New Methods For Germinating Orchid Seeds

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BIOLOGY OF THE ORCHID SEEDLING

Plant seedlings in their first stages are heterotrophic and depend entirely on nutrient reserves deposited during the maturation of the seed. The mother plant thus provides support until the seedling has developed a photosynthetic apparatus.

Orchid seeds are very small ("dust seeds"), being less than 1 mm long including the testa. A concentrated reserve of protein and lipids is stored in the embryo but is necessarily small. The embryo is a poorly differentiated body of at most 200 or 300 cells. During germination the reserves are mobilized but because the heterotrophic phase is extremely long and the seed reserves so limited, no orchid seedling develops far without external nutrient supplies. The seedling relies on an alternative source of nutrition, i.e. the breakdown of fungal hyphae on which the plant parasitizes. The radicle is completely specialized into a mycotrophic organ; until the seedling produces adventitious roots and leaves it cannot emerge above the substrate and begin photosynthesis. Terrestrial orchid seedlings usually live underground as heterotrophic organisms for several months after germination.

GERMINATION

Symbiosis with fungi is a necessity when orchid seeds germinate in nature. In culture the special requirements of the seedlings can be met by two means. Either they are grown asymbiotically on a nutrient substrate from which they can take up macro- and microelements, soluble carbohydrates, amino acids, vitamins, and hormones, or symbiotically in co-culture with a compatible fungus on a substrate that sustains the fungus, often based on starch or cellulose. Both methods require that germination takes place in vitro, although a procedure for symbiotic culture under nonsterile conditions could be developed.

Since the beginning of this century propagation in vitro has become a standard procedure in many genera of orchids but some groups still present considerable problems. Much emphasis has been placed on the heterotrophy of the seedlings. When symbiotic culture has been unsuccessful, lack of compatibility with the fungus in question has always been an available explanation. When on the other hand an asymbiotic culture has failed, whether sporadically germinating seeds or poorly growing seedlings, it has usually been ascribed to a lack of a crucial ingredient in the substrate. The efforts to provide an adequate substitute for the symbiosis have resulted in a multitude of complicated substrate recipes, often with undefined ingredients such as yeast extract or coconut milk.

NEW INFORMATION ON ORCHID SEED DORMANCY PATTERNS

During the last years, and partly through my work and that of my co-workers, the importance of non-nutritional factors in the germination of orchids has received more attention. Several dormancy mechanisms which resemble those of other plant

seeds have been found (Rasmussen, 1995). It is essential to distinguish between factors that influence seeds during germination and those pertaining to the subsistence of the seedlings. Components of the substrate (and properties of a co-cultured symbiont) may be irrelevant factors for the seeds if they are dormant.

Surface sterilization of seeds, usually with either sodium or calcium hypochlorite, is necessary before they are sown in vitro. Not only does that prevent contamination with microorganisms but long treatments in hypochlorite also raise germination percentages, presumably because of dissolution of lipids in the testa, thus facilitating water uptake and leaching of inhibiting substances, such as abscisic acid, from seed. In nature seeds can remain 6 to 7 months in the ground before they germinate (Rasmussen and Whigham, 1993). Other strongly alkaline solutions may have effects similar to hypochlorite. Optimum treatment varies strongly with the species (Fig. 1; Van Waes, 1984) and can be as long as 4 h in 5% NaOCl. Calcium hypochlorite is usually somewhat better than sodium hypochlorite.

In spite of the minute seed size there are no indications that orchid seed germination is stimulated by light. Many species tolerate light but some are negatively photoblastic. In *Dactylorhiza majalis* the seeds needed at least 14 days of darkness in the beginning of a 6-week incubation period, otherwise germination percentage was significantly reduced (Rasmussen et al., 1990). The reaction prevents seeds from germinating on the soil surface and is an adaptive reaction in seeds whose seedlings are mycotrophic and rootless. These seedlings depend on a stable moisture regime whereas light is an irrelevant growth factor.

