PROPAGATION OF WESTERN AUSTRALIAN TERRESTRIAL ORCHIDS

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INTRODUCTION

Western Australia has over 350 species of native orchids (1) and all are terrestrial with the exception of two epiphytic species, found only in the Kimberly area. The majority of these 350 species are found in the southwest corner of the state. The flowers of terrestrial orchids are usually smaller and more delicate than those of the more commonly cultivated exotic epiphytes, but have a wide variety of form and colour. Many, such as the *Thelymitra, Diuris* and *Caladenia*, would make attractive horticultural subjects. Unlike the epiphytic orchids, which are an important part of the floriculture industry, terrestrial orchids have not been commercially exploited to any significant degree. Potential growers have probably been discouraged by both the lack of efficient propagation methods and the long dormancy period.

In order to survive hot, dry summers terrestrial orchids have evolved a deciduous growth cycles (Figure 1) and spend 4 to 6 months of the driest part of the year as a dormant subterranean tuber. The single exception is *Cryptostylus ovata*, the only orchid of the southwest to retain its leaves all year round. Following the first consistent autumn rains (usually in March) the terrestrial orchid begins to grow and develop shoots and roots. As the plant develops it forms a symbiotic association with a specific mycorrhizal fungus found in the surrounding soil. This fungus breaks down complex carbohydrates from organic material in the soil to simple sugars that can be readily utilised by the orchid (2), and is also thought to protect the orchid from infection by pathogenic fungi. Once the shoot has extended and become green the plant becomes photosynthetic and the orchid begins to put down a new replacement tuber for the next season. The orchid may then, if conditions are suitable, flower and produce seed. The vegetative parts of the plant then senesce and the process is repeated the following year.

The presence of a species specific mycorrhizal fungus is thought to be necessary to initiate seed germination (3). In the wild, seed germination occurs during the cool, damp conditions of autumn and correlates with a peak of mycorrhizal fungus growth. Those seedlings which survive the winter put down tubers in the spring as days become warmer and drier. The first flowering of these

