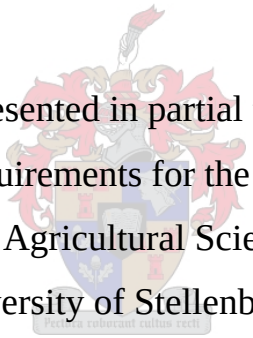
# A STUDY OF GREENHOUSE PRODUCTION TECHNIQUES FOR EVERGREEN DISAS

by

D. PIENAAR



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## DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.




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Danita Pienaar

…….

Date

**Who can estimate the elevating and refining influences and moral value of flowers with all their graceful forms, bewitching shades and combinations of colors and exquisitely varied perfumes? These silent influences are unconsciously felt even by those who do not appreciate them consciously and thus with better and still better fruits, nuts, grains, vegetables and flowers, will the earth be transformed, man's thought refined, and turned from the base destructive forces into nobler production. One which will lift him to high planes of action toward the happy day when the Creator of all this beautiful work is more acknowledged and loved, and where man shall offer his brother man, not bullets and bayonets, but richer grains, better fruit and fairer flowers from the bounty of this earth.**

- Father George Schoener (1864 -1941)

## ABSTRACT

The seven evergreen Disa species are indigenous to South Africa. These orchids grow on mountain ranges subject to winter rainfall and are found on stream banks, around waterfalls and in other damp areas. Although the Disa genus accommodates more than 130 species, by far the most commonly grown is *Disa uniflora* and *hybrids* stemming from this species. Disas have great potential as cut flowers and pot plants, but production techniques need to be further investigated since cultivation methods vary greatly between hobbyists. This study evaluated the effect of N-source, shading, root medium temperature, electrical conductivity (EC), irrigation method, foliar feeding at different plant growth stages and substrate on the growth of evergreen Disa hybrids in a controlled environment. Results showed that Disa plants can be classified as being ammonium tolerant. *Disa unidiorosa* performed best with 40% of the applied N in the $NH_4$ form, while *D.kewensis* was more tolerant towards a higher level of $NH_4$ and grew best at 60% $NH_4$. Shading levels (56% and 69%) were compared and did not differ regarding the growth of plants. A cooled root medium was found to have a negative effect on root growth and a positive effect on leaf length. High EC levels produced heavier mother plants with a bigger root:shoot ratio and a bigger stem diameter. Biomass accumulation was the best in plants receiving 'Drip' irrigation, compared to 'Ebb-and-Flood' irrigation treatments. Plants in the vegetative reproducing stage were more susceptible to leaf abscission and new leaves formed at a low rate compared to small- and potential flowering plants. Where foliar feeding is concerned plants seemed to benefit more by the presence of $NH_4NO_3$ than urea. There were no significant differences in root development between substrates in the 'hardening-off' phase. 'Hydroton' (clay pebbles) was not suitable as substrate for the cultivation of Disa plants. The growth and flowering properties of plants were optimal with sphagnum moss and peat but were negatively affected when the pH of acid peat:sand mixtures were increased. More research is needed before Disas can be cultivated on a commercial scale, while the effect of the treatments on flowering properties has to be investigated.

## UITTREKSEL

**‘n Ondersoek na kweekhuis produksie tegnieke vir immergroen Disas.**

Die sewe immergroen Disas is inheems aan Suid Afrika. Hierdie orgideë groei op berge in die winter-reënvalstreek en word op rivier oewers, digby watervalle en in ander vogtige gebiede aangetref. Alhoewel die Disa genus uit meer as 130 spesies bestaan, word *Disa uniflora* en hibriede afkomstig van hierdie spesie die meeste gekweek. Disas het baie potensiaal as snyblomme en potplante, maar die metode van kweek is nie eenvormig tussen kwekers nie en produksie tegnieke moet verder ondersoek word. In hierdie ondersoek is die effek van N-bron, skadu, wortelmedium temperatuur, elektriese geleidingspeile (EC), besproeïngsmetode, blaarvoeding tydens verskillende groeistadiums en substrate op die groei van immergroen Disas in ‘n beheerde omgewing ondersoek. Resultate het getoon dat Disa plante as ammonium tolerant geklassifiseer kan word. *Disa unidiorosa* het die beste gevaar met 40% van die toegediende N in $NH_4$ vorm, terwyl *D.kewensis* meer ammonium tolerant was en die beste groei by 60% $NH_4$ getoon het. Twee skadu peile (56% en 69%) is vergelyk en het nie betekenisvol van mekaar verskil nie. ‘n Verkoelde wortelmedium het wortelgroei negatief beïnvloed en ‘n positiewe effek op blaarlengte gehad. Hoë EC vlakke het swaarder moederplante geproduseer met ‘n groter wortel:stingel verhouding en ‘n groter stamdeursnit. Plante met ‘Drup’ besproeïng het ‘n groter toename in massa getoon as plante met ‘Ebb-en-Vloed’ behandelings. Plante in die vegetatiewe fase was meer sensitief teenoor blaarverlies en het ‘n laer blaarvormingstempo gehad vergeleke met klein- en meer volwasse plante. Wat blaarvoeding betref het plante meer gebaat by die teenwoordigheid van $NH_4NO_3$ as urea. Daar is geen betekenisvolle verskille gevind in wortel ontwikkeling tussen substrate tydens die ‘hard maak’ fase nie. ‘Hydroton’ (klei korrels) is nie ‘n geskikte substraat vir die kweek van Disa plante nie. Die groei- en blom eienskappe van plante was optimaal in ‘sphagnum’ mos en veen, maar is negatief beïnvloed indien die pH van suur veen:sand mengsels verhoog is. Meer navorsing is nodig voordat Disas op kommersiële skaal gekweek kan word, terwyl die effek van behandelings op blom eienskappe verder ondersoek moet word.

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# CHAPTER 1

**Literature review**

## INTRODUCTION

The genus Disa consists of more than 100 terrestrial orchid species found predominantly in the southern half of Africa (Orchard, 2000a). The term terrestrial is used for species that grow with their roots firmly anchored in soil (Wodrich, 1997). By far the highest concentration of species and the greatest diversity are found in South Africa (Kurzweil, 2003). The most recent revision of the Disa indicates that there are 162 species of which 131 occur in the Cape Floristic region (Vogelpoel, 2001a). The genus is also represented in Madagascar and Reunion, and one species, *D.pulchella*, extends from tropical Africa into the Arabian Peninsula (Du Plessis & Duncan, 1989). The vast majority of species are deciduous, as the above-ground part dies off at the end of the growing season (Kurzweil, 2003).

Disa displays three basic patterns of growth with which the prospective cultivator should be familiar. Firstly, the deciduous winter- growers like *D.draconis* commence growth in autumn and produce their inflorescences at the end of the growth cycle during spring or summer. The deciduous summer-growers have a growth habit which progresses much like that of the winter-growers, but for the fact that growth starts in spring, and dormancy sets in during autumn and early winter. Lastly, there is the habit of the evergreen (or almost evergreen) species exemplified by *D.uniflora*.

Except for the evergreen species, Disas are very difficult to cultivate. The deciduous species, like those of other genera, should rather be left alone in their natural state and, if it is necessary to grow them away from their natural habitat, they should always be grown in the soil obtained from it. The fact that deciduous terrestrials renew their roots annually and have to re-establish their mycorrhiza every growing season, provides at least a partial explanation for their more difficult cultivation (Du Plessis & Duncan, 1989).

*Disa uniflora* (syn. *D. grandiflora*) is the best known of the evergreen group of Disas (Northen, 1970). *D. uniflora* has been described as the 'Pride of Table Mountain', but also as the 'Ghost flower' because of the notorious difficulty experienced in its cultivation (Cywes, 1990). It is also the species that has been depleted most by picking and is now a protected flower, together with the other indigenous orchids, but plants are obtainable in the trade (Eliovson, 1984).

## DESCRIPTION

According to Terquem & Parisot (1991) Petrus Jonas Bergius described and named the genus for the first time in 1767. Several theories have been put forward regarding the name; the most appealing of these is that it comes from a Swedish version of a story from Norse mythology (Orchard, 2000a). Harry Bolus, as quoted by Terquem & Parisot (1991), states that the name may have come from the Latin *dis*, i.e. rich, in an allusion to the beauty of its flower.

These terrestrial plants have an underground tuber furnished with roots, from which a rosette of leaves and the inflorescence are formed (Terquem & Parisot, 1991). Plants are herbaceous with leaves that are cauline or radical (Stewart, Linder, Schelpe & Hall, 1982). Typically, the rootstock is a root-stem tuberoid, which is (probably always) replaced annually. The leaves may appear only at the base of the stem in a pair or rosette, or they may be regularly arranged along the stem; in many species the leaves merge into the bracts of the inflorescence (Du Plessis & Duncan, 1989). Stems are mostly erect, but sinuous and arching stems are found in a few Disa species from streamside habitats (Kurzweil, 2003).

The inflorescence is a spike, raceme or corymb. The sepals are often brilliantly coloured, and the dorsal sepal usually has a spur. The two petals are tucked inside the dorsal sepal, and the lip is usually considerably smaller than the sepals. The anther is situated above the stigma which is borne near the base of the perianth (Figure 1). Flower colour varies from white to yellow, pink, blue, purple, orange, red or green, sometimes in striking combinations (Du Plessis & Duncan, 1989). Flower colour is due to three different pigments – chlorophyll, flavonoids and carotenoids (Griesbach, 1984). This colour is enhanced by the light reflecting properties of the epidermal cells (Vogelpoel, 1991).

When an orchid flower develops, the lip usually lies facing the flower stem. Shortly before the flower opens the pedicel twists through 180° orientating the flower so the lip lies lowermost, this type of flower is called resupinate. A few species of Disa are non-resupinate and the flowers seem to be presented upside down (Wodrich, 1997). In several species the flowers are scented, and the fruit is a capsule of numerous, small seeds (Du Plessis & Duncan, 1989).

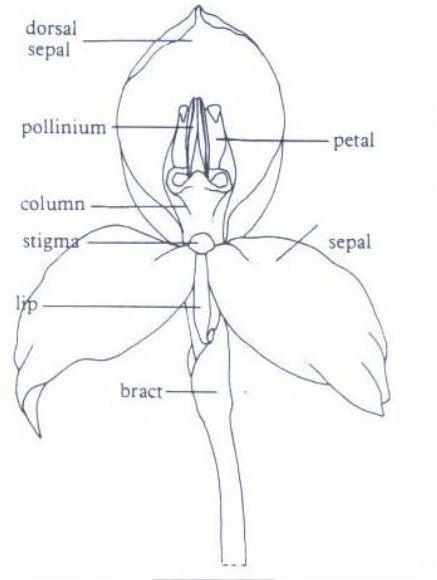



**Figure 1** The flower structure of *Disa uniflora* (Stewart, Linder, Schelpe, & Hall, 1982).

*Disa* is classified as subfamily Orchidoidae, tribe Diseae and subtribe Disinae. Disa is the largest genus in the subtribe Disinae (Linder, Johnson & Liltved, 1998). Some well-known species of Disa has previously been separated into other genera such as *Herschelia*, for instance the blue Disa, *Herschelia graminifolia* (Eliovson, 1984). Due to the short pollination-to-flowering time of three years, many hobbyists and commercial growers have built up large collections of Disa species and hybrids, producing a wide array of summer-flowering orchids with brilliance of color seldom surpassed by other orchids (Vogelpoel, 2001a).

## CONSERVATION AND CULTIVATION

Indigenous orchids are protected plants in South Africa and it is illegal to remove plants from the wild without the relevant permits and landowner's permission (Wodrich, 1997). There are several international conventions which provide conservation measures to protect orchids. Most of these, like the Convention on Biological Diversity (CBD), are primarily aimed at habitat protection rather than species conservation (Linder & Kurzweil, 1999). The convention on biological diversity (CBD) seeks to integrate conservation and sustainable use, and calls for the fair and equitable sharing of the benefits arising from the use of genetic resources (Kate & Laird, 1999).

For orchids, the most important of these conventions is the Convention on International Trade in Endangered Species (CITES) (Linder & Kurzweil, 1999). All indigenous orchids are listed in Appendix 2 of CITES, which requires certification of all plants (and related material) crossing international borders (Davenport & Ndangalasi, 2002). Appendix 2 includes species that may become threatened with extinction unless their trade is strictly regulated (Given, 1994; Addendum A). Various countries, including South Africa, are party to the convention and thus bound to the legislation concerning the international trade in endangered species (Wodrich, 1997). In CITES the wide exchange of cultivars is considered beneficial as it decreases pressure on wild populations (Kate & Laird, 1999).

Many orchids are very popular plants in horticulture, but some are or have also been used as sources of food, medicinal drugs or in superstition.

A sweet juice has been made from the roots and tubers of *Disa barbata*, *D. hians* and *D. venusta* (Linder & Kurzweil, 1999). The harvesting of orchid, including Disa tubers for food has a long history in the culture of many ethnic groups. This is particularly so in Zambia, where the resulting dish, known there as *Chikanda*, is likened to a meatless sausage. After harvesting, tubers are dried, pounded, boiled with baking soda or wood ash, and then served with a staple. Its popularity has grown and demand for the tubers has increased accordingly. This has triggered a burgeoning commercial market and has now prompted traders to seek tubers from Tanzania's Southern Highlands. It remains unclear how many species are considered edible and are thus harvested (Davenport & Ndangalasi, 2002).

*Disa chrysostachya* and *D. polygonoides* can be used for their medicinal properties. According to Linder & Kurzweil (1999), a human poison was previously made from *Disa chrysostachya*. The use of orchids in superstition is common in rural parts of Southern Africa. An infusion or body wash made from *Disa stachyoides* and *D. versicolor* is said to repel evil, while *D. aconitoides* can be used as fertility charm and *D. stachyoides* provides protection against lightning.

Disas also have great potential for cut flowers and pot plants, as they flower during the summer months when many other orchids are in vegetative growth.

The largest orchid group in Southern Africa, namely the group including the Red Disa, is a unique part of the world's orchid flora. *Disa uniflora*, although difficult to grow, is frequently cultivated in the Western Cape and abroad (Linder & Kurzweil, 1999). *D.uniflora* has without doubt made the most important contribution to horticulture of all the Southern African orchids. This species with its numerous attractive colour forms has been used by hybridisers since the late nineteenth century, when the first hybrid, named 'Veitchii', was raised in England by crossing *D.uniflora* with another Cape species, *D.racemosa* (Du Plessis & Duncan, 1989). This artificial hybrid was raised in 1891 (Schelpe, 1966). By 1922 there were 11 registered Disa hybrids and no new hybrids appeared until the 1980's (Orchard, 2000a). Since then, a wide range of spectacular hybrids have been produced involving other species, namely *D.tripetaloides*, *D.aurata, D.cardinalis*, *D.caulescens* and *D.venosa* (Du Plessis & Duncan, 1989). A flatter, rounder shape of flower is preferred to the triangular form with long hanging sepals (Kidson, 1988). These species are interfertile and form the basis of various successful and dynamic breeding programmes (Linder & Kurzweil, 1999; Vogelpoel, 2001a). To date there have been over 150 hybrids registered with the Royal Horticultural Society. All crosses have been raised from seed and serve as an example of what can and should be done in the conservation of all orchids.

New growers are encouraged to raise their own plants, thereby increasing their collections and contributing to conservation (Linder & Kurzweil, 1999). A brief glimpse at the history of Disa growing emphasizes that no grower can make progress until the cultural requirements are understood and applied. Plants die fast if these requirements are neglected, but are rewarding when given the correct growing conditions (Vogelpoel, 2001a).

Because of the comparatively easy cultivation and showy flowers of the evergreen Disas these orchids are highly desirable subjects (Linder & Kurzweil, 1999), the remainder of the chapter will thus be dedicated to them.

## THE EVERGREEN DISAS

The evergreen Disa species comprise of *Disa uniflora, D. tripetaloides, D. aurata, D. cardinalis, D. racemosa, D. venosa* and *D. caulescens* (Linder & Kurzweil, 1999; Figure 2). Distribution of these species in South Africa can be seen in Addendum B.

*Disa uniflora* has stem lengths varying from 150-1000 mm (Duckworth, 1995) and flowers from December to March (Vogelpoel, 1983). Up to twelve flowers have been recorded on an exceptionally well-grown plant (Schelpe, 1966). It has the misfortune of having a short distance between the pedicels; resulting in bunching of the flowers should more than four or five flowers be produced on a stem (Vogelpoel, 2001a). Only one flower is open at a time (Eliovson, 1984); these can measure more than 10 cm across, and vary in colour from pink through various shades of carmine, cerise and orange to red. The pure yellow form of *D. uniflora* is a rare mutant and lack the ability to produce the red and pink, water-soluble, anthocyanin pigments (Orchard, 2000a), due to an enzyme block in the bio-synthetic anthocyanin pathway (Vogelpoel, 1995). *D. uniflora*'s altitudinal range is wide, from 100 m to 1200 m above sea-level (Stewart *et al*., 1982). The curling of the lateral sepals is a dominant trait so that any parent plant will pass it on to offspring (Wodrich, 1997).

*Disa tripetaloides* is a smaller species bearing up to 50 white to pink flowers with attractive spotted markings in the dorsal sepal (Orchard, 2000a). The flowers of *D. tripetaloides* are small, usually 2 to 3 cm in width (Vogelpoel, 2001a). It grows at altitudes ranging from sea-level to 1000 m and flowers from November to January, except in the Transkei and Natal where flowering occurs from June to September (Stewart *et al*., 1982). The Natal variety can be used to produce a good quality winter flowering hybrid (Cywes, 1991). According to Vogelpoel (1992b) defects in the lateral sepals are transmitted.

*Disa aurata* is an attractive yellow species, similar in form and habitat to *D. tripetaloides* (Orchard, 2000a). *D. tripetaloides* subsp. *aurata* has been elevated to species rank and is now termed *D. aurata* (Haasbroek, 1999). *D. aurata* differs from *D. tripetaloides* in having a non-fading, rich sulphur-yellow colour in all the floral segments. There are usually fewer flowers on the stem, up to twenty. The best forms have broad, flat sepals often overlapping medially and with a particularly sparkling texture (Vogelpoel, 2001a). Flowers appear in December and January (Goldblatt & Manning, 2000).

