Asymbiotic propagation of
Tropical terrestrial orchid species

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Discussion

Very little is known about the natural pollination and subsequent germination of tropical terrestrial orchid species. Indeed it may be said that we have more research about the life cycles of the temperate terrestrials.

Studies made in our laboratory have highlighted the need for more information about wild populations. To complicate matters, terrestrials are rare in cultivation and seed is often damaged in shipment.

Despite these setbacks we have had success with our empirical methods in germinating over forty different tropical terrestrial genera.

Germination

Several researchers have found orchid seed will start limited metabolic activity and germinate in pure water, but will not sustain growth for long without additional nutrients. Most orchid seed is dispersed by the wind and is air dried before the uptake of water. What effect this natural occurrence has on the seed is not known. In some cases air dried seed germinates better than immature green capsule seed from the same mother plant. In these trials the seed was air dried at 70°F for 24 to 48 hours before processing. Excessive air drying in some subtribes killed the seed. In the tropical epiphytic subtribes *Pleurothallidinae* and *Stanhopeinae* 30 to 60 days of 70°F air drying will kill most of the seed. This makes good ecological sense since many of these species come from areas of year round moisture. It must also be said that seed of several genera, including a seasonally dry growing *Disa* species, still had a low germination percentage after ten years of air drying at room temperature. This air drying treatment has killed seed of *Disa uniflora* in as little as 60 to 90 days. From these examples it can not be concluded that taxonomic affinities are of greater importance to the researcher than knowledge of habitat. (Fib. 1) Another example comes from the genus *Stenorrhyncos*. Many attempts were made to germinate dry seed of *Stenorrhynchos speciosum*. Finally seed from an immature fruit was sown with a very high rate of germination. Another species *Stenorrhynchos lanceolatus* which grows in seasonally dry areas will germinate from dry seed with our normal treatment. (Fig. 2) This failure with dry seed prompted the use of triphenoltetrazolium chloride (TTC) stain to predict viability. Over the past five years hundreds of tropical epiphytic species have been stained. We air dry the seed for 24 to 48 hours at 70°F and stain with a .1g (TTC) powder/100ml. of deionized water solution. These preparations are placed in the dark at 80°F. When the stains dry viable embryos appear pink under magnification. After a short while it became evident that we could predict the inhibitory qualities of the testa by the rate of transmission of the stain into the embryo. The next challenge was the tropical terrestrial species. The seed of many terrestrial species are comparatively large (e.g. *Sobralia, Disa*), have a dark brown, black, or gray coloration and a relatively hard testa. In order to stain this seed a pretreatment in a 10% clorox bleach solution for 10 to 20 minutes followed by rinsing in deionized water is required. This treatment bleaches the seed and breaks down the inhibitory properties of the testa for transmission of the stain into the embryo. Another method using needles to pry the embryo from the testa, and squashing the seed under a cover glass has been effective. The inhibitory effect of the testa is surprising. It is common for seed from seasonally dry areas to need to be pretreated in order for the embryo to stain. Knowing the percentage of viable seeds in a sample is of ultimate importance for any long term study of germination and seed storage. The time it takes for the embryo to stain positive correlates directly with the length of sterilization time or the concentration of sterilant used. With these methods we have learned that some seed is very delicate and needs to be sown from the immature fruit, while other seed is very tough requiring a prolonged sterilization regime. Some seed carries internal pathogens which preclude successful sterilization by traditional techniques.

(Fig. 3) The most important strategy we employ is multi-batching the seed sowings. No matter how small the quantity of seed we always make multiple sowings. The roughened seed coat clumps and sticks the seed together. To avoid contamination small quantities of seed are mixed in a relatively large volume of 10% clorox bleach solution for ten minutes. The addition of a few drops of surfactant (Tween 40) can also break the tension between the seeds providing better coverage. The flasks are placed in the dark at 70°F for initial germination. Depending on the outcome we may need to change the time period or concentration of the sterilizing solution from one batch to another. If for instance we have a contaminated culture we simply increase the time period from 10 minutes to 15 minutes. Based on the tetrazolium tests we have a predetermined percentage of viable seeds to test our germinations against. With this information we can also prioritize rare seed for long term storage or immediate flasking.