The reaction of the seeds to ethylene could be another adaptation to prevent superficial germination and increase chances of a successful establishment of symbiosis. This gaseous plant hormone is developed from a host of microorganisms, amongst these the kind of fungi which are orchid symbionts. Soil ethylene concentrations can reach 10 ppm, rising with depth (Smith and Dowdell, 1974; Hanke and

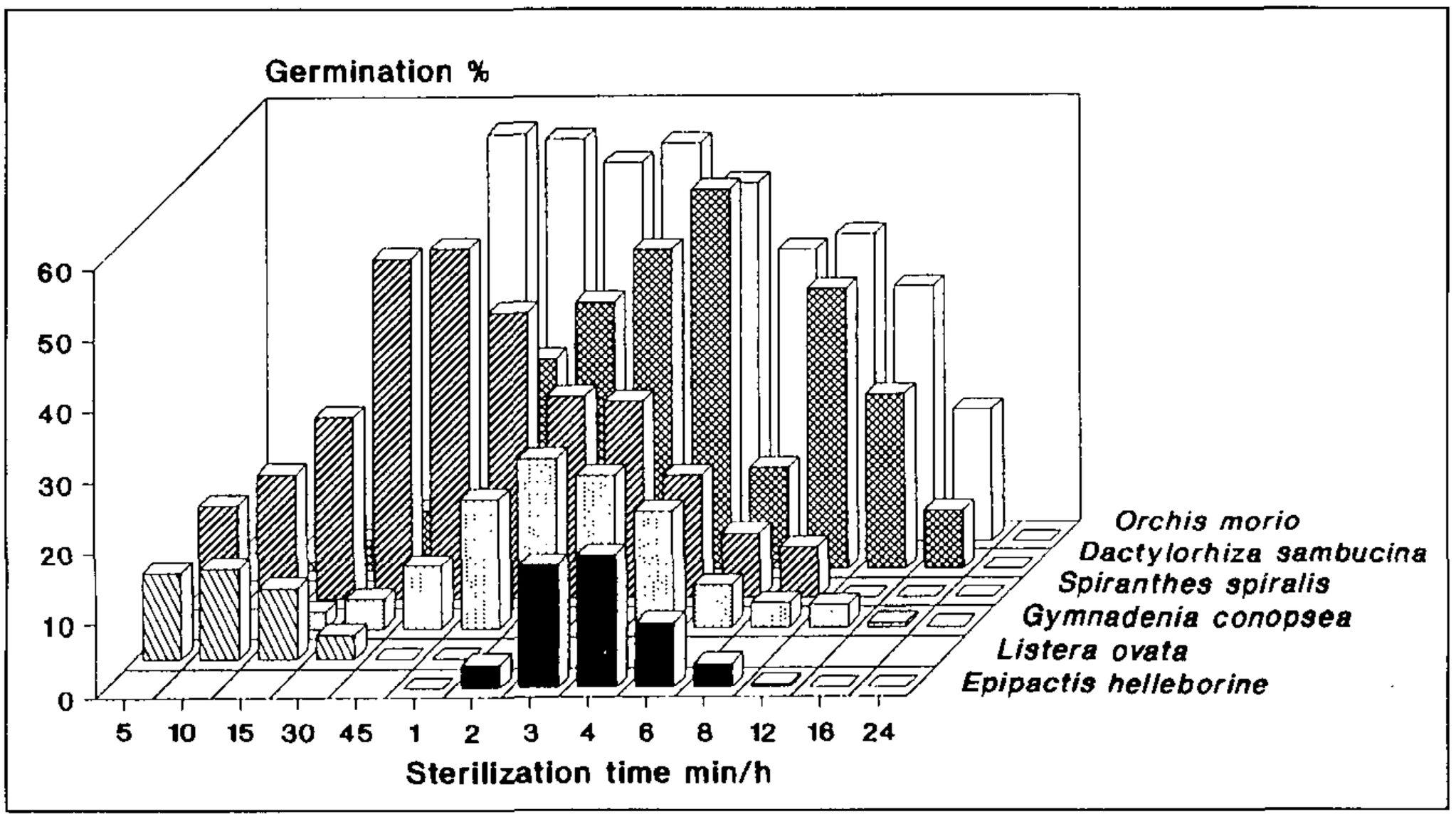

Figure 1. Optimum treatment time (scarification) for germination in a range of orchid species. The seeds were treated in 5% NaOCl with Tween 80 (1%), rinsed in sterile water, and sown on asymbiotic substrate. Data from Van Waes (1984). Figure from Rasmussen, 1995.