*Disa cardinalis*, has red flowers and is responsible for some of the reddest Disa hybrids (Orchard, 2000a). The inflorescences of *D. cardinalis* are 30 to 70 cm tall bearing eight to twenty-five flowers on a spike. The best forms have flat and wide oval sepals with little or no reflexing (Vogelpoel, 2001a). They are local along streams on the inland slopes of the Langeberg, at about 600 m, and flowers from October to December (Stewart *et al*., 1982). Recently a rare deep yellow-orange clone has been discovered (Vogelpoel, 1992c).

*Disa caulescens* flowers between November and January, at altitudes ranging between 300 and 1200 m (Stewart *et al*., 1982). Plants have up to 5-15 white flowers on an inflorescence reaching a length of 10-40 cm (Wodrich, 1997; Vogelpoel, 2001a). The petals are relatively large (3-5 mm) long, broadly oval and occupying most of the hood formed by the dorsal sepal (Vogelpoel, 2001a). *D. caulescens* is less easy to grow but transmit unique beauty to the petals of hybrids (Vogelpoel, 2001b).

*Disa racemosa* has pink to mauve flowers that appear in profusion in the first season after a fire (Orchard, 2000a). The spike length varies from 30 to 100 cm, with a slender stem bearing two to twelve or even more well-spaced resupinate flowers. The flowers are relatively small averaging 3.5 cm in natural spread (Vogelpoel, 2001a). It grows at altitudes ranging from sea-level to 1200 m and flowers in November and December (Stewart *et al*., 1982). According to Vogelpoel (1992a), there are colonies that flower in January and February.

*Disa venosa* flowers appear in November, mainly after a fire (Goldblatt & Manning, 2000). *D. venosa* is very similar to *D. racemosa*, in habitat, colour and petal markings, but *D.venosa* has narrower sepals. The best specimens have sturdy stems, up to 50 cm long, bearing five to eight flowers or more. The potential for *D.venosa* lies in those forms having deep magenta colours which are transmitted to hybrids, particularly to the petals (Vogelpoel, 2001a). This plant occurs from sea-level to 1000 m (Duckworth, 1995).

*D. racemosa* and *D. venosa*, which inhabit marshy seepage areas rather than stream-sides and waterfalls, are more difficult to maintain in cultivation and are somewhat more susceptible to fungal attack. The Disa species which hybridise with *Disa uniflora* can be grown using the same cultural requirements (Vogelpoel, 1983). Successful cultivation is based upon a thorough grasp of the characteristics of the natural habitat, growth requirements and growth/dormancy cycles (Truter, 1994).

**Figure 2** The evergreen Disas (listed from left to right, top to bottom): *Disa uniflora*, *D. venosa*, *D. uniflora* (different form), *D. racemosa* (typical form and form with dark petals), *D. caulescens*, *D. tripetaloides*, *D. aurata* and *D. cardinalis* (Linder & Kurzweil, 1999).

## Natural habitat

*Disa uniflora* is confined in nature to the sides of perennial streams, waterfalls and rockpools in the mountains of the South Western Cape (Vogelpoel, 1983; Kurzweil, 2003). They grow in niches with a humid, cool microclimate (Yang, 2001). These mountains are situated in a winter rainfall area and frequently moistened by southeaster clouds during the dry summer months, which help to sustain streams throughout the year. There is always moving air and frequent exposure to strong winds.

Disas grow in dense colonies just above the mid-summer water level, during winter they are exposed to heavy rains and often completely submerged in running water (Vogelpoel, 1983). In summer the water level sometimes sink below the plants, but their tubers never really dry out (Eliovson, 1984). They grow in various media such as sand and moss, where water is constantly moving through the root system and good drainage ensures adequate aeration. The water is always cold, even in mid-summer (Vogelpoel, 1983). These plants seem to prefer a cool, moist environment (Komoro, Yoneda & Matsumoto, 2003b). The water usually has a pH of between 5 and 6 due to the presence of humic acids (Orchard, 2000a). The prevailing temperatures for the region lie between 15 and 28°C in summer and between 6 and 17°C in winter (Wodrich, 1997). In the natural habitat of *Disa uniflora*, the mean maximum and minimum temperatures in summer are about 20°C and 10°C, respectively (Komoro, Yoneda & Matsumoto, 2002).

Nutrients are provided by decaying humus, bird and animal droppings and some trace elements are leached from the sandstone rock. Due to the excellent drainage system, these nutrients are continuously supplied in weak concentrations. Flower spikes of some species are more numerous after veld fires which clear away the shade-producing overgrowth and thus expose the plant to direct or filtered sunlight (Vogelpoel, 1983). In nature it is rare to find more than two blooms per plant, but under cultivation more flowers may develop (Stewart & Van der Merwe, 1981).

*D. uniflora* grows in a wide variety of situations and is clearly a very adaptable species (Stewart & Hennessey, 1981). The growth habit of Disa is largely determined by the climate of the natural habitat of the species (Du Plessis & Duncan, 1989).

## Growth habit

The growth habit is best explained as it progresses through the year (Wodrich, 1997).

Winter: Mature Disas have fat, plump tubers, a root system of only four or five unbranched roots and a healthy rosette of leaves. Growth is inhibited by environmental factors such as long nights, short days and low temperatures. The grower should not be feeding the plants at this time; all that is needed is attention to hygiene, provision of water with a low pH (five to six) and low in dissolved salts (less than 200 ppm), and avoidance of fungal attack.

Spring: Longer days, shorter nights, more light and a rise in temperature may break the period of slow growth. Auxins and gibberelins are switched on and a period of rapid growth ensues. A flowering spike emerges from the centre of the rosette. Growth from now on is extremely rapid culminating in a long raceme with flowers starting to bloom in 8-10 weeks.

Mid-summer: Disas in the nursery should be in full flower, at which stage feeding should be discontinued. In a matter of weeks the flowers start wilting, the leaves deteriorate and the stem starts dying back (Vogelpoel, 1993). After flowering time there is a short period of inactivity, when the plants may be considered to be relatively dormant (Du Plessis & Duncan, 1989). Presumably cytokinins are produced which react with auxins and other unknown growth factors to form new tubers as quickly as possible (Wodrich, 1997). Some young plants develop without an apparent tuberoid (Du Plessis & Duncan, 1989); a tuber at this stage will have a small apical shoot but as yet no roots. The tuber will need a month or two to reach maturity and possess active roots and a vigorous leaf shoot (Vogelpoel, 1993). The annual replacement of the entire stem and root system is genetically programmed (Wodrich, 1997). The grower should simply attend to hygiene, use good quality water and withhold feeding.

Late-summer: By this time the stems will have completely withered and plump new tubers will have formed with their healthy leaf shoots now growing rapidly. Now is the time to resume regular feeding to attain growth and vigour before the advent of winter dormancy (Vogelpoel, 1993). There is no true dormant season with the evergreen Disas; even when the flowering stem dies back and the leaves wither there is continuous activity below the surface with the formation of new stolons and tubers (Linder & Kurzweil, 1999).

# CULTURAL REQUIREMENTS

## Growing area

Almost all the cultivated species do well in a greenhouse (Terquem & Parisot, 1991). Disas have soft, thin leaves that lose moisture easily and these plants do not do well in the dry air of centrally heated houses. They are best grown in cool to intermediate greenhouses (Orchard, 2000a). A special area should be set aside for the evergreen Disas, whether the collection is small or large (Linder & Kurzweil, 1999).

A structure should give sufficient light, maintain humidity and allow for air circulation, the grower could construct or adapt a simple structure (Vogelpoel, 1983; Du Plessis & Duncan, 1989). The plants in their containers can be placed on benches filled with sand. The latter will draw excess water from the pots, maintain humidity and keep the root system cool, provided there is contact between the internal and external sand. Disas can also be grown very successfully in a hanging basket lined with hessian and containing a mixture of coarse sand and peat (Vogelpoel, 1983). The plants are best grown in pots or tubs and given a shaded position with morning sun during summer (Eliovson, 1984).

Disas can be grown successfully without resorting to hydroponics, but there are at least three different types of hydroponic systems being used with Disas; the continuous-flow system, ebb-and-flood system and aeroponics (Orchard, 2000b). The main needs for terrestrial orchids are: good drainage; lean compost, high in inorganic material; very good ventilation; and very precise attention to watering (Powell, 2002).

## Growing Medium

A variety of media can be used to grow Disas hydroponically (Orchard, 2000b). The choice of substrate depends firstly on the demands of the plant and the cultivation system (Wever, 2001). A porous medium consisting of thoroughly washed, medium to coarse river sand or live sphagnum, free of decaying organic matter has been found best, from the point of convenience and availability. Another advantage of sand is that it can be washed, dried and stored for future use.

Very fine sand and the presence of soil and particularly clay must be avoided (Vogelpoel, 1983). Disas have not done well grown in lightweight clay particles, commonly employed in hydroponics, rockwool or pumice (Orchard, 2000b). Limestone must also be avoided (Eliovson, 1984).

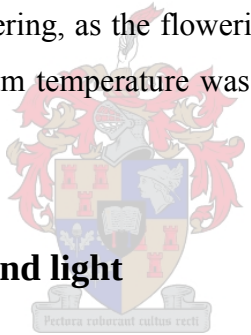
A mixture of imported peat and coarse sand (1:3), materials such as osmunda fibre and pots filled with sphagnum moss only can be used as alternatives (Vogelpoel, 1983). Fresh sphagnum moss may contain some growth hormone and is also a useful indicator because it turns brown when the water is too warm or with a build-up of salts (Yang, 2002). Unfortunately sphagnum moss goes off quite quickly, especially in contact with fertilisers (Terquem & Parisot, 1991). It is also increasingly difficult to find and rather expensive (Cywes, 1990). A mixture of peat (2 parts) and perlite or vermiculite (1 part) can also be used (Terquem & Parisot, 1991). Peat tends to acidify the draining water (Wodrich, 1997). Peat also tends to accumulate and retain salts, which may expose the plants to damage caused by excess salinity (Terquem & Parisot, 1991). The type of peat used in the root medium will affect the physical properties of the medium (Argo, 1998). Coconut fibre can also be considered as an alternative medium (Komoro *et al*., 2003b). Fern fibre contains a natural cytokinine and renders the pH of the medium acid (Yang, 2002). Good drainage is essential to prevent fatal waterlogging, "souring" and the development of toxic anaerobic conditions in the root zones. A mildly acid root environment has been found to suit Disas (Vogelpoel, 1983). There are some indications that the mutualistic relationship between *Disa uniflora* and a specific mycorrhizal fungus plays a significant role in cultivation and is a requirement for optimal growth (Du Plessis & Duncan, 1989).

Pot sizes should be kept uniform (Wodrich, 1997). The pot size for mature plants should not exceed 15 cm and to promise good internal aeration, extra holes can be drilled in the sides near the base (Vogelpoel, 1983). Plastic pots are found to give the best results (Du Plessis & Duncan, 1989).

## Temperature

A cool root environment ranging from 10ºC-20ºC is desirable. A fairly wide latitude for air temperature is permissible and an air temperature of 30ºC can be tolerated, provided that adequate humidity and free air movement are ensured (Vogelpoel, 1983). High night temperatures can be damaging to these plants (Komoro, Yoneda & Matsumoto, 2003a). The temperature should never fall below 0ºC (Terquem & Parisot, 1991) and frost is reported to be fatal (Vogelpoel, 1983). In terms of growth and flowering, Disas were found to tolerate low temperatures of 2-5ºC in autumn and winter (Hasegawa, Horiuchi, Komori & Yoneda, 2004). Plants in the 2ºC treatments had longer stems than those in the 5ºC treatments, but the number of flowers was not affected by temperature treatments (Komoro *et al*., 2002).

It is possible that temperature fluctuations could be of importance, because of the soft herbaceous nature of the plants (Stewart & Van der Merwe, 1981). Temperature may also have an effect on flowering, as the flowering dates of Disas were advanced by 13-27 days when the minimum temperature was increased to 15ºC (Hasegawa *et al*., 2004).




## Humidity, air circulation and light

The ideal humidity should be between 50% and 70% (Vogelpoel, 1983; Du Plessis & Duncan, 1989). This is achieved by standing the pots on a sand-covered bench and by regularly wetting the floor, particularly during warm weather (Du Plessis & Duncan, 1989). Over-heating combined with high humidity lead to soft stems that require staking, the plants are also more vulnerable to fungal attack.

Hot, desiccating winds are harmful (Vogelpoel, 1983). Excellent air circulation must be maintained and there should be openings below the benches to allow fresh air to circulate at all times (Powell, 2002). Stagnant air seems to induce fungal attack.

Adequate light intensity is important for Disa spike development and filtered sunlight is essential to ensure a strong, fibrous stem and optimal colour development of the flower. The full potential of colour is only realised when exposing the flower to sunlight at some stage, preferably the morning sun. Subdued sunlight giving approximately 50% shade is required in summer (Vogelpoel, 1983). They are best

grown in filtered sunlight with shading of forty to fifty percent, but can also be grown under a glass or fibreglass roof (Du Plessis & Duncan, 1989). They can be grown in full sun, provided they are given some shading during the hottest part of the day (Terquem & Parisot, 1991). Keep seedlings and young plants more shaded (Linder & Kurzweil, 1999).

## Water and pH

Rain, river or borehole water can be used (Vogelpoel, 1983). Irrigating Disas with rainwater is not essential, but water containing high concentrations of soluble salts must not be used (Du Plessis & Duncan, 1989). Municipal water can be fatal if chlorination is excessive and the pH high, water with a neutral or low pH is ideal (Vogelpoel, 1983). According to Cywes (1992) water with a relatively high pH produced plants with better growth, but according to Wodrich (1997) a pH range between 4.5 and 5.4 should be sought in irrigation water.

According to Terquem & Parisot (1991) the quality of water is of prime importance and should contain as little calcium as possible. Fluoride is also suspected of damaging Disas (Orchard, 2000a).

Disas require a moist medium, but over-watering should be avoided even where drainage is perfect. Watering depends on the growing area and must be done daily, sometimes twice a day in very hot weather. Wetting the flowers may cause unsightly spotting and fungal attack on pollen, stigma and ripening seedpods (Vogelpoel, 1983). Botrytis may also develop on the pollinia and blemishes may form on the petals (Yang, 2002). When using overhead irrigation systems, the best is a good watering in the morning, which allows the leaves and flowers to dry quickly (Vogelpoel, 1983). Watering from below reduces the incidence of fungal infection (Cywes, 1990).

## Feeding

According to Du Plessis & Duncan (1989) Disas respond well to feeding, and the general rule is to feed frequently with very dilute solutions of liquid fertilisers such as Chemicult at a rate of about one level teaspoon to ten litres of water. This is given either in the form of a foliar spray or directly into the potting medium.

According to Powell (2002) evergreen terrestrials can be fed weekly, with due allowance made for the plant's seasonal cycle. These should be supplied with a reduction during the cold winter months, when plants are almost dormant. During the winter, foliar feeds with ammonium nitrate every two weeks are beneficial (Wodrich, 1997). According to Heathcote (2004) plants that are not growing actively should not be given fertiliser.

Nutrients and trace elements given frequently in weak concentration are preferable to heavy dosages at long intervals. Inorganic fertiliser promotes growth of Disas. The best time for application of nutrients is early in the day. Although nutrients can be absorbed rapidly it is best not to water the plants for 24 hours after feeding; otherwise the nutrients will simply be washed off or leached out (Vogelpoel, 1983).

Fertiliser mixtures vary in strength but it is best to avoid those that are too concentrated since Disa leaves are easily scorched by strong solutions (Vogelpoel, 1983). There are no absolutes, but according to Orchard (2000a) a nutrient solution with an EC (Electrical conductivity) of 0.2 mS $cm^{-1}$ is preferable, but an EC of 0.3 mS $cm^{-1}$ is acceptable. According to Wodrich (1999) feeding concentrations up to a maximum of 0.6 mS $cm^{-1}$ seems to pose no problems. This high EC increases plant size, flower stem length and general vigour of the plants. One drawback that the feeding may have on recently deflasked seedlings is that many of these tend to grow well and even flower in the first year, only to perish during the rest period following flowering (Wodrich, 1999). The culture media used in flasking have quite high dissolved solids contents, some measures up to 1.6 mS $cm^{-1}$, yet Disas seem to thrive in this (Heathcote, 2004). However, according to Orchard (2000a), Disas are intolerant of salt build-up.

Excessive use of organic based nutrients must be avoided, as it increases the risk of fungal and bacterial attack, which could lead to root rot and other diseases (Vogelpoel, 1983). Komoro *et al*. (2002) studied the effects of different kinds of fertilisers on the growth and flowering of Disa orchids. In this study an organic fertiliser, rapeseed oil meal, was compared with a slow-release inorganic fertiliser (6:40:6). Difference in fertiliser type did not affect flowering date, but the survival rate of plants was higher in the group of plants receiving inorganic fertiliser.

## Re-potting

Disas are best repotted in the autumn, after flowering (Orchard, 2000a). It should not be done immediately after flowering but deferred until the new plants are vigorous (Linder & Kurzweil, 1999). This can also be done in spring or at any time if the plants look pale and sickly, possibly due to a medium which is stale, densely packed or "sour". If, on inspection, the roots appear inactive and there are a large proportion of black, dead roots, then re-potting is indicated and all food should be withheld until the root system recovers (Vogelpoel, 1983).

The best time to repot terrestrials is when they are starting into growth after dormancy or rest. Evergreen terrestrials can be repotted when new growth starts, at whatever time of year that may be (Powell, 2002). The best first-aid treatment for a sick plant is to remove it from its medium, trim off all dead roots and leaves, soak for a short while in a fungicide and then re-pot in clean riversand.

To remove a plant from a pot requires a gentle shaking of the pot in a bucket of water, removal of dead roots and old tubers, followed by careful re-potting. Re-potting seems to infuse new vigour into the plants (Vogelpoel, 1983). A collection of Disas can double or triple each year just by repotting (For a suggested potting procedure see Addendum C).

Disas should be slightly over potted with the stem area well covered (Yang, 2002). Disa can be repotted on a yearly basis (Terquem & Parisot, 1991). Mature plants require re-potting every two years but the criterion should be the condition of the root system (Vogelpoel, 1983).

# PROPAGATION

Propagation is by seed or the separation of tuberoids which have developed at the tips of stolons produced by the mature plant (Du Plessis & Duncan, 1989). According to Jorgensen & Andersen (1998), Disas can also be propagated efficiently in aseptic *in vitro* culture by the division of proliferating seedlings.

## In Nature

In nature, dense colonies of *Disa uniflora* become established by vigorous vegetative propagation. This results from a sequence of events.