Seed Quality and Media Preparations

The nutrient media used is of secondary consideration almost an afterthought. The quality of the seed and the methods used to harvest the seed are the important considerations. Researchers have published dozens of media preparations in the past 20 years and qualified them for certain genera of orchids. This practice of deliberate misinformation and media concoctions is still widespread. Modified Knudsen, half strength MS, and Thompson's media were all successful in germinating the terrestrial and epiphytic species tested. This testing included several heavily myochorrhizal dependent species (e.g. *Calochilus campestris, Hexalectris spicata, Cypripedium acaule*). The trend in our research would suggest more dilute medias are somewhat more effective for the germination of terrestrial species. By studying the seeds anatomy and trying to correlate the behavior of the seedlings in-vitro to the parent species natural environment we have been very successful in germinating these rarely grown species.

Sustained growth in-vitro

Tropical terrestrials species exhibit a number of different growth habits in flasks which simule the conditions for growth in the wild. The basic germination medias are supplemented for transflasking the small seedlings with the addition of banana homogenate or coconut water. (Fig. 4) The basic growth habits of different species include die- back to tuberoids, long stoloniferous growths and rambling. The stoloniferous and rambling types root easily and may be propagated asexually by severing the offshoots from the parent. This limits the genetic diversity of the seedling population but provides an indeterminate culture for long term storage and distribution. Species which die back to tuberoides seasonally are some of the most difficult to manage. These species will die back in culture and there is conflicting data for their treatment. In all cases we transflask the small plantlets upon initiation of the new lead growths. With the active growth cycle the plantlets grow rapidly in the fresh nutrients and the new tuberoid is produced for the next dormant period.

Deflasking Plantlets

Mature plantlets are removed from flasks after two dormant periods. At this point the mature plantlets are several years old and are adaptable to the stresses of greenhouse culture. Earlier removal results in large seedling losses which can be attributed to the size of the immature plantlets. (Fig. 5) In addition to the size, the specialized roots of terrestrial orchids, which are covered with root hairs, are more sensitive to handling than those of epiphytes; therefore, one must be especially careful of breaking the roots when removing these seedlings from flasks. Most terrestrial species resent the high concentrations of mineral salts in commercially prepared fertilizers. Specific requirements for growth must be researched on individual species since certain genera contain both seasonally dry and evergreen species. Special attention must be given to the dormant period in the life cycles of those terrestrials which go dormant. Some tuberoids grow in areas of excessive heat or seasonal fires. These tuberoids must remain completely dry and warm for several months. Evergreen species require dense shade especially after the initial removal from the flask. More detailed habit information is essential for the success of any ongoing studies of this group of the orchid family.

Conclusion

The major difficulty remains the pure rarity of these plants in cultivation. (Fig. 6) This is somewhat surprising considering the tropical terrestrial orchids contain the popular jewel orchids. We rely on seed which is kindly sent to us for study and distribution. In many cases the seed rots, is severely desiccated, or is damaged in shipment. Very few enthusiast cultivate these plants in their local habitats, and even fewer attempt propagation. We still remain optimistic and have more successes every year. We will continue our research, propagation and distribution of rare species. Individuals can make a difference. We may never be able to preserve the wild orchids because of habitat destruction, bureaucracies, laws of ignorance, and overpopulation demands. We will probably never reintroduce a cultivated species back into the wild. But our hope is to save the species for another generation, because, after all saved in cultivation is better than not saved at all. The outlook for orchids is not very good but hopefully I will still be able to show my grandchildren species orchids, and leave them with an appreciation of nature just as I learned as a young boy.