PROPAGATION OF ORCHIDS USING SYMBIOTIC FUNGI

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Abstract. The propagation of Australian terrestrial orchids from seeds or plants collected in the wild, using the fungi with which they are normally associated, is described. This technique relies heavily on the successful growth of the appropriate fungi to supply the orchids with essential nutrients for growth.

INTRODUCTION

All orchids found growing in the wild are thought to be associated with certain species of fungi (1,8). The association between orchids and fungi may persist throughout the life of the orchid, or it may be seasonally absent in some species. These fungi are thought to contribute greatly to the uptake of nutrients from the substrate in or on which the orchid is growing. Research into this aspect of the association has revealed that these fungi can convert, amongst other things, cellulose from soil into soluble carbohydrates that subsequently can be transferred to the orchids (2,13,19). These soluble carbohydrates, many of them rare natural sugars, are essential for the well being of the plants, a fact verified experimentally with plants grown *in vitro* by Ernst (11). These fungi are also thought to be responsible for the successful germination of orchids in the wild (1). This concept, since its inception by Bernard (4), has been supported by experimentation a number of times. Seeds of various orchids when sown *in vitro* on a prepared medium inoculated with certain fungi would germinate, while seeds of the same species left uninoculated but sown on an equivalent medium would, over the same time periods, barely germinate (5,6,7,8,9,2) Not everyone has agreed with this concept. Knudson (15,16) proposed an alternative concept; that orchid seeds could germinate in the absence of fungi by being able to assimilate organic and inorganic nutrients available in the substrate. In effect he proposed and experimentally showed that orchids could be grown asymbiotically *in vitro*.

Whilst this asymbiotic method of propagation has proved enormously successful, enabling the mass propagation *in vitro* of many orchid species, it still does not alter the fact that in the wild state orchids are all associated with certain fungi. Nor does it alter the early concept of Bernard. Moreover, it has been shown that seeds of not all species germinate readily under asymbiotic conditions. Attempts have been made to germinate seeds of certain species, such as *Cypripedium calceolus* L., using a specially prepared medium with limited

success (12,14). Species of Australian terrestrial orchids have also proved especially difficult asymbiotically 9,18,20). A further problem has been the difficulty of transferring asymbiotically-grown seedlings which, in some cases, may have taken up to 25 months to reach a size large enough to transfer to soil mixes. Severe losses during transfer has been the norm (Stoutamire, pers. comms.; Clements, unpublished data).

One other aspect of the association between orchids and certain fungi needs to be expanded upon before going further. This aspect is the dormant period of certain orchids. At this time plants are reduced to an underground tuberoid. During the period of dormancy, usually through the summer months, fungi are absent from most tuberoids but present in the soil as dormant spores or encysted hyphae. Exclusion of these fungi is by way of phytoalexins. These "warding off" chemicals are predominantly to be found present in the epidermal layers of tuberoids. The conclusion to be drawn from the above is that the fungi must reinfect the orchid each growing season if the association is to continue.

PROPAGATION OF ORCHIDS COLLECTED FROM THE WILD

At the Australian National Botanic Gardens there is a very large collection of species of native terrestrial orchids. There are presently some 1300 pots containing more than 400 species. Most of these plants have been collected from the wild over the past 8 years mainly by the author from many parts of Australia.

The purpose of the collection is to have as many species in cultivation as possible and for those species to be represented by a number of collections originating from various parts of the wild species range, thus providing a large genetic base for each species.

The maintenance of this collection is viewed as a long term project. The use of artificial fertilizers is thus avoided. Consequently minimization of genetic change in the plants within the collection is being aimed for. Therefore, the use of artificial fertilizers to stimulate short term growth of plants does not take priority over the long term survival of the collection.

In order to achieve these stated aims the symbiotic method of propagation has been used. This method closely fits that which occurs in the wild. The soil mix used was designed with these aims in mind. It comprises 3 parts washed river sand, forming the main body of the mix while providing good drainage; 2 parts wood shavings (soft and hardwoods), aerating the mix and providing the fungus with raw materials on

which to live; 1 part leaf mould (*Quercus* spp), providing the plant and fungus with a small amount of nutrients which are relatively freely available. This alone provides a suitable mix, in which to grow many species of terrestrial orchids. However, it has been established through trials that the addition of ½ part loam (basalt) aids the general plant growth of many species. The loam is thought to provide the plant with minor elements that may be otherwise locked up in the decaying wood shavings. Fertilizer is not added nor is the mix steam sterilized prior to use. Plastic pots are used in preference to terra-cotta as it has been established that the water retention of the latter during the summer is poor, resulting in the death of plants of many species.

This environment is apparently adequate for the fungus and plants to live in as is shown by the number of species now in cultivation.

It has also been found that it is best to remove the soil from around the tubers, otherwise decay invariably results. Most plants collected while flowering contain the fungus in their roots, which is sufficient to inoculate the potting mix.

Plants once established, using the symbiotic method of propagation, flourish for a considerable length of time. Presumably they could do so indefinitely provided they are repotted once the potting mix is spent of nutrients.