Firstly, each mature plant produces a new tuber annually; a new shoot and root system arise from the new tuber when the flowered spike and the previous year's tuber die back. This new plant is genetically identical to the old and emerges alongside the old spike in early autumn. Secondly, *D. uniflora* and its allied species produce stolons and tubers, from which new leaf growth and roots emerge (Vogelpoel, 1983). Thirdly, some plants produce a mass of new plantlets at the base of the dying flowering stem.

Reproduction from seed plays a minor role in nature, although a single seed capsule may contain a thousand or more seeds (Vogelpoel, 1983). *Disa uniflora*, *D.cardinalis*, *D.tripetaloides* and *D.caulescens* have seeds of about 1 mm length, and these are dispersed by the streams next to which the plants grow.

Various insects pollinate Disas in nature (Steiner, Whitehead & Johnson, 1994). The lip of some Disa flowers provides a landing platform for the insect (Stewart *et al*., 1982). The large red flowers of Disa are pollinated by the Mountain Pride butterfly (Johnson, Linder & Steiner, 1998). Long distance attraction of *Meneris tulbaghia* appears to be based solely on flower colour (Johnson, 1994). The spur of *D. uniflora* contains copious quantities of nectar (Johnson, 1993). The yellow form of *D. uniflora* is not visited by the pollinating butterfly and the plant can only propagate itself slowly by vegetative means (Yang, 2001). The large brightly coloured flowers of *D. racemosa* and *D. venosa* are pollinated by carpenter bees; these flowers lack nectar and rely on food deception mimicry to attract bees (Johnson *et al*., 1998). Natural hybrids between these two species have not been found, in spite of the fact that they appear to share pollinators. Species integrity seems to be maintained by a sterility barrier (Van der Cingel, 2001). Pollination by short-tongued flies occurs in *D. caulescens* (Johnson *et al*., 1998). There is no opportunity for pollen to make contact with the stigma at the base of the column; self-pollination is thus avoided (Vogelpoel, 1994).

## Growing from seed

Fertile seed is obtained by transferring ripe pollinia to the stigma of a flower at its peak. Cross-pollination by hand between species and subsequent propagation by seed is the only way to produce Disa hybrids, while intra-specific pollination can be used to improve desirable features (Vogelpoel, 1983). Self pollination by artificial means is usually the only satisfactory method for proliferation with the rare yellow form of *D. uniflora* (Crous, 1997). Polyploid plants are often selected for breeding but when a 4n clone is crossed with a 2n clone, the progeny will almost always be 3n. While these triploids are attractive, they tend to be infertile or produce very few fertile seeds blocking future breeding (Vogelpoel, 2001b).

Cross-pollination is preferred, but pollinating a flower with its own pollen brings out recessive genes of interest. *Disa uniflora* pollinia are some of the largest in the orchid family. A cutflower *Disa* can be pollinated in a vase (Vogelpoel, 1983), during this period the cut end of the stem should be trimmed and the water replenished to prevent rot at the tip (Vogelpoel, 2001b). After pollination, the flower fades rapidly and the seed capsule develops (Vogelpoel, 1983). Pollinated flowers fade after about 3 days if fertilisation takes place, and the seed takes from 4 to 6 weeks to ripen (Du Plessis & Duncan, 1989). The capsule then splits to release the seed, this is the time to harvest the capsule and place it in an open container to dry before separating the seed.

Seed gradually loses viability after a few months and should be sown soon after harvesting (Vogelpoel, 1983), to ensure maximum germination. The viability period of the seed varies from genus to genus, one of the shortest being that of *Disa uniflora* (Schelpe, 1981). However, viability of *D. uniflora* seeds increase with decreases in both temperature and moisture content (Thornbill & Koopowitz, 1992). Excellent germination and subsequent growth have been obtained with *Disa uniflora* seeds that have been stored in a deep-freeze for four years (F.J.Haasbroek, July 2004, Forellesngl 16, DBoord, Stellenbosch). Below -70ºC orchid seeds should retain 50% viability for a minimum of two centuries, thus allowing gene banking (Thornbill & Koopowitz, 1992). Sowing in late summer or early autumn has the added advantage of producing strong seedlings (Vogelpoel, 1983).

*Disa uniflora* is unique amongst the commonly grown orchids in that germination in sterile flasks on nutrient agar is not a prerequisite, germination is excellent when sown

on a damp surface of boiled sphagnum moss or imported peat moss (Wodrich, 1997; Addendum D).

Germination is rapid and protocorms start appearing 4-6 weeks after sowing. Soon the first leaf emerges and after 3 months plants will normally have 2 leaves approximately 1-2 mm in length. Feeding from below with a weak nutrient solution can then be commenced. The seedlings grow rapidly until the onset of winter, when growth appears to remain static for several months. Growth can be forced with heating, high humidity and artificial lighting, but is not recommended because of the risk of encouraging fungal attack and algal overgrowth (Vogelpoel, 1983). Flowers out of season are never quite as good and the plant is often set back (Vogelpoel, 1993).

With the arrival of spring, growth accelerates and small roots start developing and by early summer the seedlings are ready for pricking out into community pots (Addendum E). The plastic covering should be removed a few months after sowing, to prevent plants becoming spindly and to harden them before planting out. Pricking out should be done when leaf length reaches about 0.75 cm and roots are growing actively. Strong Disa seedlings with leaf lengths of 1-2 cm should be produced 9-12 months after sowing. Seedlings grow rapidly in their second year and the more vigorous growing plants will begin to multiply vegetatively (Vogelpoel, 1983).



## Vegetative propagation

Well-established plants should be divided when new daughter plants appear next to the older plant, usually in late summer and early autumn. The old plant, mostly a dying flowering stem, can be discarded and the new plants can be carefully divided and individually potted into fresh medium (Vogelpoel, 1983; Figure 3). Stolons sometimes emerge in a drainage hole, which creates problems, particularly at repotting time (Orchard, 2000a). One or more plants can be placed in a pot and allowed to grow to maturity. Always handle roots and tubers carefully as these are very brittle and fragile, the plant is set back seriously should these be broken (Vogelpoel, 1983).

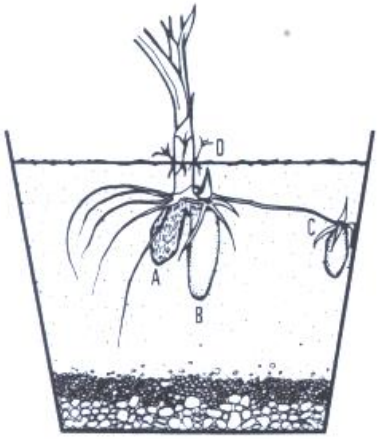



**Figure 3** Vegetative propagation: A. Old, withering tuber and roots of preceding year. B. New tuber with developing leaf shoot and roots. C. Stolon with new tuber, leaf shoots and roots at a distance from main plant. D. Plantlets at the base of old flowering stem (Vogelpoel, 1983).

## *In-vitro* Propagation

Tissue culture exploits the potential of certain meristematic tissues in the Disa to replicate vegetatively. After careful removal of the explant, sterilising against fungal and bacterial contaminants, the tissue is cultured on a sterile agar medium containing inorganic compounds and a cytokinin (Pelser, 1994). One method of obtaining an explant that can be used for micro propagation is based on cutting out the vegetatively multiplying group of cells at the apex of new shoots, from stolons and plantlets developing at the base of stems (Wodrich, 1997). Currently most Disa growers are using sections of young flower buds as explants for micro-propagation (F.J.Haasbroek, July 2004, Forellesngl 16, DBoord, Stellenbosch).

Sowing *in-vitro* produces strong seedlings at a much faster rate than sowing seed on to sphagnum or peat (Wodrich, 1997). A quarter strength modified Murashige and Skoog medium can be used for sowing (Addendum F). When a medium is prepared, green banana pulp is sometimes added because of the excess of cytokinine hormones, which regulate the cell devision in callus tissue. Charcoal powder can be added to absorb the phenols given off by the chemical compounds and to act as a buffer to stabilise the pH (Yang, 2002). Several terrestrial species have been successfully

germinated using formulations in which the nutrients are supplied in organic form (Du Plessis & Duncan, 1989).

Disa plants and propagules are becoming more readily available to would-be growers. Seed, pollen, flasks and plants are now routinely being traded and exchanged internationally (Orchard, 2000a). Due to the rapid two to three year pollination-to-flowering time, there is much scope for the newcomer (Vogelpoel, 1992d).

## DISEASES AND PESTS

Good hygiene minimises the need to use fungicides and insecticides, which could harm the plants and flower growth (Vogelpoel, 1983). Success in controlling disease depends on evasive action, early recognition and appropriate control measures (Quigley, 2000). All fungicides and insecticides should be in wettable powder form as the cuticle of the leaves can be damaged by solvents (Wodrich, 1997).

### Fungal disease

Disas are quite susceptible to root, stem and crown rotting (Orchard, 2000a). The plant rapidly disintegrates, the stem collapses and when the medium is examined, the tubers and roots have disappeared or turned into a glutinous mass. The disease can spread in epidemic proportions and wipe out a collection of seedlings and mature plants. The cause of this rot has yet to be identified. Available fungicides fail to cure this infection once it has established.

The best safeguard against fungal infection is constant vigilance and the regular use of a variety of fungicidal sprays; prevention is better than cure (Vogelpoel, 1983). Getting the watering regime right and providing good aeration are the keys to avoid damping off. Damping off is a problem with tuberous orchids and those with underground pseudobulbs (Powell, 2002). Using clean sand medium, free of decaying organic matter and regular feeding with dilute nutrient solutions to ensure vigorous, healthy plants, drops the incidence of disease markedly. Fungal attack is apt to occur in plants with poor root systems or plants growing in a badly drained medium.

Furthermore, cleanliness and good hygiene, repotting of weak plants, removal of dying leaves from the base of the stem and prompt removal, isolation or even destruction of all diseased plants and avoidance of over-watering help prevent fungal diseases (Vogelpoel, 1983). *Cylindrocladium, Rhizoctonia* and *Cylindrocarpon* had been detected in infected plants (Orchard, 2000a). When a new fungicide is used it must be ensured that the fungicide does not kill the plant. Dithane, Kaptan, Funginex and Benlate are safe to use, but Benlate given in full strength can result in severe chlorosis of the leaves of seedlings and young plants (Vogelpoel, 1983). If Benlate is used, it should be at a rate of 2.5 g per 10 litres, which is less likely to cause damage (Yang, 2002).

## Bacterial disease

Terrestrials are very vulnerable to rots. Many produce tight rosettes of fleshy leaves at ground level and this may be susceptible to bacterial infection, should it be exposed to water (Powell, 2002). Bacterial Soft Rot (*Erwinia carotovora*) cause amber coloured spots to develop on leaf blades, which then turns brown. If not arrested, the rot may involve the entire plant and destroy it (Vogelpoel, 1983). Bacteria can cause leaf spots of stems and rootstocks (Du Plessis & Duncan, 1989). This infection usually occurs only through wounds and care should be taken not to bruise plants. The relevant bacterium has yet to be determined in Disas. Physan 20 is an excellent bactericide, but could burn the delicate Disa plant and should be used with extreme caution (Vogelpoel, 1983).

## Viruses

These have never been recognised in Disas and could not be identified in suspect plants (Vogelpoel, 1983).

## Insect pests

Some growers have reported severe damage from red spider mite and thrips, with the occasional damage resulting from greenfly attack (Vogelpoel, 1983). The result of a greenfly infestation is stunted growth and deformed flowers, leaves and buds (Wodrich, 1997). Systemic insecticides are best avoided when the flower spike is developing, since pedicel and bud growth may be affected and malformed flowers may result. A most serious problem is damage caused by the larvae of a gallmidge fly, belonging to the genus *Cecidomyidae*. From early spring onwards, the flies lay their eggs on the young flowering stalks or in the undeveloped flower bud. Small, bright yellow larvae cause the plant tissue to collapse and the plant then becomes rapidly susceptible to fungal attack. If the larvae are not destroyed, the tip of the flower spike may be seriously damaged, with resultant malformation of the flower buds.

The only effective treatment appears to be Lebacyd (fention), which should be administered at monthly intervals from August onwards (Vogelpoel, 1983). As preventative of gallmidge fly a few insectivorous plant species should be grown with the Disas (Wodrich, 1997). Attack from scale insects has not been encountered (Vogelpoel, 1983). Mites are controlled by Pentac (dienochlor), aphids by Primor (Peremicarb) and thrips by Ultracide (methomyl) (Yang, 2002). A variety of caterpillars, including boll-worm, can be eliminated by dusting with contact insecticides (Vogelpoel, 1983).

## Snails and slugs

They enjoy the flower buds and leave nasty perforations on them. Slugs hide under the pots or amongst the stones in the bottom of the pots. Snailbait is best applied around the pots (Vogelpoel, 1983).

## CONCLUSION

For even moderate success with *D. uniflora* the plants must have perfect drainage and buoyant, cool and moist conditions throughout the year (Schelpe, 1966). Disas seem to require that whatever the growing conditions, their roots remain cool and moist. The cultural requirements should be adjusted to suite local conditions. Disas are not that difficult to grow, merely particular about their growing conditions. With good composts, ventilation and watering, the evergreen terrestrials are just as easy to grow as other orchids, but they are generally less tolerant of mistakes (Powell, 2002). Many growers agree that the single most important factor in success with Disas is the water, this includes the quality and quantity of water, in addition to how and when the plants are watered (Orchard, 2000a).

Constant attention should be given to detail, cleanliness, hygiene and provision of the basic requirements throughout the seasons; this should minimise fungal and bacterial rots and avoid damage from mites, thrips, insect larvae and caterpillars (Linder & Kurzweil, 1999). Yang (2001) states that, in summary, the cultivation principle are to keep them cool, moist and shaded.

Many growers are discouraged by the bad reputation of Disas in cultivation and are unwilling to persist after initial losses. Production techniques need to be further investigated since only a few growers produce Disas as a hobby and a lot of variation is found in their production techniques. Not all growers in SA use hydroponic systems, but hydroponics can produce lush growth and is very close to the way many Disas grow in nature, with their roots in running water.

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# CHAPTER 2

**The effects of N-source, shading and root medium temperature on the growth of two evergreen Disa hybrids (*Disa unidiorosa* and *D. kewensis*).**

## ABSTRACT

Although the Disa genus accommodates more than 130 species, by far the most commonly grown is *Disa uniflora* and hybrids stemming from this species. The evergreen Disas are indigenous orchids and have enormous potential to be marketed as cutflowers or potplants. However, before this potential can be utilised, production techniques need to be further investigated. This study has evaluated the nutritional needs of two Disa hybrids under varying environmental conditions. Plants were cultivated in a controlled environment and growth was evaluated after 266 days. Plants were fertigated according to guidelines provided by Naaldwijk for Cymbidiums, but included different $NH_4$:$NO_3$ ratios with the same amount of total nitrogen. Two levels of shading were provided (56% and 69%); root medium temperature was lowered and compared to the growth of plants in un-cooled media. Results showed that *Disa unidiorosa* performed best with 40% of the applied N in the $NH_4$ form. *D.kewensis* was more tolerant towards a higher level of $NH_4$ and grew best at 60% $NH_4$. Shading was found to have no main effect on plants but was involved in interactions with $NH_4$:$NO_3$ ratios and root medium temperature. A cooled root medium was found to have a negative effect on root growth and a positive effect on leaf length, while the two Disa hybrids reacted differently to the treatments.

# INTRODUCTION

The evergreen Disas, of which *Disa uniflora* is the most renowned, comprise seven species well known in cultivation and indigenous to the Western Cape. One species, though centered in the Western Cape, ranges into the Eastern Cape and Kwazulu-Natal. The evergreen Disa species are indigenous orchids which comprise of *Disa uniflora*, *D.tripetaloides*, *D.aurata*, *D.cardinalis*, *D.racemosa*, *D.venosa* and *D.caulescens*. Because of the comparatively easy cultivation and showy flowers of the evergreen Disas these orchids are highly desirable subjects (Linder & Kurzweil, 1999).

Cultivation of indigenous orchids has recently had a resurgence of interest, particularly as artificially raised plants of the more common species are becoming available (Linder & Kurzweil, 1999). A brief glimpse at the history of Disa growing emphasizes that no grower can make progress until the cultural requirements are understood and applied (Vogelpoel, 2001a). Successful cultivation is based upon a thorough grasp of the characteristics of the natural habitat, growth requirements and growth/dormancy cycles (Truter, 1994).

*Disa uniflora* is confined in nature to the sides of perrenial streams, waterfalls and rockpools in the mountains of the South Western Cape (Vogelpoel, 1983; Kurzweil, 2003). They grow in various media, where cold water is constantly moving through the root system and good drainage ensures adequate aeration (Vogelpoel, 1983). *D. uniflora* grows in a wide variety of situations and seems to be a very adaptable species (Stewart & Hennessey, 1981).

The Disa species which hybridise with *Disa uniflora* can be grown using the same cultural requirements (Vogelpoel, 1983). Adequate light is important for Disa spike development and filtered sunlight is essential to ensure a strong, fibrous stem and optimal colour development of the flower (Vogelpoel, 1983). They are best grown in filtered sunlight with shading of forty to fifty percent, but can also be grown under a glass or fibreglass roof (Du Plessis & Duncan, 1989). According to Terquem & Parisot (1991) they can be grown in full sun, provided they are given some shading during the hottest part of the day.

A cool root environment ranging from 10°C to 20°C is desirable (Vogelpoel, 1983). Responses to root temperature may have a considerable effect on the growth and yield of greenhouse crops (Savvas & Passam, 2002).

According to Du Plessis & Duncan (1989) Disas respond well to feeding, and the general rule is to feed frequently with dilute solutions of liquid fertilisers such as Chemicult (Reg. no.K2025Act36-1947). In reviewing the available literature, it was noted that a lack of information concerning the nutrient requirements of Disas exists.

Plants absorb nitrogen as nitrate or ammonium (Wilkens, 1984). For most crops it is sufficient to have about 10% of the total N as $NH_4$, but this quantity will vary with crop, growth stage and growing conditions (Savvas & Passam, 2002). The response of plant species or hybrids varies widely, depending on the nitrogen source (Lasa, Frechilla, Aparicio-Tejo & Lamsfus, 2002).