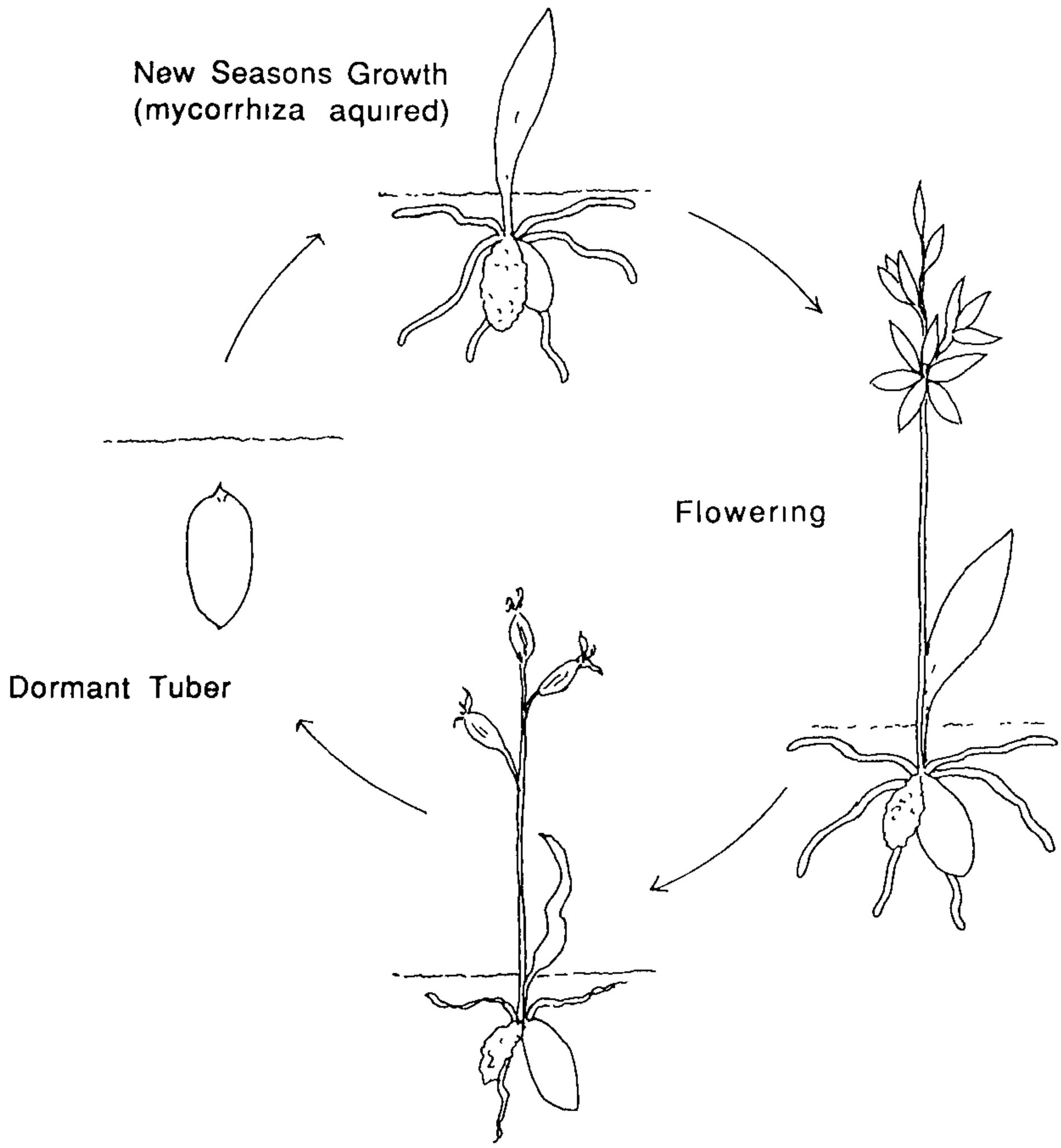

Figure 1. Growth cycle of a Western Australian terrestrial orchid.

new orchids does not normally occur for at least 2 to 3 flowering seasons.

METHODS OF PROPAGATION

At present terrestrial orchids are propagated by orchid growers primarily by vegetative means and through seed germination. The first of these methods relies on the natural propensity of certain species to produce more than one replacement tuber each year. By carefully removing the new tuber from the plant after it has formed, the orchid can be induced to produce another new tuber. This can be repeated so as to produce several new tubers in one growing season (4).

Germination of orchid seed under natural circumstances requires the presence of a species specific mycorrhizal fungus; this presence may be assured by sprinkling orchid seed around the base of the parent plant in autumn. A few of these many thousands of seeds will germinate and produce seedlings, some of which will survive to produce adult plants. Greater efficiency in seed germination and production of seedlings can be achieved through the use of *in vitro* techniques.

In vitro propagation of terrestrial orchids has been achieved through the following techniques:

i) symbiotic germination—co-cultivation of seed with a species of specific mycorrhizal fungus,

ii) asymbiotic germination—germination under sterile conditions, and

iii)tissue culture—micropropagation from the vegetative parts of the orchid.

SYMBIOTIC SEED GERMINATION

The first step in propagating from seed is the isolation of the specific mycorrhizal fungus from each orchid species to be propagated (Figure 2). The infected parts of the orchid, usually the roots, leaf collar or the underground stem, are collected from the wild during the full flush of vegetative growth (5). The plant tissue is surface sterilized, the intracellular fungal coils or pelotons removed with a very find glass needle and placed on fungal isolation medium (6). Alternatively the infected section of tissue can be cut into small cubes and placed on a fungal isolation medium; the fungus will then grow out of the plant tissue into the surrounding medium. The fungus is then subcultured to obtain a pure culture and tested for mycorrhizal efficacy through its ability to stimulate germination and growth of orchid seed. Mycorrhizal agents for each orchid species are then maintained in cold storage for future use.

