Disa Orchids and Their Ex Vitro Seed Culture©

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INTRODUCTION

Before I retired I was employed as Technical Officer in The Fernery in Pukekura Park, New Plymouth. Part of my job was to look after the very diverse orchid collection that the Fernery is known for. This extensive collection has been assembled over many years by George Fuller and others. This paper will look at some of the background in the development of the terrestrial orchid species *Disa uniflora* and its hybrids as export cut flower and pot plant crops in New Zealand (N.Z.).

THE PLANTS

Disa is a genus of over 100 species of terrestrial orchids that mainly occur in southern Africa in habitats that range from swamp to seasonally dry areas. Many of the species have small flowers and are of collector value only. They can also be a real challenge to grow ex situ.

Disa uniflora, also known as "Pride of Table Mountain," is centred on the Table Mountain area, where it grows in damp and or other seasonally wet sites such as stream sides or swamps and can even be found peeping out from under waterfalls. Although the species name is "uniflora" because the first plant described only had a single flower on each stem, other plants were later found to be multiflowered. They can have quite large flowers that range from red/orange to orange/gold with the occasional gold/yellow. The flower count per stem can be from 1 to 12 or so, with stem lengths from 15 cm up to 1 m.

As a consequence of the efforts of a small group of enthusiastic *Disa* orchid breeders including George Fuller and others in New Zealand and a number in South Africa, Australia, and the U.S.A., the range of flower colour has widened to include lilacs, reds, yellows, and pale yellows, and over the last couple of years near whites. There are also a number of pastels within the colour range. Since the early 1980s George has been distributing seed and selected plants to many growers in N.Z. to try out.

The cut flowers travel quite well and have a good vase life, and flowering pot plants last well. When grown as a pot plant or as cut flower we have found that they often benefit from being repotted each year when dormant.

Disa uniflora is a species that is winter dormant and almost deciduous (depending on growing conditions and winter temperatures), and produces annual carrot-shaped tubers that are replaced each year, but not all these replacement tubers are flowering size. Flower stems develop from the terminal of flowering-size tubers to flower in mid summer with stems 30–80 cm tall, usually carrying from one to five flowers. Some of the hybrids produce flower stems between 0.5 m and 1 m with a dozen or so flowers.

The replacement tubers develop on short lateral rhizome-type growths, often called droppers and have a similar growth sequence to the Australasian terrestrial orchid genera *Pterostylis* and *Thelymitra.*

SOME HISTORY

In the early 1980s I began helping George Fuller with some ongoing seed germination work he was doing with *D. uniflora* and various crosses with other *Disa* species such as *D. cardinalis*, *D. racemosa*, *D. tripetaloides*, and various hybrids. At that time George was Curator of Pukekura Park, including its extensive orchid collection. I was running the Parks Department nursery, where much of the orchid collection was housed when not in flower or on display in the Fernery.

Most of this seed work revolved around using *D. uniflora* as a species and as a primary parent in the breeding of the seed lines used. Although in vitro culture was being done at that time and still is with *Disa*, George was interested in the possibility of ex vitro germination of the seed, with it sown on sterilised or unsterilised media, and kept permanently damp but not covered. He still continues with this interest. Various combinations of untanalised fresh *Pinus radiata* sawdust, peat, composted pine bark, sand, sphagnum moss, charcoal and pumice, etc. were trialed.

In general most of the mixes worked and the seed germinated well but there were problems with the competitive growth of liverwort, mosses, and ferns outgrowing the very small *Disa* seedlings. As a result of George's work a number of other people were experimenting with growing *Disa* from seed without the use of a tissue lab. Some of these people were quite successful and still are. From these small beginnings the current export cut flower and pot plant trade has developed.