## MATERIAL AND METHODS

### Plant material

Plants were cultivated in a glasshouse at Welgevallen, an experimental farm of the University of Stellenbosch. The temperature inside the glasshouse was regulated at 18°C during the day and 13°C at night, with the average humidity at 50-60%. Plantlets were transplanted on 17/06/2003 and harvested on 08/03/2004, after 266 days of growth. Plants were grown in 10 cm diameter white plastic containers filled with silica sand, obtained locally from Consol Glass (Pty) Ltd. Three grades of sand were used; firstly a layer of coarse sand (3-6 mm) was placed at the bottom 20% of each pot, then a mixture of 1:1 fine (1 mm): medium sand (1-3 mm) was used to fill up the pot and lastly a final layer of coarse sand was placed on top. Before pots were filled with this medium, a small piece of gauze was placed at the bottom to prevent sand from falling through the drainage holes. Successful culture of Disas should be in step with the dramatic seasonal bio-rhythms (Vogelpoel, 1993), thus nutrients should be supplied at lower concentrations during the cold winter months, when plants are almost dormant (Wodrich, 1997). Plants received nutrient solutions as shown in Table 1, but diluted with an electrical conductivity (EC) of 0.24 mS $cm^{-1}$ during the first two months of the trial (during winter) and an EC of 0.41 mS $cm^{-1}$ for the

remainder of the trial. The pH of the tanks was kept between 5.0 and 6.5 by the addition of $H_2SO_4$ and KOH when needed. Plants were irrigated using the 'ebb-and-flood' method. At irrigation time (once daily in winter at 12h00 and twice daily in summer at 11h00 and 13h00) water slowly rose submerging three-quarters of the pots and was then allowed to drain to waste. General maintenance included spraying plants every three weeks with fungicides, a mixture of Dithane and Kaptan (1 g $l^{-1}$).

**Treatments**

Four $NH_4^+$:$NO_3^-$ ratios, two shading levels, two root medium temperatures and two cultivars were used in a 4×2×2×2 factorial experiment. Four nutrient solutions, containing different $NH_4^+$:$NO_3^-$ ratios (0:100, 20:80, 40:60, 60:40) with the same total N concentration were used (Table 1). Micro-nutrients were supplied at standard ratios in all four nutrient solutions, using guidelines provided by Naaldwijk for Cymbidiums (Table 2). For this trial the glasshouse was equipped with shadenet to provide two levels of shading, 56% and 69% (Addendum G). Half of the pots involved in this trial were equipped with a glass spiral, which was connected to a water cooler that circulated water of 14°C in an attempt to reduce the temperature of the root medium (Addendum H). Two Disa hybrids, namely *Disa kewensis* and *D.unidiorosa* 'Rosy Face' (Figure 1, Addendum I), were used.

**Table 1** Composition of the four basic (undiluted) nutrient solutions containing various $NH_4^+$:$NO_3^-$ ratios at the same total N (5 $mmol_c$) level and at an electrical conductivity (EC) of 0.73 mS $cm^{-1}$.

| | $NH_4^+$: $NO_3^-$ ratios (%) | | | |
|---|---|---|---|---|
| Salt ($mmol_c$) | 0:100 | 20:80 | 40:60 | 60:40 |
| $KNO_3$ | 0.94 | 1.8 | 0.36 | 0 |
| $K_2SO_4$ | 1.74 | 1.0 | 2.0 | 1.92 |
| $KH_2PO_4$ | 0.56 | 0 | 0 | 0 |
| $NH_4H_2PO_4$ | 0 | 0.8 | 1.04 | 1.28 |
| $NH_4NO_3$ | 0 | 0.2 | 0.96 | 0.64 |
| $(NH_4)_2SO_4$ | 0 | 0 | 0 | 1.08 |
| $Ca(NO_3)_2.2H_2O$ | 2.32 | 2.0 | 1.68 | 1.36 |
| $MgSO_4.7H_2O$ | 0 | 1.5 | 1.26 | 1.02 |
| $Mg(NO_3)_2.6H_2O$ | 1.74 | 0 | 0 | 0 |

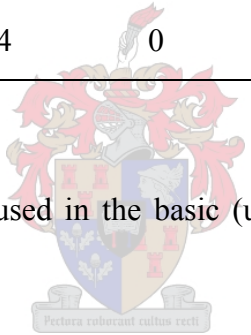

**Table 2** Micro-nutrient levels used in the basic (undiluted) nutrient solutions (see Table 1).

| **Chemicals used to supply micro-nutrients** | | **Nutrient solution composition** | |
|---|---|---|---|
| Source | Concentration (%) | Micro-nutrient | Level ($mg.kg^{-1}$) |
| Libfer (Fe-EDTA) | 13.0 | Fe | 0.43 |
| Manganese sulphate | 24.7 | Mn | 0.55 |
| Zink sulphate | 22.5 | Zn | 0.27 |
| Solubor | 20.5 | B | 0.21 |
| Copper sulphate | 25.2 | Cu | 0.03 |
| Ammonium molibdate | 54.3 | Mo | 0.04 |

*D.kewensis* = *D.uniflora* × *D.tripetaloides*

*D.unidiorosa* = *D.diorosa* × *D.uniflora*

*D.diorosa* = *D.diores* × *D.racemosa*

*D.diores* = *D.veitchii* × *D.uniflora*

*D.veitchii* = *D.uniflora* × *D.racemosa*

**Figure 1** A diagram showing the back-crossing involved in creating the two hybrids used in this trial (Vogelpoel, 2001b).

**Experimental design**

The trial was conducted using a completely randomized design. A single plant was considered as an experimental unit and the treatments were replicated twice. Two-way analyses of variance were performed using SAS version 8.2 (SAS, 1999). Student's t-Least Significant Differences (LSD) was calculated at a 5% significance level to compare treatment means. Shapiro-Wilk's test was performed to test for non-normality (Shapiro & Wilk, 1965). There was no evidence against normality, therefore no transformation was needed.

**Measurements**

The following parameters were measured at the beginning and end of the trial: Plant mass, the dimension (diameter and length) of the plant, stem diameter, number of leaves and roots, as well as the diameter and length of leaves and roots. The roots and leaves were counted, while plant diameter was measured as the widest distance between two leaftips and plant length was measured as the distance between the tip of the longest root and the tip of the longest leaf. Stem diameter was measured halfway between the roots and the leaves, while the average length and diameter of both roots and leaves were calculated. Plant growth was monitored every two weeks by means of noting the amount of leaves, the average diameter and length of leaves and the mass of plants. Additional measurements were made at the end of the trial: The mother plant was cut halfway between the roots and the leaves to compare the mass of

above-ground- to below-ground plant parts. New plants were separated from the mother plant and cut in a similar way. Where tubers were present their mass and dimensions were measured. To narrow down the amount of parameters measured, some parameters were combined. The following parameters were calculated: Change in plant mass as a percentage; total root length at the beginning and end; root growth as a percentage; root: shoot ratio for mother plant; root: shoot ratio for newly formed plants; shape of tuber [tuber length/tuber diameter]; single leaf area [leaf length × leaf diameter × 0.745]; plant leaf area [Single leaf area × number of leaves].

## RESULTS

### Shape and mass of plants

The 40:60 % $NH_4$:$NO_3$ ratio produced the longest plants with the biggest diameter, but the thickest stems formed at 60:40. The treatments containing less than 40% $NH_4$ were significantly (P = 0.05) inferior to the two treatments containing 40% and 60% $NH_4$ (Table 3). The two hybrids were significantly different in plant length, with *D.unidiorosa* producing longer plants. Shading and cooling of root media had no significant effect on the shape of the plants.

Plant mass (roots and shoots) was affected by a highly significant interaction (P = 0.01) between $NH_4$:$NO_3$ ratios and the two hybrids (Figure 2). The optimum $NH_4$ level for *D.unidiorosa* seemed to be at 40% $NH_4$, with a higher $NH_4$ percentage having a negative effect on plant mass. *D.kewensis* showed tolerance to high $NH_4$ levels, with plant mass the highest at 60% $NH_4$.

**Table 3** The effects of different $NH_4$:$NO_3$ ratios, levels of shading, root medium temperature and hybrids on the shape of evergreen Disa plants.

| **Treatments** | **Plant dimensions (cm)** | | |
|---|---|---|---|
| | Plant diameter | Plant length | Stem diameter |
| $NH_4$:$NO_3$ (%) | | | |
| 0:100 | 6.32 c | 14.49 b | 0.510 c |
| 20:80 | 6.94 bc | 14.63 b | 0.582 bc |
| 40:60 | 10.94 a | 18.59 a | 0.755 ab |
| 60:40 | 8.76 b | 16.52 ab | 0.848 a |
| LSD (P = 0.05) | 1.834 | 3.386 | 0.1747 |
| Shade (%) | | | |
| 56 | 8.04 a | 15.86 a | 0.702 a |
| 69 | 8.58 a | 16.37 a | 0.663 a |
| LSD (P = 0.05) | NS | NS | NS |
| Root media | | | |
| Not-cooled | 7.81 a | 15.66 a | 0.675 a |
| Cooled | 8.77 a | 16.53 a | 0.689 a |
| LSD (P = 0.05) | NS | NS | NS |
| Hybrids | | | |
| *D.unidiorosa* | 8.70 a | 20.13 a | 0.670 a |
| *D.kewensis* | 7.88 a | 11.67 b | 0.695 a |
| LSD (P = 0.05) | NS | 2.390 | NS |

Means followed by the same letters are not significantly different at the 5% probability level.
NS = Not Significant

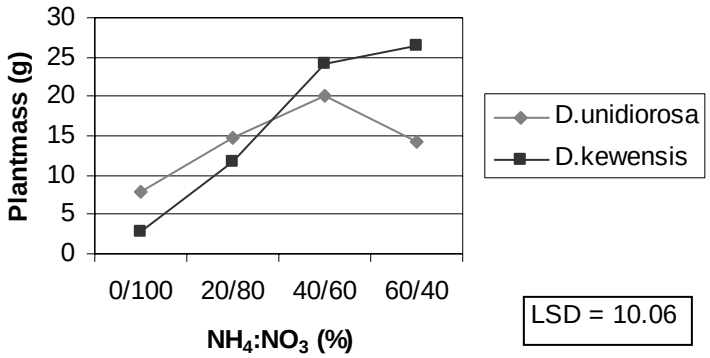


**Figure 2** Plant mass affected by an interaction (P = 0.01) between two evergreen Disa hybrids and different $NH_4$:$NO_3$ ratios.

The mother plant was separated from newly formed plants and weighed separately as shown in Table 4. The mass of the mother plants was significantly higher in the two treatments with ammonium concentrations of 40% and 60% $NH_4$. *D.unidiorosa* produced heavier plants than *D.kewensis* (Table 4) due to the fact that *D.unidiorosa* plants had a higher root mass (results not shown).

A highly significant interaction (P = 0.01) between $NH_4$:$NO_3$ ratios and the two hybrids affected the mass of newly formed plants (Figure 3). *D.unidiorosa* performed the best at 20 to 40% $NH_4$, while the plant mass of newly formed plants of *D.kewensis* increased linearly up to the 60% $NH_4$ level.

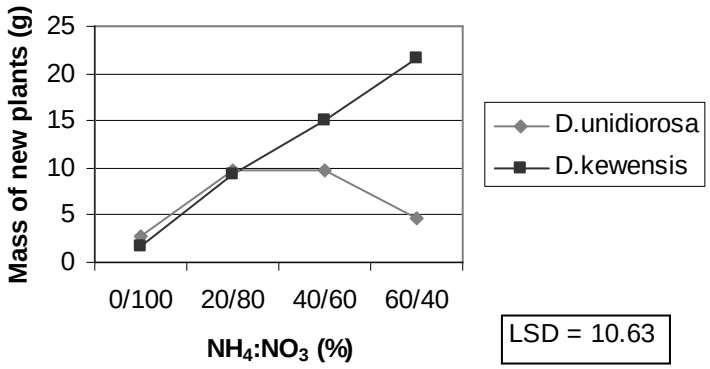


**Figure 3** Plant mass of newly formed plants affected by an interaction (P = 0.01) between two evergreen Disa hybrids and different $NH_4$:$NO_3$ ratios.

The root:shoot ratio of mother plants was not affected by the treatments, while the root:shoot ratio of newly formed plants was affected by an interaction between $NH_4$:$NO_3$ ratios and hybrids. The ratio declined in both hybrids with rising $NH_4$, but a more drastic effect was found on *D.unidiorosa* (Figure 4).

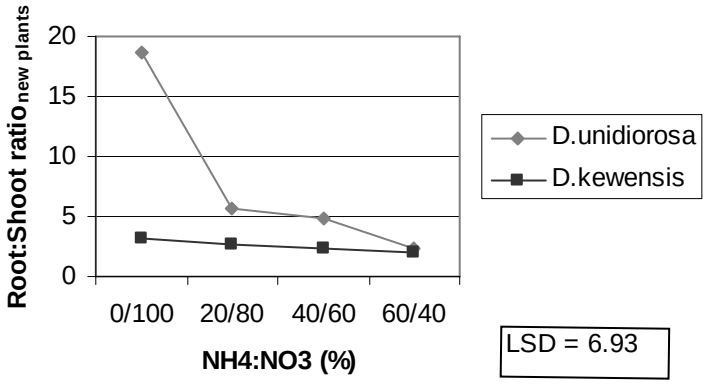


**Figure 4** An interaction (P = 0.01) between $NH_4$:$NO_3$ ratios and cultivars affecting the root:shoot ratio of newly formed plants from two evergreen Disa hybrids.

The change in plant mass over the growth period was found to be significantly lower in the 0% $NH_4$ treatment, compared to the treatments with some ammonium added (Table 4). Plants grown in un-cooled root media gained significantly more mass than plants in the cooled root media (Table 4).

**Table 4** The effects of different $NH_4$:$NO_3$ ratios, levels of shading, root medium temperature and hybrids on plant mass of evergreen Disas.

| **Treatments** | **Plant mass** | | |
|---|---|---|---|
| | Mother plant at harvesting (g) | Tuber mass at harvesting (g) | Change in plant mass (%) |
| $NH_4$:$NO_3$ (%) | | | |
| 0:100 | 3.4 b | 1.1 b | 228 b |
| 20:80 | 3.6 b | 1.5 b | 867 a |
| 40:60 | 9.8 a | 2.5 a | 1352 a |
| 60:40 | 7.3 a | 1.5 b | 1363 a |
| LSD (P = 0.05) | 3.60 | 0.78 | 538.2 |
| Shade (%) | | | |
| 56 | 6.3 a | 1.6 a | 990 a |
| 69 | 6.0 a | 1.7 a | 978 a |
| LSD (P = 0.05) | NS | NS | NS |
| Root media | | | |
| Not-cooled | 6.4 a | 1.8 a | 1195 a |
| Cooled | 5.9 a | 1.6 a | 794 b |
| LSD (P = 0.05) | NS | NS | 380.0 |
| Hybrids | | | |
| *D.unidiorosa* | 7.5 a | 2.3 a | 860 a |
| *D.kewensis* | 4.6 b | 1.0 b | 1121 a |
| LSD (P = 0.05) | 2.54 | 0.55 | NS |

Means followed by the same letters are not significantly different at the 5% probability level.
NS = Not Significant

**Root development**

Total root length, measured at the end of the trial, was the highest with 40% $NH_4$, as shown in Table 5, roots were found to have a bigger diameter in plants receiving the nutrient solution containing 40% and 60% $NH_4$. Shading, root medium cooling and cultivars had no significant influence on the diameter of roots. Root length expansion was not consistent. Cooling of the root media had a significant negative effect on total root length and the percentage root growth. *D.unidiorosa* had a higher total root length than *D.kewensis* (Table 5), due to significant differences in both the amount and length of roots (results not shown). The root length of *D.unidiorosa* expanded more than *D.kewensis* (Table 5).

As shown in Table 4 the tuber mass of plants grown with the 40% $NH_4$ treatment was significantly higher than the other treatments and *D.unidiorosa* produced heavier tubers than *D.kewensis*. Tuber shape was influenced by a highly significant interaction (P = 0.01) between cultivars, $NH_4$:$NO_3$ ratios and cooling of the root medium (Figure 5). Although *D.unidiorosa* seemed to produce longer shaped tubers than *D.kewensis*, this was especially true where the 20% $NH_4$ treatment was combined with a cooled root medium. However, where the 60% $NH_4$ treatment was combined with a cooled root medium, the tuber shape (length/diameter) of *D.kewensis* was significantly higher than *D.unidiorosa*.

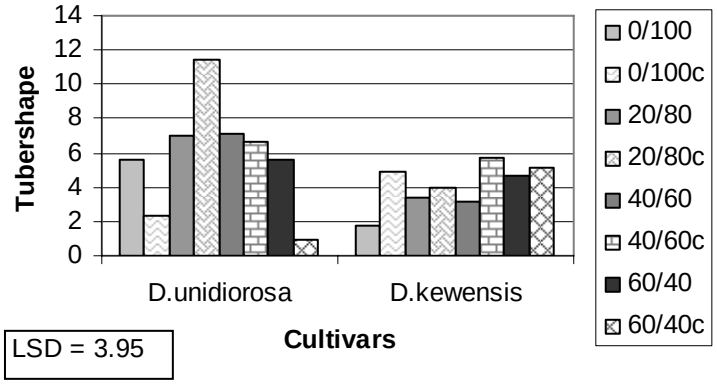


**Figure 5** An interaction (P = 0.01) between cultivars, $NH_4$:$NO_3$ ratios and cooling of the root medium affecting tuber shape of two evergreen Disa hybrids. c = cooled root media.

**Table 5** The effects of different $NH_4$:$NO_3$ ratios, levels of shading, root medium temperature and hybrids on root development of evergreen Disas.

| **Treatments** | **Root development** | | |
|---|---|---|---|
| | Total root length (cm) | Root diameter (cm) | Root growth (%) |
| $NH_4$:$NO_3$ (%) | | | |
| 0:100 | 59.2 ab | 0.17 b | 230 ab |
| 20:80 | 47.0 b | 0.18 b | 176 b |
| 40:60 | 80.8 a | 0.25 a | 514 a |
| 60:40 | 56.4 b | 0.26 a | 422 ab |
| LSD (P = 0.05) | 23.98 | 0.035 | 317.0 |
| Shade (%) | | | |
| 56 | 59.1 a | 0.21 a | 343 a |
| 69 | 62.5 a | 0.22 a | 338 a |
| LSD (P = 0.05) | NS | NS | NS |
| Root media | | | |
| Not-cooled | 71.1 a | 0.22 a | 489 a |
| Cooled | 51.5 b | 0.21 a | 207 b |
| LSD (P = 0.05) | 16.93 | NS | 223.8 |
| Hybrids | | | |
| *D.unidiorosa* | 89.1 a | 0.23 a | 514 a |
| *D.kewensis* | 29.5 b | 0.20 a | 148 b |
| LSD (P = 0.05) | 16.93 | NS | 223.8 |

Means followed by the same letters are not significantly different at the 5% probability level.
NS = Not Significant

**Leaf development**

The leaf area of a single leaf as well as the total leaf area per plant was found to be significantly higher with 40% and 60% $NH_4$ compared to the lower $NH_4$ treatments (Table 6), mainly due to bigger leaves. Unlike the supressing effect of a cooled root medium on roots, leaf area significantly increased with cooling (Table 6). A cooled root medium produced plants with significantly more and broader leaves than an un-cooled medium. Shading had no effect on leaf area. In most cases bigger leaves were associated with higher leaf areas per plant. An exception was that *D.unidiorosa* had a higher plant leaf area than *D.kewensis* (Table 6), due to *D.unidiorosa* producing significantly more leaves than *D.kewensis* (results not shown).