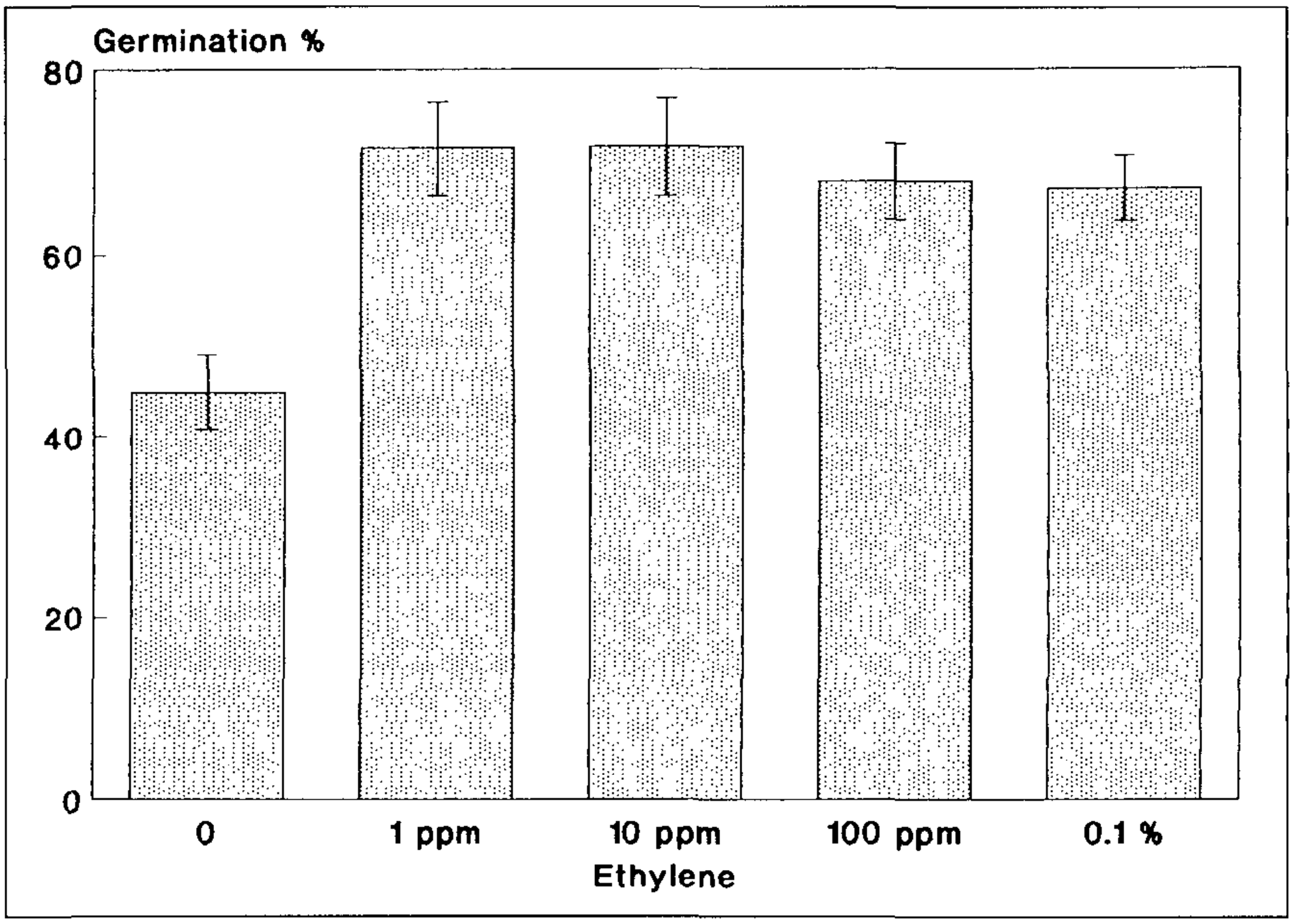

Figure 2. Effects of ethylene added to the atmosphere above ventilated Petri dishes with incubated seeds. *Dactylorhiza majalis* was sown on oat medium and inoculated with a fungal strain identified as *Tulasnella deliquescens* and incubated in darkness at 20C. Treatment began immediately after sowing and was interrupted after 21 days by ventilation with sterile ambient air. Germination was recorded after 42 days. Means of 16 to 18 samples with 95% confidence intervals. All treatments are higher than the control ($P<0.01$, arcsin transformation). Figure from Rasmussen, 1995.

Dollwet, 1976). In *D. majalis* germination percentage increased significantly in response to the addition of 1 ppm ethylene and there was a great tolerance towards high ethylene concentrations (Fig. 2).

Species differ greatly with respect to seed dormancy patterns. Cold stratification is required by some. Seeds of *Epipactis palustris* (marsh helleborine) reached the highest germination percentage when treated for 12 weeks at 6 to 8C after a warm incubation for about 6 weeks at 20C. There was an additional strong increase in the temperature reactions when the seeds were co-cultured with a compatible fungus (Rasmussen, 1992). Most likely other species, amongst these the horticulturally most interesting species of *Cypripedium* (lady's slipper orchids), need cold stratification. Although these species have been subjected to many germination trials the temperature reactions have been little studied. Such reactions may furthermore be difficult to detect since the seeds germinate sparsely without inoculation and the compatible fungi are still unknown.

Besides traditional dormancy mechanisms the seed also reacts to inoculation. A 20% germination in seeds of *Platanthera chlorantha* (greater butterfly orchid) could be increased by inoculation with a range of symbionts to levels between 20% and 80% (Fig. 3). Those fungal strains that stimulated germination most were not always those that would establish the best symbiosis with the seedlings (Rasmussen, 1995). The kind of signalling that takes place between the fungi and the seeds is still