SEED

The species *D. uniflora* has what are possibly the largest seeds amongst orchids. It and many of its hybrids have quite large seeds that can be seen individually with the naked eye and easily felt with a finger tip and rolled around on a sheet of paper. Due to their size and the fact that they are not adapted to fly very far (being heavier than most epiphytic and other orchid seed and having no wings), and following discussions that I had with some visiting South African growers, it appears that seed dispersal in the wild is of the pepper-pot style in windy conditions and just gravitational when it is calm. The seed will also float, so water can help in its distribution along watercourses. Due to the weight of the seeds and the fact that they can roll helps to explain why the plants in the wild appear as colonies growing out of long grass and other low vegetation, along water courses and other damp areas, even beside waterfalls.

As a follow on from George's work and part of the discussions that I had with the visiting South African growers, I decided to have a look at the effect of shade on the germination of disa seed and the comparative growth rates of liverwort, mosses, and ferns along with any effect from mycorhizal inoculation of the seed-sowing media.

The South Africans had indicated that *D. uniflora* plants generally grew in the litter layer on top of permanently damp/wet soil, with the litter layer made up of leaf, root, and other plant debris including the remains from previous years' *Disa* plants.

They can also be found growing in humus-filled cracks or pockets in rocks that are part of waterfalls. The disa plants also have to compete with other low-growing vegetation for nutrients and the *Disa* plant bases are often shaded by the surrounding vegetation.

MATERIALS AND METHODS

Medium. As I had only a limited amount of room to run a simple trial I decided to stick with one type of medium. The medium used was a fine grade of very well

composted *Pinus radiata* bark that had been nitrogen stabilised with calcium ammonium nitrate. The grade used was 2–3 mm with all the dust sieved and washed out to reduce any potential fern, moss, and liverwort spore load. There was no other sterilisation carried out. Small pots and seedling punnets were filled with 4 cm of compacted fine bark, watered from below in a watering bath to prevent any disturbance of the surface of the bark, and were then ready for seed sowing.

Preparing and Sowing the Seed. It is very easy to sow *Disa* seed too thickly, especially with their tendency to roll and even bunch up. Overcrowded seedlings are not easy to prick out, and are more susceptible to damage and then damping off and other pathogenic organisms. Such seedlings are best pricked out in clumps and then separated at the first repotting when dormancy has occurred.

Seed pods were collected from plants that had been hand pollinated several months before. As soon as the pods started to split they were removed from the parent plant and placed in unsealed paper sachets to finish splitting and shedding their seed.

The seed was allowed to dry for several days to ensure that it would flow freely for the next stage. Viable *Disa* seed is easier to sort when it is shed, as it is not light and does not "float in the air" like the seed of many other orchids. I used a small domestic flour sieve to do the job and ended up with samples of seed that were free flowing, easy to sow, and had the best chance of fairly even germination. With most *Disa* seed it would be possible to use an air winnowing system to separate out any small undeveloped seeds and other chaff.

This screening meant that I could see with the naked eye individual viable seeds and control how thickly I was sowing them. The seed was sprinkled evenly over the pots of bark and gently watered with the aid of a hand mister to prevent any disturbance of the bark.

At present most commercial cut flower growers use some form of hydroponic system for their flowering plants. At the Fernery we had a hydroponic table that we grew the flowering-size *Disa* plants in to develop their full flowering potential. Constant access to water appears to be a requirement for the full development potential of the flower stems. This trough was kept flooded with water to a depth of 2 cm with a constant recycling system. This was where the pots of seed were stood for the duration of the trial, so most of the watering was capillary in nature. Over several years previously I had carried out small germination trials using a range of media with very varied results.

Inoculation. I am not yet fully convinced that all orchids have symbiotic fungi species specific to each species of orchid. It is very possible that there may be several universally occurring fungi that can live symbiotically with many orchid species.

I inoculated half of the pots of seed with a proprietary mycorrhiza mix available in most garden centres in powder form (commercially available *Trichoderma* preparation in powder form). In the inoculated pots the resultant seedlings were greener than the uninoculated ones, grew slightly faster, but were quickly outgrown by the very lush moss, etc., that developed. Application of the inoculant was done with the aid of a watering can.