During the trial some lower leaves deteriorated and abscised. This rate was monitored as some plants seemed to be more susceptible. It was found that the amount of leaves removed was significantly higher in the 0% $NH_4$ treatment, compared to 40% and 60% $NH_4$. Significantly more leaves were also removed from *D.kewensis* (Table 6).

An interaction between shading, root medium cooling and hybrids affected leaf lengths (Figure 6). The leaflength of *D.unidiorosa* was not significantly affected by rootmedium temperature at both levels of shading, while *D.kewensis* produced significantly longer leaves due to cooling of the root zone at the 69% shading level.

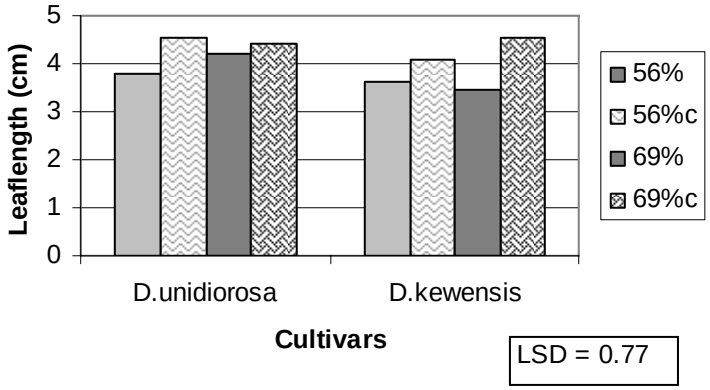


**Figure 6** An interaction (P = 0.01) between the level of shading and root medium temperature on the leaf length of two evergreen Disa hybrids. c = cooled root media.

Shading, root medium cooling and hybrids affected the total leaf area per plant (Figure 7). At the 56% shading level *D.unidiorosa* produced larger leaf areas with root zone cooling, while cooling the root zone had a negative effect on total leaf surface of this cultivar at 69% shade. The only significant difference was found at the 69% shading level where *D.kewensis* reacted positively to root zone cooling.

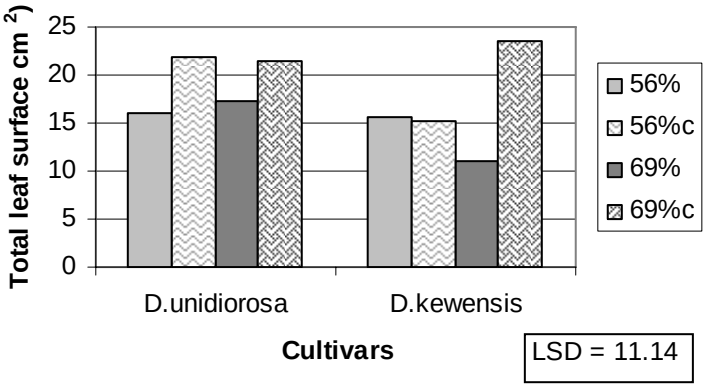


**Figure 7** An interaction (P = 0.01) between the level of shading and root medium cooling on the plant leaf area of two evergreen Disa hybrids. c = cooled root media.

**Table 6** The effects of different $NH_4$:$NO_3$ ratios, levels of shading, root medium temperature and hybrids on leaf development of evergreen Disas.

| **Treatments** | **Leaf development** | | |
|---|---|---|---|
| | Single leaf area ($cm^2$) | Plant leaf area ($cm^2$) | *Fraction of leaves removed |
| $NH_4$:$NO_3$ (%) | | | |
| 0:100 | 1.91 b | 13.9 b | 0.352 a |
| 20:80 | 1.95 b | 14.2 b | 0.298 ab |
| 40:60 | 2.72 a | 20.9 a | 0.254 b |
| 60:40 | 2.72 a | 22.2 a | 0.256 b |
| LSD (P = 0.05) | 0.425 | 3.79 | 0.0612 |
| Shade (%) | | | |
| 56 | 2.32 a | 17.2 a | 0.311 a |
| 69 | 2.33 a | 18.4 a | 0.268 a |
| LSD (P = 0.05) | NS | NS | NS |
| Root media | | | |
| Not-cooled | 2.04 b | 15.0 b | 0.294 a |
| Cooled | 2.61 a | 20.6 a | 0.286 a |
| LSD (P = 0.05) | 0.301 | 2.68 | NS |
| Hybrids | | | |
| *D.unidiorosa* | 2.46 a | 19.2 a | 0.224 b |
| *D.kewensis* | 2.20 a | 16.5 b | 0.356 a |
| LSD (P = 0.05) | NS | 2.68 | 0.0433 |

Means followed by the same letters are not significantly different at the 5% probability level.
NS = Not Significant
*Fraction x 100 = Percentage

## DISCUSSION

The growth of plants fertigated with the nutrient solution containing 0% $NH_4$ were inferior to all other $NH_4$:$NO_3$ ratios, thus it seems as if Disa plants prefer to be fertigated with a relatively high percentage of nitrogen in the form of $NH_4$. According to Jones (1997), the presence of ammonium in nutrient solution stimulates the uptake of nitrate. A study by Cao & Tibbitts (1993) demonstrates that potato plants can more effectively utilize mixed ($NH_4$ and $NO_3$) N-forms than a single nitrogen form.

A $NO_3$:$NH_4$ ratio of 1:1 was found to be best for the growth of young tomato plants under a wide range of root temperatures (Ganmore-Neumann & KafKafi, 1980). Enhanced growth of potatoes with mixed nitrogen sources was best at 8% to 20% $NH_4$-N (Cao & Tibbitts, 1993). Most greenhouse crops are grown with 3 to 9% $NH_4$-N (De Kreij, Voogt, Van den Bos & Baas, 1999). Disa plants can thus be classified as being ammonium tolerant. Differences found between spinach (ammonium-sensitive species) and pea (ammonium-tolerant species) seem to be related to differences in the site of ammonium assimilation as well as to the assimilation route (Lasa *et al.*, 2002).

The pH level maintained in the root zone is influenced by the form of nitrogen, $NO_3^-$ or $NH_4^+$, utilized for fertilization. The advantages in growth for a particular nitrogen form can be associated with more favourable pH conditions being maintained in the growth medium (Cao & Tibbitts, 1994).

Ammonium can be toxic to plants when it is the major source of N, resulting in slow growth and development (Jones, 1997). A higher $NH_4$: $NO_3$ ratio stimulates the uptake of cations and suppresses the uptake of anions at the same level of total N uptake (Savvas & Passam, 2002). According to Cao & Tibbitts (1993) an increased Ca supply may be desirable to prevent a possible Ca deficiency with $NH_4^+$ additions. Ammonium may be included in the nutrient solution during the early vegetative growth period but should then be excluded from flower initiation to the end of the growth cycle (Jones, 1997). The results obtained in our study, that 40 to 60% of the total N-supply should be in the ammonium form for best development before flowering, was obtained under a 'drain-to-waste' irrigation system. Further research is needed on Disas to investigate the effect of $NH_4$: $NO_3$ ratios on the quality of flowers.

Cooling of the root medium had a negative effect on plant mass and root length, while it had a positive effect on leaf area. Average root zone temperatures were 16.8°C for cooled and 21.4°C for the un-cooled media. The greenhouse was relatively cool, set at 18/13°C day/night temperatures. Due to this the root zone temperatures were relatively cool, thus limiting the beneficial effects expected with lower root zone temperatures (respiration). According to Jones (1997), the optimum root temperature may vary somewhat with plant species, but in general, root temperatures below 20°C may reduce growth, although root system size does not necessarily translate into top growth and yield. Ammonium was shown to be an undesirable source of nitrogen for tomato and strawberry plants at root-zone temperatures above 30°C (Ganmore-Neumann & KafKafi, 1980; Ganmore-Neumann & KafKafi, 1983).

During this trial leaves were removed when they turned brown and after closer inspection, the causes of these symptoms were found to be *Cylindrocarpon* sp. *D.kewensis* was more susceptible to this organism, although the organism can be saprophytic or parasitic. The fact that a low ammonium level increased the incidence of this leaf loss is of importance for Disa growers.

Differences between the two hybrids are likely to be of genetic origin, mainly as part of adaptation to environmental conditions in their natural habitat. Except for *D.uniflora* backcrossing in *D.kewensis* involved *D.tripetaloides* and in *D.unidiorosa* involved *D.racemosa*. *D.tripetaloides* is found to grow mostly on the banks of perrenial streams, while *D.racemosa* inhabits marshy seepage areas rather than streamsides and waterfalls (Vogelpoel, 1983). The conversion of ammonium-N to nitrate by microbial action is slow in acid soils and plants adapted to these conditions may be tolerant to ammonium (Cooke, 1982).

The fact that the vegetative growth of the two tested Disa species was best at relatively high ammonium levels is an indication that the nutritional needs of the evergreen Disas may differ substantially from the rest of the cultivated orchids, regarding their N-source needs. According to De Kreij *et al*. (1999), the standard requirement of $NH_4$ for Cymbidiums is 10 to 20%.

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# CHAPTER 3

**Influence of electrical conductivity and irrigation method on the growth of two evergreen Disa hybrids (*Disa unidiorosa* and *D. kewensis*).**

## ABSTRACT

The evergreen Disa species is the largest indigenous orchid group in Southern Africa, forming a unique part of the world's orchid flora. The evergreen Disas have enormous potential to be marketed as cutflowers or potplants. However, production techniques need to be further investigated before this potential can be utilised. In this study, different concentrations of a standard Cymbidium nutrient solution were applied to two Disa hybrids. Different nutrient solution electrical conductivity (EC) levels were provided for two Disa hybrids using two irrigation methods. The plants were cultivated in a controlled environment and growth was evaluated after 294 days. Results showed that high EC levels produced heavier mother plants, with a bigger root:shoot ratio and a bigger stem diameter. Irrigation methods affected the biomass accumulation of plants and was involved in interactions with EC levels and hybrids, affecting total leaf loss. *Disa unidiorosa* produced heavier and longer plants compared to *D.kewensis*.

# INTRODUCTION

The evergreen Disa species are indigenous orchids which comprise of *Disa uniflora*, *D.tripetaloides*, *D.aurata*, *D.cardinalis*, *D.racemosa*, *D.venosa* and *D.caulescens*. *Disa uniflora*, although difficult to grow, is frequently cultivated in the Western Cape and abroad. Because of the comparatively easy cultivation and showy flowers of the evergreen Disas these orchids are highly desirable subjects (Linder & Kurzweil, 1999).

A brief glimpse at the history of Disa growing emphasizes that no grower can make progress until the cultural requirements are understood and applied. Plants die fast if these requirements are neglected, but are rewarding when given the correct growing conditions (Vogelpoel, 2001). Successful cultivation is based upon a thorough grasp of the characteristics of the natural habitat, growth requirements and growth/dormancy cycles (Truter, 1994).

In nature *Disa uniflora* is confined to the sides of perennial streams, waterfalls and rockpools in the mountains of the South Western Cape (Vogelpoel, 1983; Kurzweil, 2003). Disas grow in dense colonies just above the mid-summer water level, during winter they are exposed to heavy rains and often completely submerged in running water (Vogelpoel, 1983). In summer the water level sometimes sink below the plants, but their tubers never really dry out (Eliovson, 1984). Nutrients are provided by decaying humus, bird and animal droppings and some trace elements are leached from the sandstone rock. Due to the excellent drainage system, these nutrients are continuously supplied in weak concentrations (Vogelpoel, 1983). *D. uniflora* grows in a wide variety of situations and seems to be a very adaptable species (Stewart & Hennessey, 1981). The Disa species which hybridise with *Disa uniflora* can be grown using the same cultural requirements (Vogelpoel, 1983).

According to Du Plessis & Duncan (1989) Disas respond well to feeding, and the general rule is to feed frequently with dilute solutions of liquid fertilisers such as Chemicult (Reg. no.K2025Act36-1947). Successful culture of Disas should be in step with the dramatic seasonal bio-rhythms (Vogelpoel, 1993), thus feeding should be supplied with a reduction during the cold winter months, when plants are almost dormant (Wodrich, 1997). Fertiliser mixtures vary in strength but it is best to avoid those that are too concentrated since Disa leaves are easily scorched by strong

solutions (Vogelpoel, 1983). There are no absolutes, but according to Orchard (2000a) a nutrient solution with an electrical conductivity (EC) of 0.2 mS $cm^{-1}$ is preferable, but an EC of 0.3 mS $cm^{-1}$ is acceptable. However, Wodrich (1999) used feeding concentrations up to a maximum of 0.6 mS $cm^{-1}$. According to Heathcote (2004), the culture media used in flasking have quite high dissolved solids contents, some measures up to 1.6 mS $cm^{-1}$, yet Disas seem to thrive in this.

There are at least three different types of hydroponic systems being used with Disas; the continuous-flow system, ebb-and-flood and aeroponics (Orchard, 2000b). Drip irrigation is one of various types of irrigation systems used in greenhouse production (Savvas & Passam, 2002).

## MATERIAL AND METHODS

### Plant material

Plants were cultivated under 69% shade in a glasshouse at Welgevallen, an experimental farm of the University of Stellenbosch. The temperature inside the glasshouse was regulated at 18°C during the day and 13°C at night, with the average humidity at 50-60%. Plantlets were transplanted on 17/06/2003 and harvested on 02/04/2004, after 294 days of growth. Plants were grown in 10 cm diameter white plastic containers filled with silica sand, obtained locally from Consol Glass (Pty) Ltd. Three grades of sand were used; firstly a layer of coarse sand (3-6 mm) was placed at the bottom 20% of each pot, then a mixture of 1:1 fine (1 mm): medium sand (1-3 mm) was used to fill up the pot and lastly a final layer of coarse sand was placed on top. Before pots were filled with this medium, a small piece of gauze was placed at the bottom to prevent sand from falling through the drainage holes. Nutrients were supplied at standard ratios using guidelines provided by Naaldwijk for Cymbidiums (De Kreij, Voogt, Van den Bos & Baas, 1999). The composition of the basic nutrient solution (EC = 0.73 mS $cm^{-1}$) was compiled by dissolving (mg $l^{-1}$) $KNO_3$ = 181.8, $K_2SO_4$ = 87, $NH_4H_2PO_4$ = 92, $NH_4NO_3$ = 16, $Ca(NO_3)_2.2H_2O$ = 200, $MgSO_4.7H_2O$ = 184.5, Fe-EDTA = 3.31, manganese sulphate = 2.23, zinc sulphate = 1.20, Solubor = 1.02, copper sulphate = 0.12 and sodium molibdate = 0.07. Irrigation times were at 12h00 in winter and at 11h00 and 13h00 in summer. The pH of the tanks was kept

between 5.5 and 6.5 by addition of $H_2SO_4$ or KOH when needed. General maintenance included spraying of plants every three weeks with fungicides, a mixture of Dithane and Kaptan (1 g $l^{-1}$).

**Treatments**

Four electrical conductivity (EC) levels, two irrigation methods and two cultivars were used in a 4×2×2 factorial experiment. Plants received the same nutrient solution at different dilutions and EC level combinations: 0.25-0.41 mS $cm^{-1}$; 0.25-0.60 mS $cm^{-1}$; 0.44-0.80 mS $cm^{-1}$ and 0.44-1.11 mS $cm^{-1}$. The first EC level in all combinations was provided to plants during the first two months of the trial (in winter) and the second EC level was provided for the remainder of the trial. Plants were irrigated using a closed (re-circulating) irrigation system, with the tanks being cleaned and the contents replaced every two weeks. Half of the plants involved in this trial were irrigated using the 'Ebb-and-Flood' system and the other half using drip irrigation. In the 'Ebb-and-Flood' system, water was allowed to submerge three-quarters of pots before it drained away. Two Disa hybrids, namely *Disa kewensis* and *D.unidiorosa* 'Rosy Face', were used (Genetic composition of hybrids explained in Chapter 2). The growth pattern of both hybrids can be seen in Addendum J.



**Experimental design**

The trial was conducted using a completely randomized design. A single plant was considered as an experimental unit and the treatments were replicated four times. Two-way analyses of variance were performed using SAS version 8.2 (SAS, 1999). Student's t-Least Significant Differences (LSD) was calculated at a 5% significance level to compare treatment means. Shapiro-Wilk's test was performed to test for non-normality (Shapiro & Wilk, 1965). There was no evidence against normality and therefore no transformation was needed.

### Measurements

The following parameters were measured at the beginning and end of the trial: Plant mass, the dimension (diameter and length) of the plant, stem diameter, number of leaves and roots, as well as the diameter and length of leaves and roots. The roots and leaves were counted, while plant diameter was measured as the widest distance between two leaftips and plant length was measured as the distance between the tip of the longest root and the tip of the longest leaf. Stem diameter was measured halfway between the roots and the leaves, while the average length and diameter of both roots and leaves were calculated. Plant growth was monitored every two weeks by means of noting the amount of leaves, the average diameter and length of leaves and the mass of plants. Additional measurements were made at the end of the trial: The mother plant was cut halfway between the roots and the leaves to compare the mass of above-ground- to below-ground plant parts. New plants were separated from the mother plant and cut in a similar way. Where tubers were present their mass and dimensions were measured. To narrow down the amount of parameters measured, some parameters were combined. The following parameters were calculated: Biomass accumulation as a percentage; total root length at the beginning and end; root growth as a percentage; root:shoot ratio for mother plant; root:shoot ratio for newly formed plants; shape of tuber [tuber length/tuber diameter]; single leaf area [leaf length × leaf diameter × 0.745]; plant leaf area [single leaf area × number of leaves] and total leaf loss.

