal symbiont on germination

Our experiments demonstrate that a fungal symbiont does not have to be present for seed germination to occur but the presence of symbiotic fungi can have a positive influence on germination. Similar results have been found for *Goodyera pubescens*, a North American species (Whigham *et al.* 2006). Of the different mycorrhizal fungi used in the experiments, fungi isolated from roots of *Coeloglossum viride* and protocorms of *Goodyera pubescens* supported germination (Fig. 2 and Table 1), as did a fungus

isolated from adult *Dactylorhiza lapponica* plants (Fig. 3). In most instances, fungi isolated from adult *D. lapponica* did not enhance germination. However, such symbiotic relationship *in vitro* may have little bearing on what takes place under natural conditions, and thus are not evidence of an association between *D. lapponica* and the current fungi. Many *in vitro* germination experiments in terrestrial orchids have shown highly variable results when seeds are exposed to fungi. It is a general view that there is a low fungal specificity in green orchids like our study species (Taylor *et al.* 2002), although McCormick *et al.* (2004) found high fungal specificity in three green North American species. Muir (1989) found enhanced germination in species of *Dactylorhiza*, *Orchis* and *Serapias* from several fungal isolates, especially on the early stages of development. Others found that fungal isolates from adult orchids of one species stimulated germination of seeds from another orchid species but not seeds from the species the fungus was extracted from (Harvais & Hadley 1967a, Hadley 1970, Harvais 1974, Tokunaga & Nakagawa 1974; *see* also Taylor *et al.* 2002, Rasmussen 1995, 2002 for other references).

It is important to distinguish between associations found under experimental conditions *in vitro* and those that are possible and competitive under natural conditions. Thus germination percentages found under very favourable conditions *in vitro* can not be compared to those found *in situ*. Further tests *in situ* or under realistic conditions *in vitro* are necessary in order to establish whether there is a high degree of fungal specificity in *D. lapponica*. More important in our results is the great variation in asymbiotic germination *in vitro*, indicating that the amount and type of available nutrients (*see* above) is equally or more important than the presence of a fungal symbiont in the earliest stages of development. Thus, the lack of germination *in situ* could be a question of nutrient availability, where the purpose of a fungal symbiont is to facilitate the germination either by enhancing the supply or availability of nutrients, e.g. through decomposing organic matter (Rasmussen & Whigham 1998a), or as supplier of certain metabolites that the seeds need in order to grow and develop into a protocorm (Purves & Hadley 1976, Hadley

& Pegg 1989). *In vitro*, where these elements can be added, germination occurs readily. However, detailed experiments varying the supply of specific elements and conducted under more natural like conditions, are necessary in order to reveal which elements are involved. Lack of an embryo in many of the seeds can also explain the low *in situ* germination percentage. Andronova (2003) experienced this in a germination experiment with *D. maculata*. More than 80% of the seeds were found to be incapable of germination because they lacked embryo.

There is a clear effect of fungal symbionts on the growth of the germinated seeds of *D. lapponica* and development into the earliest protocorm stage. Without a fungal partner this process is very slow, even when the seeds are grown on substrates rich in nutrients. We can not tell from the results of the growth experiments *in vitro*, if seedlings are unable to grow beyond the early stages and cease to develop entirely unless they become associated with an appropriate fungus. On the other hand, we did not find any seedlings that had grown beyond this early stage in the *in situ* germination experiments. It could be that the seedlings of *D. lapponica* behave in much the same way as seedlings of *Goodyera pubescens* (Rasmussen & Whigham 1998b, Whigham *et al.* 2002), that germinate more or less independent of external supplies, but with great mortality during subsequent development until a relationship with an appropriate symbiont is established. However, further studies of *in situ* germination are needed to confirm this.

Germination *in situ* and consequences for population input

The *in situ* germination percentages for *D. lapponica* were very low, and extremely few seemed to survive and develop further given the large amount of seeds that were deployed in the seed packets. Similar results were found by Van der Kinderen (1995a) in his study of *D. maculata* using the same sowing technique. After a fast initial germination, later development was very slow, and an increasing amount of dead and parasitized seedlings were observed. He concluded that only a few of several thousand seeds would

reach the autotrophic stage. Clearly, there is a large year-to-year variation, as both seed quality (viability), and the degree of dormancy are influenced by weather conditions (Rasmussen 1995, Andronova 2003). This is evident in our material, in the difference between the maximum germination percentage of seeds collected in 2003 (Fig. 2) and the viability found in seeds collected in 2004 (Fig. 3). Results from a TTC test should be interpreted with caution, as the test is sensitive to variation in the permeability of the seed coat (Vujanovic *et al.* 2000). However, all seeds from 2004 were chilled in our experiments, which should potentially increase the viability percentage compared to those that did not. The differences we observed should therefore be reliable. The method using seed packets also limits the possibilities for further development of the seedling (Rasmussen & Whigham 1998b). The number of seedlings reaching the autotrophic stage is thus difficult to estimate from the results of the seed sowing study. On the other hand, preliminary results from our monitoring programme at Sølendet (unpubl. data) show that "new" seedlings emerging above ground make up about 5%–10% of the population of *D. lapponica* in vegetation similar to the areas where the *in situ* germination studies were carried out.

Without a long-lived seed bank the production of seedlings in *D. lapponica* will be directly dependent on the seed produced in the preceding season. However, our knowledge of further development and survival of the protocorms is very limited. There could be a "protocorm bank" functioning as a more or less persistent seed bank. A number of studies reports that terrestrial orchids spend several years underground before emerging as green plants (for references *see* Rasmussen 1995), but we are not aware of any studies confirming that a "protocorm bank" exists in these species. Leeson *et al.* (1991) sampled soil cores and looked for protocorms of *D. fuchsii*. They found that the number of protocorms decreased rapidly in the winter, and no protocorms were found in the spring. They explained this by rapid transition to later developmental stages and high mortality. Their results suggest that there is no "protocorm bank" in these species, but further investigations and experiments are necessary to clarify this.

Our results, showing low and highly variable germination, short-lived seeds, variable seed quality and virtually no seed bank, have increased our knowledge on the population dynamics of *D. lapponica* in the upper-boreal areas of northern Europe. The results also emphasise the critical importance of recruitment in the population dynamics of this orchid. Recruitment is low and highly variable unless an unknown and substantial protocorm bank exists, and survival of the "perennial" plants is crucial to the fate of the population. This also supports earlier findings of a strategy that gives priority to survival of the "perennial" plant (Øien & Pedersen 2005). High mortality among "adults" could therefore extirpate a population in the long run, and re-establishing a population after major environmental perturbations that wipe out many "adults" will be slow and difficult.

Acknowledgements

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