After exflasking of tissue-cultured orchid plants, they are often grown on in chopped sphagnum moss (the fresher the better) for a while before their final potting into a standard potting mix. Fresh and good quality sphagnum has the reputa-

tion of having some antiseptic properties, so I would expect that it would have an effect on any mycorrhizal fungal establishment, yet the often small orchid plants grow away well.

Shading. Based on the information the South African growers had given to me, and observations made over previous years, I decided to provide a fair degree of shading for the trial with only a limited number of uncovered control pots. This proved to be a breakthrough. To try to emulate the climate that the seed would be subjected to in the wild the seed was sown in two lots during May and July (N.Z. late autumn and mid winter). There was no apparent benefit in the late sowing. The trial was done in an unheated fibreglass-roofed shadehouse (that would go down to near freezing in the winter), so there was a degree of shading already in place from the structure.

The punnets of seed were placed in small plastic propagation trays, (standing in 2 cm of water) with another tray inverted over the top. These inverted trays were of the type with the bottom perforated with fine drainage slots that allowed restricted light to get to the seed.

RESULTS

After about 3 weeks it was already possible to see initial signs of germination with the aid of a hand lens, and after 6 weeks some seedlings could be seen with the naked eye. At this stage all the pots were given a very dilute 5 : 4 : 11 (NPK) seaweed-based liquid feed with a hand mister. This was done weekly for 3 weeks. After this it was repeated once a week using a watering wand as part of the regular liquid feeding routine of the flowering-size plants. Any high-potash seaweed-based liquid feed appears suitable for the flowering plants as long as it is well diluted. The micronutrients and alginates derived from the seaweed appear to be therapeutic to most orchids.

Under the level of shade applied the development of moss and liverwort was very slow, but on the control pots the speed of development was very noticeable, especially on those inoculated. Over the next 12 weeks the shading was gradually reduced. The seedlings in the shaded pots generally grew ahead of the moss, etc.

Between 12–18 months after sowing, and depending on the vigour of individual seed lines, the surviving seedlings were pricked out (usually when 10–20 mm tall). The pricking-out medium was milled fresh spaghnum moss in which the seedlings stayed until the next spring when they were put into the normal potting mix of aged bark, drainage chip, and milled spaghnum moss. With the hybrids being developed now it is possible to get seedlings to flower 2 to 3 years from sowing.

As the genetic base is getting wider and more complex the range of flower colours being produced can be quite wide so careful selection of distinct colours and forms is very important. Once a good plant is selected it can be tissue cultured to bulk up numbers, as natural multiplication can be quite slow.

MOSSES, LIVERWORTS, AND FERNS

Because this trial was being done in New Plymouth in North Taranaki with its natural high humidity and huge fern, moss, and liverwort windborne-spore loadings it did not take long for all three to make their presence known. The ferns almost certainly had an effect on the number of seedlings that survived to pricking out from those pots that had been inoculated. Mosses and especially ferns have long

been suspected of causing growth suppression of orchids and other pot plants when they occur as weeds.

POTTING MEDIA

The main challenge for anyone growing these orchids has been that they go against the "norm" when it comes to potting media or mixes when compared to most other orchids that are commonly grown. The medium needs to be moisture retentive but also free draining to emulate the growing conditions in the wild.

Many ingredients on their own or in various combinations have been used. They include fresh coarse sawdust, sand and fine gravel chip, peat, pumice, spaghnum moss (long fibre, chopped or rubbed), various grades of potting bark, and synthetic products or similar such as polystyrene chips, coco fibre, the list goes on!! Many cut flower growers are now successfully using straight sphagnum in varying grades, crushed composted bark, or with these used in various combinations that suit individual growing environments.

THE FUTURE

The ongoing development of *Disa* as a cut flower crop continues with markets in Europe, North America, and various Asian countries. The range of flower colours and flower count and stem length is improving. The main harvesting time in New Zealand is early November to late February (late spring to late summer) with a few cultivars outside of that time period. Although tissue culture is used to speed the bulking up of selected cultivars, growers are using vegetative multiplication for most. At the same time a number are using seed to increase the range of colours and forms available.