## RESULTS

### Shape and mass of plants

As can be seen in Table 1, *Disa unidiorosa* produced significantly longer plants compared to *D.kewensis*, while the other treatments had no significant effect on plant length. Stem diameter was affected by EC levels, with the 0.44-0.80 mS $cm^{-1}$ and 0.44-1.11 mS $cm^{-1}$ treatments producing significantly larger stem diameters. Irrigation method had no significant effect on the shape of plants (Table 1). Plant diameter was not significantly influenced by any of the treatments in this trial (results not shown).

**Table 1** The effects of different EC levels, irrigation method and hybrids on the shape and mass of evergreen Disa plants.

| **Treatments** | | **Plant dimensions** | |
|---|---|---|---|
| | Total plant mass (g) | Plant length (cm) | Stem diameter (cm) |
| EC (mS $cm^{-1}$) | | | |
| 0.25-0.41 | 36.92a | 20.33a | 1.000b |
| 0.25-0.60 | 24.03a | 16.19a | 0.953b |
| 0.44-0.80 | 35.03a | 22.49a | 1.579a |
| 0.44-1.11 | 32.67a | 20.26a | 1.961a |
| LSD (P = 0.05) | NS | NS | 0.5568 |
| Irrigation method | | | |
| 'Ebb-and-Flood' | 28.67a | 18.07a | 1.234a |
| 'Drip' | 35.45a | 21.35a | 1.445a |
| LSD (P = 0.05) | NS | NS | NS |
| Hybrids | | | |
| *D.unidiorosa* | 37.00a | 23.02a | 1.418a |
| *D.kewensis* | 26.24b | 15.76b | 1.250a |
| LSD (P = 0.05) | 9.781 | 5.061 | NS |

Means followed by the same letters are not significantly different at the 5% probability level.
NS = Not Significant

The two hybrids were significantly different regarding total plant mass, with *D.unidiorosa* producing heavier plants (Table 1). The mother plant was separated from the newly formed plants and weighed separately as shown in Table 2. The mass of the mother plants were significantly higher in the 0.44-0.80 mS $cm^{-1}$ and 0.44-1.11 mS $cm^{-1}$ EC treatments compared to the 0.25-0.60 mS $cm^{-1}$ treatment. The biomass accumulation over the growth period was found to be significantly lower in the 'Ebb-and-Flood' irrigation treatment, with plants receiving drip irrigation gaining more mass over the growth period (Table 2).

**Table 2** The effects of different EC levels, irrigation method and hybrids on the mass of evergreen Disa plants.

| **Treatments** | **Plant mass** | | |
|---|---|---|---|
| | Mother plant (g) | Tuber mass (g) | Biomass accumulation (%) |
| EC (mS $cm^{-1}$) | | | |
| 0.25-0.41 | 16.7ab | 1.3a | 2416a |
| 0.25-0.60 | 7.5b | 1.0a | 1429a |
| 0.44-0.80 | 22.6a | 1.2a | 2148a |
| 0.44-1.11 | 24.4a | 0.7a | 1852a |
| LSD (P = 0.05) | 13.13 | NS | NS |
| Irrigation method | | | |
| 'Ebb-and-Flood' | 13.4a | 1.2a | 1476b |
| 'Drip' | 21.3a | 0.9a | 2412a |
| LSD (P = 0.05) | NS | NS | 742.7 |
| Hybrids | | | |
| *D.unidiorosa* | 19.2a | 1.5a | 2268a |
| *D.kewensis* | 15.2a | 0.4b | 1612a |
| LSD (P = 0.05) | NS | 0.82 | NS |

Means followed by the same letters are not significantly different at the 5% probability level.
NS = Not Significant

A significant interaction (P = 0.05) between irrigation method and EC levels affected the mass of newly formed plants (Figure 1). With the 'Ebb-and-Flood' irrigation method, the mass of newly formed plants was highest at an EC of 0.25-0.41 mS $cm^{-1}$ and lowest at 0.25-0.60 mS $cm^{-1}$. With the drip irrigation method the mass of newly formed plants were highest at an EC of 0.25-0.60 mS $cm^{-1}$ and low at 0.25-0.41 mS $cm^{-1}$. However, the mass of newly formed plants only differed significantly at the 0.25-0.41 mS $cm^{-1}$ treatment using 'Ebb-and-Flood' compared to drip irrigation. The average mass of new daughter plants tended to decrease with increasing concentrations of applied nutrient solutions (Figure 1).

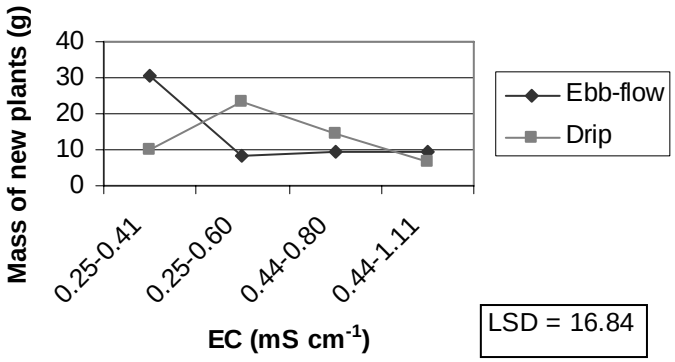


**Figure 1** Mass of newly formed plants affected by an interaction (P = 0.05) between irrigation method and different EC levels.

**Root development**

*Disa unidiorosa* produced heavier tubers than *D.kewensis*, while the other treatments had no effect on tuber mass (Table 2). Regarding tuber shape there were no significant differences between treatments (results not shown). As shown in Table 3 the total root length of plants grown at 0.25-0.41 mS $cm^{-1}$ was significantly greater than plants grown at 0.25-0.60 mS $cm^{-1}$. *D.unidiorosa* had a significantly greater total root length compared to *D.kewensis*. Irrigation method had no significant effect on root development (Table 3), as was also found with the number of roots (results not shown). The root:shoot ratio of mother plants were significantly higher at 0.25-0.60 mS $cm^{-1}$ compared to the 0.44-0.80 mS $cm^{-1}$ and 0.44-1.11 mS $cm^{-1}$ treatments (Table 3). The root mass of mother plants was not significantly affected by any of the treatments, while the leaf mass of mother plants were significantly lower at 0.25-0.60 mS $cm^{-1}$ compared to the 0.44-0.80 mS $cm^{-1}$ and 0.44-1.11 mS $cm^{-1}$ treatments (results not shown). The root:shoot ratio of newly formed plants was significantly influenced by EC level, with 0.25-0.41 mS $cm^{-1}$ producing a significantly higher root:shoot ratio compared to 0.44-0.80 mS $cm^{-1}$ and 0.44-1.11 mS $cm^{-1}$ (Table 3).

**Table 3** The effects of different EC levels, irrigation methods and hybrids on the root development of evergreen Disa plants.

| **Treatments** | **Root development** | | |
|---|---|---|---|
| | Total root length (cm) | Root:Shoot ratio of mother plants | Root:Shoot ratio of newly formed plants |
| EC (mS $cm^{-1}$) | | | |
| 0.25-0.41 | 127.7a | 2.01ab | 2.38a |
| 0.25-0.60 | 58.9b | 2.63a | 1.54ab |
| 0.44-0.80 | 92.5ab | 1.25b | 0.94b |
| 0.44-1.11 | 75.4ab | 0.95b | 0.94b |
| LSD (P = 0.05) | 68.76 | 1.310 | 1.148 |
| Irrigation method | | | |
| 'Ebb-and-Flood' | 70.0a | 2.04a | 1.56a |
| 'Drip' | 108.0a | 1.49a | 1.42a |
| LSD (P = 0.05) | NS | NS | NS |
| Hybrids | | | |
| *D.unidiorosa* | 119.5a | 2.04a | 1.67a |
| *D.kewensis* | 52.9b | 1.40a | 1.27a |
| LSD (P = 0.05) | 48.74 | NS | NS |

Means followed by the same letters are not significantly different at the 5% probability level.
NS = Not Significant

Root diameter was influenced by a significant interaction (P = 0.05) between EC levels and hybrids (Figure 2). *D.unidiorosa* had a significantly bigger root diameter at EC levels of 0.44-0.80 mS $cm^{-1}$ and 0.44-1.11 mS $cm^{-1}$, compared to *D.kewensis*. At lower EC levels the two cultivars showed no significant differences.

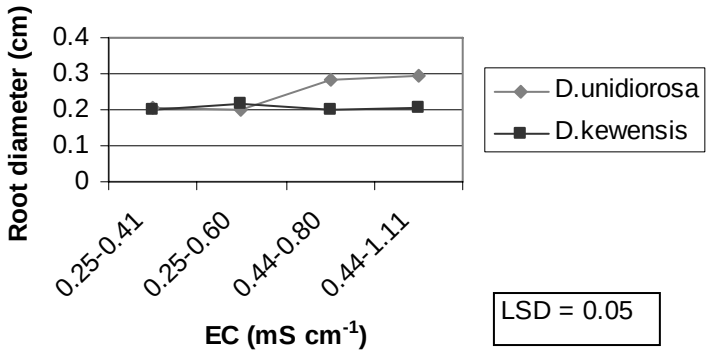


**Figure 2** An interaction (P = 0.05) between different EC levels and hybrids affecting the root diameter of Disa plants.

**Leaf development**

Single leaf area was found to be significantly higher with an EC of 0.44-1.11 mS $cm^{-1}$ compared to the 0.25-0.41 mS $cm^{-1}$ and 0.25-0.60 mS $cm^{-1}$ EC levels. The single leaf area was also significantly higher at 0.44-0.80 mS $cm^{-1}$ than at 0.25-0.60 mS $cm^{-1}$. The leaf area per plant was significantly higher at 0.44-0.80 mS $cm^{-1}$ and 0.44-1.11 mS $cm^{-1}$ compared to 0.25-0.60 mS $cm^{-1}$. Irrigation method and hybrids had no significant effect on leaf area (Table 4).

During the trial some lower leaves deteriorated and abscised. This was monitored as plants from some treatments seemed to be more susceptible. Irrigation method had no significant effect on the number of abscised leaves, while significantly more leaves were lost at the 0.44-0.80 mS $cm^{-1}$ and 0.44-1.11 mS $cm^{-1}$ EC treatments compared to the lower EC levels. Significantly more leaves abscised from *D.kewensis* (Table 4).

**Table 4** The effects of different EC levels, irrigation method and hybrids on the leaf development of evergreen Disa plants.

| **Treatments** | **Leaf development** | | |
|---|---|---|---|
| | Single leaf area ($cm^2$) | Plant leaf area ($cm^2$) | Abscised leaves |
| EC (mS $cm^{-1}$) | | | |
| 0.25-0.41 | 2.77bc | 27.8ab | 0.344b |
| 0.25-0.60 | 2.58c | 19.3b | 0.379b |
| 0.44-0.80 | 3.39ab | 34.4a | 0.483a |
| 0.44-1.11 | 3.73a | 39.3a | 0.523a |
| LSD (P = 0.05) | 0.664 | 12.25 | 0.0945 |
| Irrigation method | | | |
| 'Ebb-and-Flood' | 2.97a | 26.1a | 0.414a |
| 'Drip' | 3.23a | 33.8a | 0.446a |
| LSD (P = 0.05) | NS | NS | NS |
| Hybrids | | | |
| *D.unidiorosa* | 3.26a | 29.6a | 0.331b |
| *D.kewensis* | 2.94a | 30.5a | 0.538a |
| LSD (P = 0.05) | NS | NS | 0.0668 |

Means followed by the same letters are not significantly different at the 5% probability level.
NS = Not Significant

An interaction (P = 0.05) between irrigation method, EC levels and hybrids affected total leaf loss (Figure 3). The total leaf loss of *D.unidiorosa* was not significantly influenced by irrigation method, but *D.kewensis* under drip irrigation produced a significantly higher total leaf loss at 0.25-0.41 mS $cm^{-1}$ and 0.44-0.80 mS $cm^{-1}$ compared to the 'Ebb-and-Flood' irrigation method.

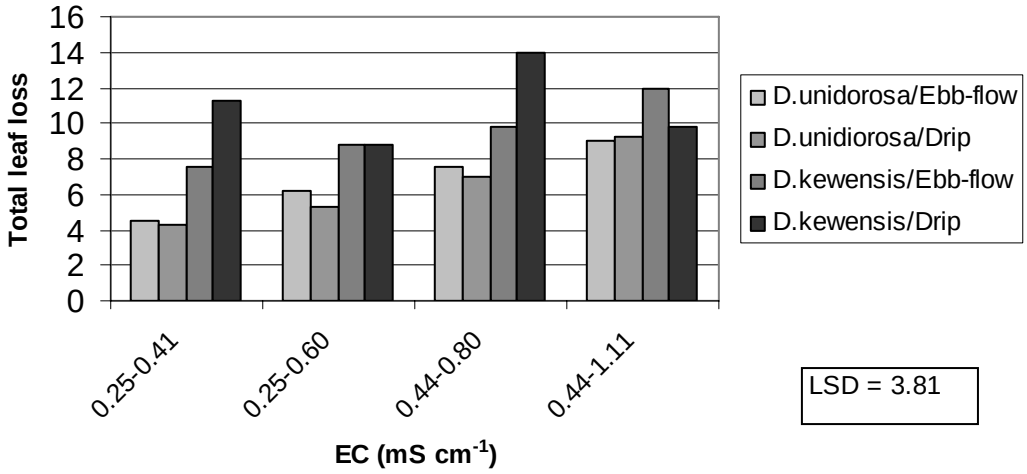


**Figure 3** An interaction (P = 0.05) between different EC levels and irrigation methods affecting total leaf loss of two Disa hybrids.

# DISCUSSION

The only significant difference between irrigation methods was found within the percentage biomass increase, with plants receiving drip irrigation gaining more mass than plants receiving 'Ebb-and-Flood' irrigation (Table 2). According to Jones (1997), 'Ebb-and-Flood' nutrient solution systems are difficult to manage and are very inefficient in its use of water and essential elements. This was also found in this study. Just before the Disa plants were harvested, the daily water use per gram biomass was 0.414 ml $g^{-1}$ for 'Drip, compared to 0.427 ml $g^{-1}$ for the 'Ebb-and-Flood' system. For the homeowner and hobbyist, this system of growing is relatively easy to construct and operate on a small scale and gives reasonably good plant performance with a moderate level of care (Jones, 1997). However, there is a tendency for salt to accumulate in the upper portion of the root zone when using subsurface irrigation, 'Ebb-and-Flood' irrigation. Apart from water losses due to the drying of wet surfaces after irrigations with the 'Ebb-and-Flood' system, this could also have contributed to increase salt concentrations in this study, a possible explanation for the relative poor performance of the 'Ebb-and-Flood' system. Drip irrigation is a surface irrigation system, designed for the low volume delivery of water (Savvas & Passam, 2002). No difference in growth was found between sub irrigated and top irrigated poinsettias (Argo & Biernbaum, 1995). Using drip irrigation for Disa production needed extra care, since Disa plants are sensitive to crown-rot and the drippers had to be checked regularly to prevent water dripping on the crowns of the plants.

Results of this trial showed that Disa plants can tolerate a high EC level, this is in contrast with literature stating that Disas prefer a lower EC value and that Disas can tolerate a maximum of 0.6 mS $cm^{-1}$ (Orchard, 2000a; Wodrich, 1999). For a range of floral crops, the average recommended EC for nutrient solutions is about 1.1 mS $cm^{-1}$ (De Kreij, Voogt & Baas, 1999). The fertilization regime used in Dutch commercial horticulture for *Cymbydiums* includes an EC of 0.4 mS $cm^{-1}$ from April to July and 1.0 mS $cm^{-1}$ for the remainder of the year (De Kreij & Van den Berg, 1990). Anthurium is perhaps the most sensitive flower crop in terms of salinity, and EC values of 0.7 mS $cm^{-1}$ in the applied solution is recommended (Sonneveld & Voogt, 1993).

The trial was ended before the effect of EC could be determined on flower quality. According to Savvas & Passam (2002), the hypothesis that a high EC in the nutrient solution will result in a better product quality does not apply for ornamental plants. Higher EC values reduce the uptake of water and nutrients, particularly calcium (Savvas & Passam, 2002). According to De Kreij & Van den Berg (1990), a high EC gave more spikes per $m^2$, but fewer per shoot and the spikes were shorter in *Cymbidium*.

Schwarz & Grosch (2003) stated that the shoot/root ratio of tomato plants decreases with increasing EC. Opposed to this, our study found that with Disa plants an increase in nutrient solution concentration lowered the root:shoot ratio significantly (Table 3), reduced the production of tubers (Table 2) and reduced the production of daughter plants (Figure 1). According to Wodrich (1999), a high EC increases plant size, flower stem length and general vigour of Disa plants, but that recently deflasked seedlings grow well and even flower in the first year, only to perish during the rest period following flowering. The results of this study is in agreement with the results of Wodrich (1999) and the reduction in tuber production, associated with high EC nutrient solutions, may explain the reduction in regrowth potential in the following season.

During this trial leaves were removed when they turned brown and after closer inspection, the causes of these symptoms were found to be *Cylindrocarpon* sp. *D.kewensis* plants and plants irrigated with a high EC level were more susceptible to this organism.

Differences between the two hybrids are likely to be of genetic origin, as direct result of the breeding criteria used to produce these hybrids, with *D.unidiorosa* having the ability to produce bigger and heavier plants in general. If these hybrids were to be used commercially, *D.unidiorosa* would be an ideal hybrid for cutflower production and *D.kewensis* would be more suitable as a pot plant.

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# CHAPTER 4

**Additional research: Short communications.**

**Part 1: The effect of different nitrogen sources used in foliar feeding at different plant growth stages of *Disa kewensis*.**

## ABSTRACT

The evergreen Disas are highly desirable subjects due to their showy flowers. These plants are indigenous orchids and have enormous potential to be marketed as cut flowers or pot plants, but production techniques need to be further investigated before this potential can be utilised. In this study, the effect of foliar nutrient solution sprays were evaluated at three different plant growth stages of *D.kewensis*. Plants were cultivated in a controlled environment and growth was evaluated after 3 months. Plants were fertigated according to guidelines provided by Naaldwijk for Cymbidiums, but included four foliar spray treatments containing different N-sources (water, complete solution, solution without $NH_4NO_3$, solution without urea). Results showed that vegetative reproducing plants were more susceptible to leaf abscission and formed less leaves compared to small- and flowering plants. Plants sprayed with the complete foliar spray solution and solution without urea produced significantly more leaves than where $NH_4NO_3$ was omitted. Comparing the omission of equal amounts of nitrogen in the form of urea and $NH_4NO_3$, the loss of urea-N had no significant effect compared to a significant reduction in growth with omission of $NH_4NO_3$.