Once an effective mycorrhizal fungus has been isolated it is used to germinate seed. Surface sterilized orchid seed is spread thinly over a sterile filter paper on low nutrient medium (0.25% oatmeal agar). Several small cubes of mycorrhizal fungus on potato dextrose agar (PDA) are then placed on the edge of the filter paper, the plate sealed and incubated at RT in the dark. The seed will usually germinate within 4 to 6 weeks at 20° C.

ASYMBIOTIC SEED GERMINATION

If a suitable mycorrhizal fungi is unavailable, as is the case for many *Thelymitra* species, orchid seed can be germinated asymbiotically on a complete nutrient media. Various complex media including Knudson C, Vacin and Went, Fast, and modified Burgeff's Pa5, (7, 8) have been used. The orchid seed is surface sterilized, spread thinly over the nutrient agar then the plate sealed and stored in the dark at 20° C until germination occurs. This process is considerably slower than symbiotic germination, commonly taking 2 to 4 months, with some species taking 8 months or more.

In both procedures, once seeds have germinated and protocorms formed they are subcultured onto the appropriate medium and placed under lights at 20° C. When symbiotically germinated seedlings have grown to about 10mm and begun to form droppers they are deflasked into pasteurised perlite: sheoak (*Allocasuarina fraseriana)* mulch, under fog and in 70% shade.

Asymbiotically germinated seedlings are not deflasked until droppers are well formed and tubers have begun to develop. After 4 to 6 weeks under fog the seedlings are transferred to mist and 70% shade where they remain for several months. Dormancy is induced at the beginning of summer by gradually drying out the trays of seedlings. The tubers that form are then collected and repotted in a well draining potting mix with 2 to 3 cm of sheoak mulch on the surface.

TISSUE CULTURE

The micropropagation of epiphytic orchids has been achieved from most vegetative parts of the plant: shoot and protocorm apices, flower stalk nodes, root and leaf tips (9). Terrestrial orchid species however produce very little vegetative growth on each plant that is suitable for explants. The underground parts, the primary shoot (produced underground), the underground stem, and the roots are heavily infected with symbiotic bacteria and fungi, making sterilization of this tissue extremely difficult. Leaf material is also often heavily contaminated as terrestrial orchids are affected