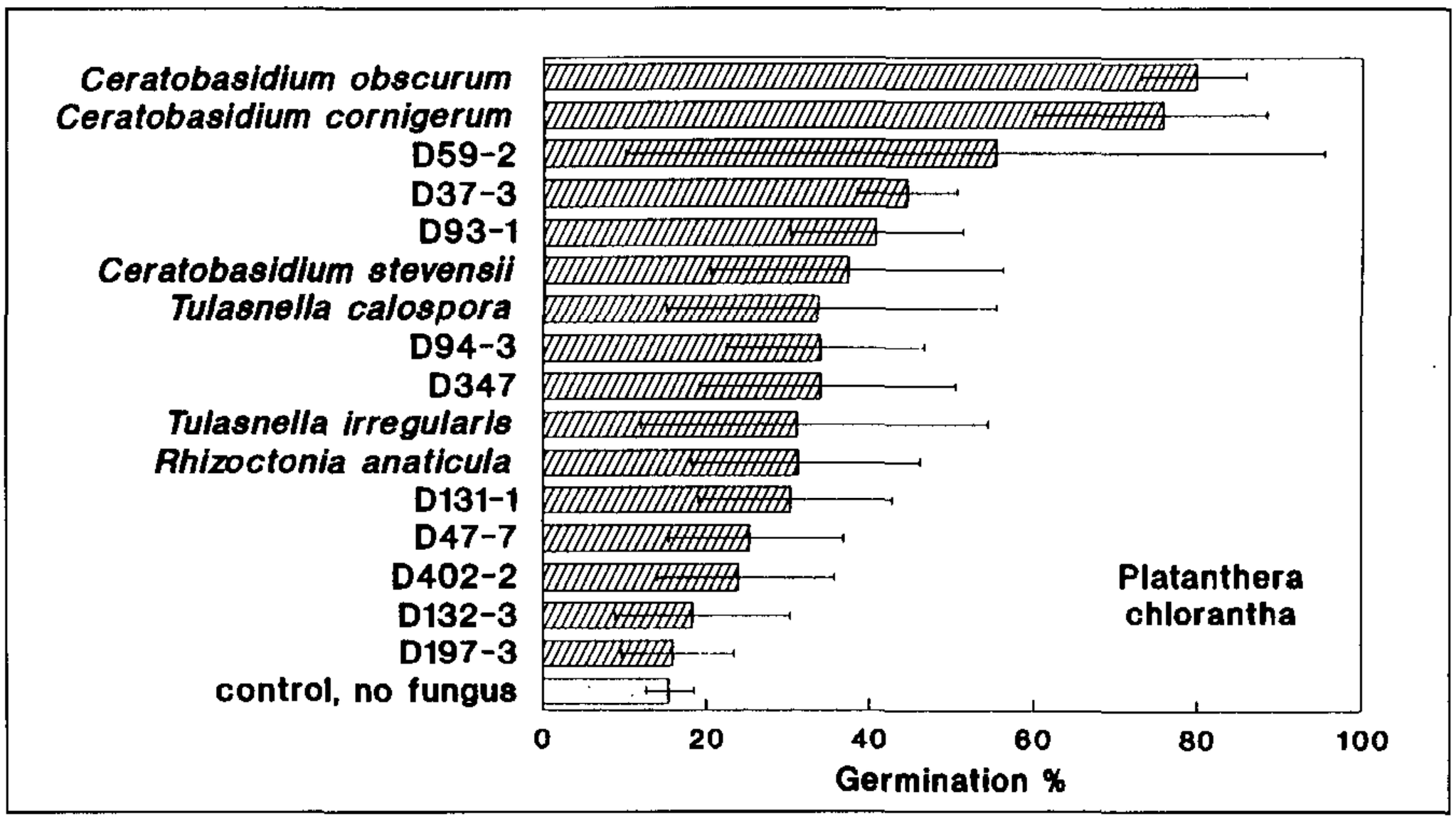

Figure 3. Germination in *Platanthera chlorantha* with a range of isolates and reference cultures of orchid symbionts. Germination percentage after 42 days in darkness at 20C on oat medium. Mean of five samples with 95% confidence intervals, arcsin transformed data. Figure from Rasmussen, 1995.

unknown; the detection of an effective stimulator of seed germination could potentially be of great practical importance.

CONCLUSION

Production of symbiotic plants presents a number of advantages. Not only germination percentage and rate but also development of the seedlings are usually improved, and a better rate of success on transfer to nonsterile conditions is achieved. Conservation of species and biological diversity are often an important consideration when orchids are concerned. Obviously, the fungal symbiont that controls the establishment of new seedlings is required if the plant species is to be preserved in natural populations. Symbiotic propagation has thus gained increasing application in the last decades.

Asymbiotic orchid culture will remain of major commercial importance for propagation of those species that respond well to this method, mainly tropical taxa. The symbiotic technique is a supplement for those species that previously have been very difficult to propagate. This technique has made it possible to reveal a number of seed dormancy mechanisms that are unrelated to the special nutritional system of the seedlings. Such mechanisms must be dealt with to obtain a rational propagation procedure. An extension of the assortment of species offered on a commercial scale is thus within reach.

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