# INTRODUCTION

The evergreen Disas, of which *Disa uniflora* is the most renowned, comprise seven species well known in cultivation and indigenous to the Western Cape. Because of the showy flowers of the evergreen Disas these orchids are highly desirable subjects (Linder & Kurzweil, 1999). A brief glimpse at the history of Disa growing emphasizes that no grower can make progress until the cultural requirements are understood and applied (Vogelpoel, 2001).

According to Du Plessis & Duncan (1989) Disas respond well to feeding, and the general rule is to feed frequently with dilute solutions of liquid fertilisers given either in the form of a foliar spray or directly into the potting medium. At times foliar fertilisers are vital supplements to standard plant nutrition, especially under unfavourable conditions. The salts of both macro- and micro-nutrients are taken up easily by the leaves, thus foliar spraying gives a very quick response (Stancheva, Mitova & Petkova, 2004).

Plants may absorb fertilizer-derived nitrogen in the form of $NO^{3-}$, $NH^{4+}$, urea and amino acids. When comparing different N-sources, the majority of experiments with upland crops indicate that surface-applied urea gives lower yields than ammonium nitrate (Rengel, 1998). The response of plant species or hybrids varies widely, depending on the nitrogen source (Lasa, Frechilla, Aparicio-Tejo & Lamsfus, 2002). It was found that Disa plants prefer to be fertigated with a relatively high percentage of nitrogen in the form of $NH_4$ and that *D.kewensis* grew best at 60% $NH_4$ (Chapter 2). The aim of this study was to determine whether foliar feeding enhances plant growth under normal conditions and to compare the sensitivity of Disa plants at various growth stages towards different N-sources.

# MATERIAL AND METHODS

## Plant material

An evergreen Disa hybrid, *D.kewensis*, was used in this trial. *Disa kewensis* is a primary hybrid resulting from a *D.uniflora* × *D.tripetaloides* crossing. Plants were cultivated under 56% shade in a glasshouse at Welgevallen, an experimental farm of the University of Stellenbosch. The temperature inside the glasshouse was regulated at 18°C during the day and 13°C at night, with the average humidity at 50-60%. Plantlets were transplanted on 15/12/2003 and harvested on 11/03/2004, after 3 months of growth. Plants were grown in 10 cm diameter white plastic containers filled with silica sand, obtained locally from Consol Glass (Pty) Ltd. Three grades of sand were used; firstly a layer of coarse sand (3-6 mm) was placed at the bottom 20% of each pot, then a mixture of 1:1 fine (1 mm): medium (1-3 mm) sand was used to fill the pot and lastly a final layer of coarse sand was placed on top. Before pots were filled with this medium, a small piece of gauze was placed at the bottom to prevent sand from falling through the drainage holes. Pots were sub-irrigated using a standard nutrient solution as prescribed by Naaldwijk for Cymbidiums (De Kreij, Voogt, Van den Bos & Baas, 1999). The basic nutrient solution (EC = 0.73 mS $cm^{-1}$) was compiled by dissolving (mg $l^{-1}$) $KNO_3$ = 181.8, $K_2SO_4$ = 87, $NH_4H_2PO_4$ = 92, $NH_4NO_3$ = 16, $Ca(NO_3)_2.2H_2O$ = 200, $MgSO_4.7H_2O$ = 184.5, Fe-EDTA = 3.31, manganese sulphate = 2.23, zinc sulphate = 1.20, Solubor = 1.02, copper sulphate = 0.12 and sodium molibdate = 0.07. Plants received dilutions of this nutrient solution at an electrical conductivity (EC) of 0.37 mS $cm^{-1}$ and the pH of the tanks was kept between 6.1 - 6.5 by addition of $H_2SO_4$ or KOH when needed. Plants were fertigated with a closed (re-circulating) system using the 'Ebb-and-Flood' method, with the tank being cleaned and the contents replaced every two weeks. At irrigation times, twice daily at 11h00 and 13h00, water slowly rose submerging three-quarters of the pots and was then allowed to drain. General maintenance included spraying plants every three weeks with a mixture (50:50) of Dithane and Kaptan (1 g $l^{-1}$).

**Treatments**

Four foliar feeds and three growth stages were used in a 4×3 factorial experiment. The foliar feeds contained different N-sources and were classified as follows: A control solution (tap water), complete foliar feed (used by Duckitt Nurseries), the complete feed without $NH_4NO_3$ and the complete feed without urea (Table 1). The foliar sprays were applied on a daily basis at 10h00. Plants were also divided into three different growth stages; the first group contained only small plants, the second group consisted of mature flowering plants and the third group of mature plants that did not flower but instead showed signs of vegetative reproduction.

**Table 1** Composition of the four foliar sprays used in this trial (control = tap water, solution 1 = complete feed without $NH_4NO_3$, solution 2 = complete feed without urea, solution 3 = complete foliar feed)

| | Foliar sprays | | | |
|---|---|---|---|---|
| Salt ($mmol_c$) | Control | Solution 1 | Solution 2 | Solution 3 |
| $KNO_3$ | 0 | 0.73 | 0.73 | 0.73 |
| $K_2SO_4$ | 0 | 0.92 | 0.92 | 0.92 |
| $KH_2PO_4$ | 0 | 0.66 | 0.66 | 0.66 |
| $NH_4H_2PO_4$ | 0 | 0.87 | 0.87 | 0.87 |
| $NH_4NO_3$ | 0 | 0 | 10 | 10 |
| $CH_4N_2O$ | 0 | 10.75 | 0 | 13.34 |

**Experimental design**

The trial was conducted using a completely randomized design. Four plants were considered as an experimental unit and the treatments were replicated three times. Two-way analyses of variance were performed using SAS version 8.2 (SAS, 1999). Student's t-Least Significant Differences (LSD) was calculated at a 5% significance level to compare treatment means. Shapiro-Wilk's test was performed to test for non-normality (Shapiro & Wilk, 1965). There was no evidence against normality and therefore no transformation was needed.

**Measurements**

The following parameters were measured at the beginning and end of the trial: The number of leaves was counted; the diameter and length of leaves were measured and the average length and diameter of leaves per plant were calculated. Additional measurements were made at the end of the trial: Plant mass; the mass of above-ground- and below-ground plant parts (plants were cut halfway between the roots and leaves); number of leaves removed and a flowering index was awarded to the plants (1 = plant not going to flower, 2 = plant shows signs of bud formation, 3 = flower bud visible in crown of plant, 4 = flower stem visible, 5 = plant produced a flower). Some parameters were combined to form new parameters: Single leaf area [leaf length × leaf diameter × 0.745]; plant leaf area [single leaf area × number of leaves]; root:shoot ratio of plants; percentage increase in leaf number [(number of leaves (end) – number of leaves (start) + number of leaves removed)/ number of leaves (start)] and percentage abscised leaves [number of leaves removed/ number of leaves (end)].

## RESULTS

The differences between treatments regarding plant dimensions can be seen in Table 2. The plant mass of mature plants was significantly higher compared to the mass of small plants. There were no significant differences between the complete foliar spray and the solution without urea, but both solutions produced significantly heavier plants than the control treatment. The root:shoot ratio of vegetative reproducing plants was significantly higher than the other two stages of growth. A significantly lower root:shoot ratio was found with the complete foliar feed and the solution without urea, while the solution without $NH_4NO_3$ did not differ significantly from the other treatments (Table 2).

**Table 2** The effects of different N-sources, used as foliar sprays, on the plant dimensions of evergreen Disa plants during three different growth stages.

| **Treatments** | **Plant dimensions** | | |
|---|---|---|---|
| | Plant mass (g) | Root:Shoot ratio | Increase in leaf number (%) |
| Stages of growth | | | |
| Small plants | 5.89b | 2.56b | 81.54a |
| Flowering plants | 18.15a | 2.44b | 96.77a |
| Vegetative reproducing plants | 18.51a | 3.32a | 56.90b |
| LSD (P = 0.05) | 4.617 | 0.678 | 15.500 |
| Foliar sprays | | | |
| Control | 10.02b | 3.45a | 60.66b |
| Solution without $NH_4NO_3$ | 12.66ab | 2.74ab | 67.71b |
| Solution without Urea | 16.48a | 2.54b | 92.99a |
| Complete solution | 17.76a | 2.25b | 110.25a |
| LSD (P = 0.05) | 5.325 | 0.782 | 17.991 |

Means followed by the same letters are not significantly different at the 5% probability level.

Plants reproducing vegetatively formed significantly less leaves than plants in the other growth stages, while plants sprayed with the complete solution and solution without urea produced significantly more leaves compared to the other foliar spray treatments (Table 2).

As can be seen in Table 3, both the single leaf area and plant leaf area were significantly higher in mature plants than in small plants. Different foliar spray treatments did not affect the single leaf area, but the total leaf area of plants receiving the complete solution was significantly higher than the solution without $NH_4NO_3$ and the control treatment (Table 3). This was due to a significantly lower number of leaves in the control treatment, compared to the complete solution and the solution without urea (results not shown). The total leaf area of plants receiving the solution without urea was not significantly different from the other treatments (Table 3).

During the trial some lower leaves deteriorated and abscised. This was monitored as plants from some treatments seemed to be more susceptible. The foliar spray treatments had no significant effect on the number of abscised leaves, while significantly more leaves abscised from vegetative reproducing plants compared to the other growth stages (Table 3). Small plants were found to have a significantly lower flower index when compared to mature plants (results not shown).

**Table 3** Leaf properties of Disa plants at three different growth stages, after receiving additional foliar feed treatments for three months.

| **Treatments** | Single leaf area ($cm^2$) | Plant leaf area ($cm^2$) | Abscised leaves (%) |
|---|---|---|---|
| Stages of growth | | | |
| Small plants | 1.86b | 25.6b | 23.24b |
| Flowering plants | 2.98a | 66.0a | 26.81b |
| Vegetative reproducing plants | 2.62a | 67.6a | 39.08a |
| LSD (P = 0.05) | 0.605 | 15.39 | 9.727 |
| Foliar sprays | | | |
| Control | 2.27a | 38.2b | 33.73a |
| Solution without $NH_4NO_3$ | 2.43a | 48.1b | 31.57a |
| Solution without Urea | 2.46a | 55.7ab | 25.07a |
| Complete solution | 2.84a | 70.9a | 24.74a |
| LSD (P = 0.05) | NS | 17.75 | NS |

Means followed by the same letters are not significantly different at the 5% probability level.
NS = Not Significant



## DISCUSSION

At the beginning of the trial the three growth stages obviously differed regarding their single leaf area and plant leaf area; with vegetative reproducing plants having the largest leaf area, flowering plants the second largest leaf area and small plants the smallest leaf area (Data not shown). At the end of the trial, vegetative reproducing plants and flowering plants were equal regarding single leaf area and plant leaf area, while small plants still had significantly smaller leaf areas (Table 3). From this, it can be concluded that flowering plants were more positively affected by foliar feeding than the vegetative reproducing plants.

According to Stancheva *et al*. (2004) the highest values of fresh biomass, leaves, stems and roots were observed with foliar feeding applied at budding and flowering

stages of garden beans. The absorption, translocation and assimilation of urea, nitrate and ammonium were measured in tomato plants within 24 hours after $^{15}N$ labelled compounds were applied at four different growth stages. Results showed that at the reproductive growth stage of tomatoes, the absorption, translocation and assimilation of hydroponically applied urea were greatly improved compared to the seedling stage (Tan, Ikeda & Oda, 2002).

In this trial foliar application of the complete solution resulted in a bigger plant leaf area, as a result of the significantly higher increase in leaf number, compared to the control solution and the solution without $NH_4NO_3$. Plants seemed to benefit more by the presence of $NH_4NO_3$ than urea. According to Cooke (1982), urea sprayed on the leaves has proved to be an effective way of getting nitrogen into apple trees in grassed-down orchards where crops often suffer from nitrogen deficiency. Although the Disa control plants were daily sub-irrigated with nutrient solution, significant plant mass (Table 2) and total leaf area (Table 3) increases occurred where extra ammonium nitrate was applied (solution without urea and complete solution). Clearer results could have been obtained if plants were not sub-fertigated and only received nutrients as foliar spray treatments.

Plants that received foliar sprays containing ammonium nitrate had lower root:shoot ratios. This did not significantly affect the flower index, but the regrowth of plants could have been detrimentally affected. Mother plants with a low root:shoot ratio produced less daughter plants (Chapter 3). Some leaves abscised from plants during this trial and the cause was found to be *Cylindrocarpon* sp. Vegetative reproducing plants were more susceptible to this organism, although it is not known whether the organism was saprophytic or parasitic in this case. Vegetative reproducing plants had a significantly higher root:shoot ratio and formed less leaves compared to the other growth stages. This growth stage seems to be important in nature but is not needed for greenhouse production. The root:shoot ratio can be lowered with high levels of N, especially when given as $NH_4NO_3$, rather than urea

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**Part 2: The effect of different sphagnum peat moss substrates on the root growth of two *Disa uniflora* hybrids ('Cape Salmon' and 'Kalahari Sunset').**

## ABSTRACT

Although the Disa genus accomodates more than 130 species, by far the most commonly grown is *Disa uniflora* and hybrids stemming from this species. The evergreen Disas have enormous potential to be marketed as cut flowers or pot plants, but production techniques need to be further investigated before this potential can be utilised. In this trial plantlets were 'hardened-off' in a controlled environment and growth was evaluated after four months. Plants were fertigated according to guidelines provided by Naaldwijk for Cymbidiums using overhead irrigation. Two peat moss types were devided into four moss:sand mixtures and two *D.uniflora* hybrids, namely 'Cape Salmon' and 'Kalahari Sunset', were used. There were no significant differences in root development between the substrates, but plants accumulated more biomass in the 50:50 (mixed) NZ-sphagnum moss:sand treatments compared to the green moss:sand mixtures. The two *D.uniflora* hybrids were not significantly different.

# INTRODUCTION

*Disa uniflora* is the best known of the evergreen group of Disas (Northen, 1970). *D. uniflora* has been described as the 'Pride of Table Mountain', but also as the 'Ghost flower' because of the notorious difficulty experienced in its cultivation (Cywes, 1990). Disas have great potential as cut flowers and pot plants, as they flower during the summer months when many other orchids are in vegetative growth (Linder & Kurzweil, 1999).

A brief glimpse at the history of Disa growing emphasizes that no grower can make progress until the cultural requirements are understood and applied. These plants die fast if these requirements are neglected, but are rewarding when given the correct growing conditions (Vogelpoel, 2001). Successful cultivation is based upon a thorough grasp of the characteristics of the natural habitat, growth requirements and growth/dormancy cycles (Truter, 1994). In nature Disas grow in various media such as sand and moss, where water is constantly moving through the root system and good drainage ensures adequate aeration (Vogelpoel, 1983). *D. uniflora* grows in a wide variety of situations and is clearly a very adaptable species (Stewart & Hennessey, 1981). The Disa species which hybridise with *Disa uniflora* can be grown using the same cultural requirements (Vogelpoel, 1983).

A variety of substrates can be used to grow Disas hydroponically (Orchard, 2000). The choice of substrate depends firstly on the requirements of the plant and the cultivation system (Wever, 2001). A porous medium consisting of thoroughly washed, medium to coarse river sand or live sphagnum, free of decaying organic matter has been found best, from the point of convenience and availability (Vogelpoel, 1983).

In this study, different substrates were evaluated during a four month 'hardening-off' period, following the deflasking of tissue cultured plantlets.

# MATERIAL AND METHODS

## Plant material

Tissue cultured plantlets were transplanted into a seedling tray and placed under 80% shade in a glasshouse at Welgevallen, an experimental farm of the University of Stellenbosch. The temperature inside the glasshouse was regulated at 18°C during the day and 13°C at night, with the average humidity at 50-60%. Plantlets were purchased from ALBA labs (Pty) and transplanted on 26/03/2004; they were harvested after 4 months of 'hardening-off' (on 26/07/2004). During the first week of the trial plants were sprayed twice with nutrient solution at an electrical conductivity (EC) of 0.73 mS $cm^{-1}$, while for the remainder of the trial plants received the nutrient solution with daily irrigations at an EC of 0.34 mS $cm^{-1}$. Nutrients were supplied at standard ratios using guidelines provided by Naaldwijk for Cymbidiums (De Kreij, Voogt, Van den Bos & Baas, 1999). The composition of the basic nutrient solution (EC = 0.73 mS $cm^{-1}$) was described in Chapter 4.1. Plants were irrigated daily at 12h00 by means of an overhead sprinkler system, while the pH of the tank varied between 7.1 and 7.6. General maintenance included spraying of plants every three weeks with a 50:50 mixture of Dithane and Kaptan (1 g $l^{-1}$).



## Treatments

Eight substrates and two hybrids were used in an 8×2 factorial experiment. Two *Disa uniflora* hybrids, namely 'Cape Salmon' and 'Kalahari Sunset', were used, while the eight substrates consisted of different sphagnum peat moss and sand (1-3 mm) mixtures. Two sphagnum peat moss types were used ['Green moss' and 'New-Zealand sphagnum (petal) moss'], in four different mixtures [50% moss:50% sand (not mixed – with sand in the bottom half of the pot and sphagnum moss in the top half), 75% moss:25% sand (mixed), 50% moss:50% sand (mixed), 25% moss:75% sand (with sphagnum placed on top, around the plant)]. Differences between water extracts from the two sphagnum peat moss types can be seen in Table 1.