It is usually more convenient to repot the terrestrial orchids during their dormant period in summer. As the fungus is known to be invariably absent from the tuber during this period, it is necessary to transfer some of the old potting mix to the new lot so that the fungus is not lost. Some species are short lived (5 to 10 years) in the wild and this is also the case with these same species in cultivation. It is therefore desirable to replace those plants with seedlings grown *in vitro* from seed collected from the adults before the latter die and replacement from a wild source is the only alternative.

THE SYMBIOTIC PROPAGATION OF ORCHIDS FROM SEED

The propagation of terrestrial orchids from seed using the symbiotic method of germination, similar to that described by Bernard (5,6,9), has been used with great success at the National Botanic Gardens (9,10). Over one hundred species have been raised from seed. A number of these species are classified as rare and endangered (17). The symbiotic method of germinating orchid seeds also duplicates, *in vitro*, that which occurs in the wild. Seeds are sown on a low nutrient medium (powdered rolled oats, 3.5 g; Difco yeast extract, 0.1 g; Davis agar, 10 g; and distilled water, 1000 ml) after surface steriliza-

tion with a 0.5% solution sodium hypochlorite. The medium is then inoculated with an appropriate fungal isolate; the test tube sealed with a sterile cotton wool bung before being placed in an environmental growth cabinet where the 16-hour day temperature is maintained at 20°C ± 3°C, and the night temperature is maintained at 15°C ± 3°C. Under this controlled environment the fungus first grows and covers the entire surface of the agar between 10 to 20 days from sowing. During growth the fungus infects the orchid seeds. Germination of these seeds soon follows providing the correct fungus has been used in the experiment. If not, the seeds either fail to germinate or swell only slightly and never manage to produce viable seedlings.

It takes 6 to 9 months, from sowing, for a seedling to grow, develop, and produce a tuberoid. This rate of development is comparable to that observed in the wild (Clements, unpublished data). It is then a relatively easy matter to transfer seedlings that have reached this state of development to the environment of a potted plant. Results to date show approximatley a 75% success rate in establishing plants grown symbiotically *in vitro* when transferred to a soil mix. In some cases 100% of seedlings have successfully been transferred to the soil mix. These latter successes have occurred when extreme care has been taken to ensure that the plants suffer a minimum water stress at transfer.

The first seedlings germinated symbiotically *in vitro* flowered in cultivation last year. Plants of *Diuris punctata* Smith var. *longissima* Benth., flowered just two seasons after being planted out from the test tube.

DISCUSSION

The symbiotic method of propagating orchids from seed offers the grower a number of advantages that are otherwise unavailable when seedlings are raised in the absence of the fungus. Firstly, seedlings grown *in vitro* already contain the appropriate fungus when they are transferred to the soil mix. It is, therefore, not left to chance as to whether spores of the appropriate fungus fall into and inoculate the soil mix and eventually the orchid. Secondly, it is known that these fungi normally found associated with the orchids actually protect the orchid tissue from being infected by a number of other pathogenic organisms. Although this particular state of control may exist only for the period while the orchid is healthy and actively growing it, nevertheless, assists greatly during the period of establishing the seedlings grown *in vitro*. Thirdly, symbiotically grown seedlings invariably take on the same morphological structure of the plants from seeds germinated in

nature. There is a very short period of adjustment between deflasking and renewal of growth. Consequently larger tubers are established during the growing season before the plants are forced to go into dormancy at the onset of summer.

The symbiotic method of germinating orchid seeds also offers the grower a method of obtaining seedlings of many species that until recently have proved impossible or extremely difficult to grow *in vitro*. At a time when more and more species of plants are being lost to science, the symbiotic method of propagation offers some hope in the conservation of many orchids.

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TISSUE CULTURE OF *EUCALYPTUS*

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Abstract. Clones of several eucalypt species have been propagated *in vitro*, enabling the utilization of genotypes which have been selected as superior individuals. By this means it is possible to produce clonal populations showing superior growth rate, form, and adaptation to specific sites. Large numbers of hybrids exhibiting a marked degree of hybrid vigour can also be grown.

Close cooperation between researchers and the horticultural and forestry industries will be needed to fully exploit the commercial potential of this technology. The clonal propagation of high value horticultural specimens, such as *E. caesia* and *E. macrocarpa*, offers obvious and immediate commercial benefit. Extension of this practice to plantation forestry will require lower production costs but the large demand for plants will stimulate the development of improved and cheaper techniques.

INTRODUCTION

Eucalypts, like most forest trees, have long generation times (from years to decades), are very difficult to cross-pollinate to produce large quantities of seed of hybrids, and selection of genotypes for important characteristics like growth rate and form usually takes several years. All of the above features make tree breeding a slow and costly process.

The vegetative propagation of superior clones can assist in overcoming some of these problems as selected clones can be rapidly propagated for commercial plantations. Since vegetative propagation enables the cost of breeding and selection to be spread over a large number of clonal individuals then advanced breeding techniques (e.g. hybrids between inbred lines, interspecific hybrids, and back-crossing) could become as practical for tree breeding as they are with the breeding of annual crops.

There are several methods of vegetatively propagating eucalypts (35) but for forestry plantations stem cuttings and tis-