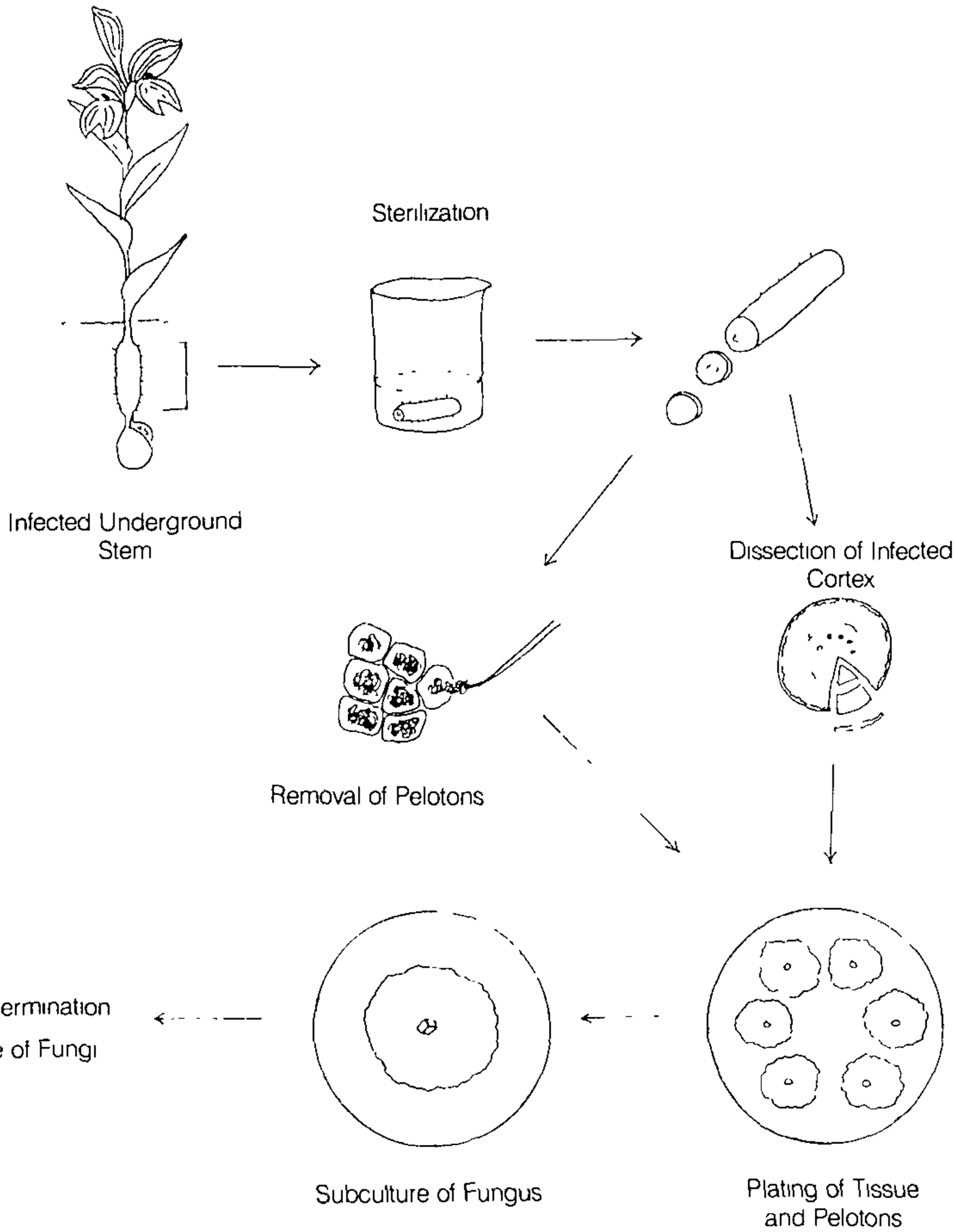

Figure 2. Isolation of mycorrhizal fungus

by rusts and prone to insect damage. As a result very little work has been done in this area and only two Western Australian terrestrial species, *Diuris longifolia* and *Thelymitra crinita* have been successfully tissue-cultured (8).

Protocorm apices from asymbiotically germinated seed and flower spikes of *Thelymitra crinita*, and flower spikes of *Diuris longifolia* were used as explants. The slices of tissue were placed on modified asymbiotic germination medium (Burgeff Pa5) containing added vitamins and the cytokinin, benzyladenine. The formation of protocorm-like bodies (plb's) and multiple shoots occurred within 50 days from protocorm apices. Flower stalks proved to be less productive, as neither stem nodes nor stem slices formed callus or multiple shoot cultures. A few nodes cut from the base of very immature flower buds have however produced plb's for both *T. crinita* and *D. longifolia*. Larger shoots and plb's have been subcultured onto Burgeff's Pa5 to induce root production. No *T. crinita* and only a few *D. longifolia* have produced roots in vitro, but no plants have been deflasked.

CONCLUSIONS

Although there have been significant developments in techniques for propagation of terrestrial orchids, at present these procedures are still slow and inefficient at producing large numbers of viable adult plants. Although efficient seed germination has been achieved with a number of species, the mycorrhizal fungi have not been isolated from many other species and some of these will not germinate asymbiotically. As a result some of the most horticulturally desirable plants are unavailable by any means.

Additionally, there have proved to be considerable problems with deflasking. Most terrestrial species suffer large losses on deflasking; for instance, with the genus *Thelymitra*, roughly 75% of seedlings fail to produce tubers, while for the *Caladenia* this is closer to 95%. Also those plants which do survive their first year often do not survive the following year. Losses over the second season have proven to be equally large (approximately 75%). As a result there is a need for a considerable amount of research into optimising media and techniques before an efficient propagation procedure has been developed.

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