**Table 1** Extracts from substrates (1:1.5 Volume extract method) showing differences between the two sphagnum peat moss types used in this trial (Data supplied by: Production Technology Laboratory, Department Agriculture, Elsenburg).

| Substrate contents | Green moss | NZ-sphagnum moss |
|---|---|---|
| pH | 4.5 | 5.0 |
| Conductivity (mS $cm^{-1}$) | 0.59 | 0.10 |
| Nitrate (meq $l^{-1}$) | 0.21 | 0.32 |
| Chloride (meq $l^{-1}$) | 1.40 | 0.28 |
| Sulphate (meq $l^{-1}$) | 2.85 | 0.09 |
| Bicarbonate (meq $l^{-1}$) | 0.05 | 0.10 |
| Phosphorus (meq $l^{-1}$) | 0.87 | 0.02 |
| Ammonium (meq $l^{-1}$) | 0.01 | 0.22 |
| Potassium (meq $l^{-1}$) | 3.37 | 0.15 |
| Sodium (meq $l^{-1}$) | 0.55 | 0.20 |
| Calcium (meq $l^{-1}$) | 0.72 | 0.10 |
| Magnesium (meq $l^{-1}$) | 1.12 | 0.12 |
| Cations (meq $l^{-1}$) | 5.77 | 0.79 |
| Anions (meq $l^{-1}$) | 5.38 | 0.81 |
| Iron (mg $l^{-1}$) | 1.31 | 0.09 |
| Manganese (mg $l^{-1}$) | 0.59 | 0.02 |
| Zinc (mg $l^{-1}$) | 0.14 | 0.03 |
| Boron (mg $l^{-1}$) | 0.06 | 0.21 |
| Copper (mg $l^{-1}$) | 0.04 | 0.01 |

**Experimental design**

The trial was conducted using a completely randomized design. A single plant was considered as an experimental unit and the treatments were replicated eight times. Two-way analyses of variance were performed using SAS version 8.2 (SAS, 1999). Student's t-Least Significant Differences (LSD) was calculated at a 5% significance level to compare treatment means. Shapiro-Wilk's test was performed to test for non-normality (Shapiro & Wilk, 1965). There was no evidence against normality and therefore no transformation was needed.

**Measurements**

Plant mass was measured at the beginning and end of the trial, while additional measurements were made at the end of the trial: The number, length and diameter of roots were measured; the average for every plant calculated and the mortality rate was noted. The root measurements were combined to form a new parameter: Root index (root length × root diameter × number of roots).



## RESULTS

As can be seen in Table 2 the biomass accumulation of plants in the green moss and sand mixtures were not significantly different from each other, while plants in the 75:25 (mixed) and 50:50 (mixed) NZ-sphagnum moss and sand mixtures accumulated significantly more biomass than the 25:75 (not mixed) NZ-sphagnum moss treatment. When the two sphagnum peat moss types are compared, plants in the 50:50 (mixed) NZ-sphagnum moss treatments accumulated significantly more biomass than the four green moss:sand mixtures. The 50:50 (not mixed) NZ-sphagnum moss treatment did not differ significantly from the other medium treatments in this trial. The root index of plants was not significantly affected by the different substrates due to no differences in the number, diameter and length of roots.

The two hybrids were not significantly different (Table 2) except for the mortality rate, which was 37.5% for 'Kalahari Sunset' and 76.6% for 'Cape Salmon' (results not shown).

**Table 2** The effect of different sphagnum peat moss substrates on the biomass accumulation and root development of two *D.uniflora* hybrids.

| **Treatments** | Biomass accumulation (g) | Root index ($cm^2$) |
|---|---|---|
| Substrates | | |
| Green moss:sand (%) | | |
| 50:50 (not mixed) | -0.403c | 0.351a |
| 75:25 (mixed) | -0.186bc | 0.864a |
| 50:50 (mixed) | -0.356c | 0.833a |
| 25:75 (not mixed) | -0.124bc | 0.543a |
| NZ-sphagnum moss:sand (%) | | |
| 50:50 (not mixed) | 0.238abc | 1.077a |
| 75:25 (mixed) | 0.582ab | 1.047a |
| 50:50 (mixed) | 0.808a | 1.620a |
| 25:75 (not mixed) | -0.332c | 0.560a |
| LSD (P = 0.05) | 0.8874 | NS |
| Hybrids | | |
| 'Kalahari Sunset' | 0.065a | 0.883a |
| 'Cape Salmon' | 0.390a | 1.107a |
| LSD (P = 0.05) | NS | NS |

Means followed by the same letters are not significantly different at the 5% probability level.
NS = Not Significant

## DISCUSSION

Results showed that, in terms of biomass accumulation, NZ-sphagnum moss:sand mixtures gave the best results (Table 2). The average mortality rate of plants in the NZ-sphagnum moss:sand mixtures (48.4%) were also found to be lower in comparison with the Green moss:sand mixtures (65.6%). The green moss was identified as *Funaria hygrometrica*.

According to Savvas & Passam (2002) sphagnum moss is the most desirable form of organic matter for the preparation of growth media. Sphagnum peat moss contains a fibrous structure and has a very high surface charge density and cation exchange capacity, which helps to reduce leaching of applied nutrients. Sphagnum is usually characterised by an acidic pH, which may be an advantage for some acid-loving plants (Savvas & Passam, 2002). In our study pH could not have been a contributing factor (Table 1).

According to Jones (1997) particle size and distribution in a soilless organic mix are important, as they determine both the water-holding capacity and aeration of the mix. In agreement with this statement, it was found that the biomass accumulation in plants differed between the 50:50 (not mixed), 75:25 (mixed), 50:50 (mixed) and 25:75 (not mixed) treatments (Table 2). The highest mortality rate (81.3%) was found in the 25:75 (not mixed) NZ-sphagnum moss treatment and the lowest mortality rate (25%) in the 50:50 (not mixed) NZ-sphagnum moss treatment. The water-holding capacity of the substrates was: Green moss 30 – 40%, NZ-sphagnum moss 70 – 80% and sand 5 – 10%. Results seem to indicate that Disa plants prefer a substrate with high water-holding capacity. Jones (1997) states that with more than 50% of a mix as sand, mass and a reduced water-holding capacity may be a problem.

Although NZ-sphagnum did extremely well as substrate in our study, literature shows that there are a few negative sides to using sphagnum moss as a substrate. According to Terquem & Parisot (1991), sphagnum moss decomposes quickly, especially in contact with fertilisers. It is also increasingly difficult to find and rather expensive (Cywes, 1990). Financial analysis of substrate culture systems is important in order to decide whether this method of cultivation can be recommended to growers (Savvas & Passam, 2002). It is also important to note that substrate performance is

dependant on environmental conditions, other substrates should thus be considered and tested before sphagnum moss is recommended.

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**Part 3: The effect of different substrates on the growth of *Disa unidiorosa* 'Rosy Face'.**

## ABSTRACT

The evergreen Disa species is the largest indigenous orchid group in Southern Africa, forming a unique part of the world's orchid flora. The evergreen Disas also have enormous potential to be marketed as cutflowers or potplants. However, before this potential can be utilised, production techniques need to be further investigated. This study has evaluated the growth of an evergreen Disa hybrid, *D.unidiorosa*, in different substrates after 222 days. Plants were cultivated in a polycarbonate tunnel equipped with a 'pad-and-fan' system, while they were fertigated according to guidelines provided by Naaldwijk for Cymbidiums. Eight substrates were used in this trial: 'Green moss':sand [50:50 (mixed / not mixed)]; 'New-Zealand sphagnum moss':sand [50:50 (mixed / not mixed)]; Hydroton (with layer acid peat / palm peat on top); and Acid peat:sand [50:50 (normal pH / increased pH)]. Results showed that Hydroton was not suitable for the cultivation of Disa plants, while no differences were found between the green moss:sand and NZ-sphagnum moss:sand mixtures. Plants in the acid peat:sand (normal pH) treatment yielded better results in terms of growth and flowering properties when compared to the acid peat:sand (increased pH) treatment.

# INTRODUCTION

The evergreen Disas, of which *Disa uniflora* is the most renowned, comprise seven species well known in cultivation and indigenous to the Western Cape. These species are interfertile and form the basis of various successful and dynamic breeding programmes (Linder & Kurzweil, 1999; Vogelpoel, 2001). Cultivation of indigenous orchids has recently had a resurgence of interest, particularly as artificially raised plants of the more common species are becoming available (Linder & Kurzweil, 1999).

A brief glimpse at the history of Disa growing emphasizes that no grower can make progress until the cultural requirements are understood and applied (Vogelpoel, 2001). Successful cultivation is based upon a thorough grasp of the characteristics of the natural habitat, growth requirements and growth/dormancy cycles (Truter, 1994). In nature *D.uniflora* grows along stream banks, where the water is tinged brown by the peat content. This species is also found inhabiting the vertical sides of cliff seepage areas, covered in fibrous growth and sphagnum moss (Wodrich, 1997). They grow where water is constantly moving through the root system and good drainage ensures adequate aeration (Vogelpoel, 1983).

A variety of substrates can be used to grow Disas hydroponically (Orchard, 2000). The choice of substrate depends firstly on the requirements of the plant and the cultivation system (Wever, 2001). A porous medium consisting of thoroughly washed, medium to coarse river sand or live sphagnum, free of decaying organic matter has been found best, from the point of convenience and availability (Vogelpoel, 1983). The aim of this study was to determine if substrate choice had any affect on the growth of *Disa unidiorosa* plants.

# MATERIAL AND METHODS

## Plant material

Plantlets from ALBA labs (Pty) were 'hardened-off' for 4 months before they were used in this trial. Plants of the evergreen Disa hybrid, *D.unidiorosa*, were cultivated at Elsenburg in a polycarbonate tunnel, equipped with a 'pad-and-fan' system. Plants were grown in 10 cm diameter brown plastic containers, under 78% shade and were subjected to temperatures ranging between 11.5 °C and 25.5 °C. Plants were transplanted on 07/04/2004 and the trial ended on 15/11/2004, after 222 days of growth. During the first 5 months of the trial plants received nutrient solution at an electrical conductivity (EC) of 0.4 mS $cm^{-1}$, after which the EC was raised to 0.8 mS $cm^{-1}$ and during the last month of the trial plants received an EC of 0.5 mS $cm^{-1}$. Nutrients were supplied at standard ratios using guidelines provided by Naaldwijk for Cymbidiums (De Kreij, Voogt, Van den Bos & Baas, 1999). The composition of the basic nutrient solution was supplied in Chapter 4.1. Plants were fertigated with a closed (re-circulating) system using the 'Ebb-and-Flood' method, with the tank being cleaned and the contents replaced every two weeks. At irrigation time (12h00 daily) water slowly rose submerging three-quarters of the pots and was then allowed to drain. General maintenance included spraying of plants, every three weeks, with a 50:50 mixture of Dithane and Kaptan (1 g $l^{-1}$). Every two weeks tap water was used for an overhead irrigation to remove accumulated salts from the substrates.

## Treatments

In this experiment eight substrates were used as treatments. The substrates consisted of: 50% 'Green moss':50% sand (not mixed, with sand in the bottom half of the pot and moss in the top half); 50% 'Green moss':50% sand (mixed); 50% 'New-Zealand sphagnum (petal) moss':50% sand (not mixed); 50% 'New-Zealand sphagnum (petal) moss':50% sand (mixed); 50% Acid peat:50% sand (normal pH); 50% Acid peat:50% sand (increased pH); Hydroton with a layer of palm peat on top and hydroton with a layer of Acid peat on top. The pH of the acid peat was altered by adding agricultural lime (5.6 g $l^{-1}$).

**Experimental design**

The trial was conducted using a completely randomized design. A single plant was considered as an experimental unit and the treatments were replicated twenty-five times. Two-way analyses of variance were performed using SAS version 8.2 (SAS, 1999). Student's t-Least Significant Differences (LSD) was calculated at a 5% significance level to compare treatment means. Shapiro-Wilk's test was performed to test for non-normality (Shapiro & Wilk, 1965). There was no evidence against normality and therefore no transformation was needed.

**Measurements**

Plant mass was measured at the beginning and end of the trial, while additional measurements were made at the end of the trial: Flower stems were cut (nearest to the crown of leaves) and weighed, plants were awarded a flower index (0 = no flower stem visible, 1 = bud formation visible, 2 = produced flower stem, 3 = plant in flower) and the mortality rate was noted.




## RESULTS

As can be seen in Table 1, there were no significant differences between the green moss:sand and the NZ-sphagnum moss:sand mixtures regarding biomass accumulation. Plants in the four moss and sand mixtures accumulated significantly more biomass compared to plants in the acid peat:sand (increased pH) and hydroton treatments. The green moss:sand (mixed) treatment accumulated more biomass than the hydroton and acid peat:sand treatments. The mortality rate of plants in the hydroton treatments were significantly higher compared to the other treatments (Table 1).

**Table 1** The effect of different substrates on the biomass accumulation and mortality rate of *Disa unidiorosa* plants.

| **Substrates** | Biomass accumulation (g) | Mortality rate |
|---|---|---|
| **Green moss:sand** | | |
| Mixed | 27.98a | 0.00b |
| Not mixed | 26.66ab | 0.04b |
| **NZ-sphagnum moss:sand** | | |
| Mixed | 25.15ab | 0.00b |
| Not mixed | 25.86ab | 0.00b |
| **Hydroton** | | |
| With Palm peat | 6.16d | 0.52a |
| With Acid peat | 8.20cd | 0.40a |
| **Acid peat:sand** | | |
| Normal pH | 21.64b | 0.04b |
| Increased pH | 13.21c | 0.00b |
| LSD (P = 0.05) | 6.107 | 0.152 |

Means followed by the same letters are not significantly different at the 5% probability level.



The effect of different substrates on the flowering properties of *D.unidiorosa* can be seen in Table 2. The flower stem mass of plants in the acid peat:sand (normal pH) treatment was significantly higher compared to the other treatments, except for plants in the green moss:sand (mixed) and NZ-sphagnum moss:sand (not mixed) treatments which were not significantly different from any treatment. The acid peat:sand (normal pH) treatment produced plants with a significantly higher flower index compared to the acid peat:sand (increased pH), green moss:sand (not mixed) and hydroton treatments (Table 2).

**Table 2** Flowering properties of *Disa unidiorosa* as affected by different substrates.

| **Substrates** | Flower stem mass (g) | Flower index |
|---|---|---|
| **Green moss:sand** | | |
| Mixed | 1.08ab | 0.96ab |
| Not mixed | 0.26b | 0.50bcd |
| **NZ-sphagnum moss:sand** | | |
| Mixed | 0.63b | 0.92ab |
| Not mixed | 1.32ab | 0.76abc |
| **Hydroton** | | |
| With Palm peat | 0.36b | 0.17d |
| With Acid peat | 0.54b | 0.33cd |
| **Acid peat:sand** | | |
| Normal pH | 1.96a | 1.04a |
| Increased pH | 0.37b | 0.20d |
| LSD (P = 0.05) | 1.270 | 0.519 |

Means followed by the same letters are not significantly different at the 5% probability level.

## DISCUSSION

Results showed that hydroton is not suitable for the cultivation of Disa plants. Plants in the hydroton treatment accumulated less biomass, had a higher mortality rate and their flowering properties were amongst the lower scoring treatments (Table 1& 2). This is in agreement with literature. According to Orchard (2000) Disas have not done well grown in lightweight clay particles commonly employed in hydroponics, and the presence of clay must be avoided (Vogelpoel, 1983). Results showed that placing a layer of acid peat on top of the hydroton instead of palm peat had no significant effect on the growth or flowering of *D.unidiorosa*.

A preliminary trial done at the same location showed that green moss:sand, NZ-sphagnum:styrofoam and Chilli sphagnum:styrofoam mixtures produced plants with the highest mean volume, while the other substrates yielded poor results (Addendum K). In this trial the green moss:sand and NZ-sphagnum moss:sand mixtures were not significantly different regarding the measured parameters. The mixed moss:sand mixtures were not significantly different compared to the 'not mixed' treatments. Although these substrates yielded good results in terms of growth and flowering properties there are negative factors that need to be considered. According to Terquem & Parisot (1991) sphagnum moss goes off quite quickly, especially in contact with fertilisers. It is also increasingly difficult to find and rather expensive (Cywes, 1990).

Although the acid peat:sand treatments did not accumulate the most biomass, the flowering properties of the acid peat:sand (normal pH) treatment scored the highest and was significantly better than the acid peat:sand (increased pH) treatment. According to Terquem & Parisot (1991) peat tends to accumulate and retain salts, which may expose the plants to damage caused by excess salinity. It also makes compost rather heavy and resists air circulation. A well-drained medium such as sand mixed with palm fibre 10:1 is found best for Disas (Linder & Kurzweil, 1999). Conventionally the pH of Acid peat is altered by adding 5.6 g $l^{-1}$ agricultural lime to a 1:10 Acid peat:sand mixture. Although the same amount of agricultural lime was used in this trial, the substrate consisted of 50% Acid peat. If a 1:10 Acid peat:sand mixture was used the 'increased pH' treatment may have shown better results.

The composition of the compost, although important, is not the sole determining factor in the successful cultivation of orchids; taking into account other cultural imperatives such as light, temperature, fertilisation, etc. (Terquem & Parisot, 1991).

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# CHAPTER 5

## General Discussion and Conclusions

The seven evergreen Disa species: *D.uniflora*, *D.tripetaloides*, *D.aurata*, *D.cardinalis*, *D.racemosa*, *D.venosa* and *D.caulescens*; are indigenous to South Africa. They are terrestrial plants, with underground tuberoids from which a leafy rosette arises. *Disa uniflora* is the best known of the evergreen group of Disas and has been described as the 'Pride of Table Mountain', but also as the 'Ghost Flower' because of the notorious difficulty experienced in its cultivation. Plenty of variation is found regarding the cultural practices used for Disas, mostly because the method of cultivation is not consistent between hobbyists and growers. The evergreen Disa species are cultivated because they are interfertile and form the basis of successful and dynamic breeding programmes. Disas also have great potential as cut flowers and pot plants, as they flower during the summer months when many other orchids are in vegetative growth.

Successful cultivation is based upon knowledge of the natural habitat, growth requirements and growth cycles. These orchids grow on mountain ranges subject to winter rainfall and are found on stream banks, around waterfalls and in other damp areas. Growth in winter is inhibited by environmental factors such as long nights, short days and low temperatures. In spring the period of slow growth is broken and by mid-summer Disas are in full flower. After this the flowerstem and mother plant will have completely withered, while plump new tubers have formed as basis for the production of daughter plants with rapidly growing leaf shoots.

Disas are best grown in cool greenhouses or inside a shadehouse, making sure the structure allows sufficient light penetration, maintains humidity and allows for air circulation. These plants can be grown successfully without resorting to hydroponics, although the latter method of cultivation yielded satisfactory results in our study.

Various substrates can be used to grow Disas, this includes: River sand, sphagnum moss, peat, perlite, vermiculite, coconut fibre, fern fibre and New-Zealand sphagnum moss.

When comparing Green moss:sand and NZ-sphagnum moss:sand mixtures in our study, no differences were found in root growth after deflasking (during the 'hardening-off' phase) and also between the growth of mature plants. A new tendency for Disa growers is to grow plants in an unmixed substrate, with the inorganic component in the bottom half of the pot and the organic component on top. In our study no significant differences were found between mixed and not mixed substrates. Where different percentages of organic matter were added to silica sand it was found that the treatment containing the least organic matter yielded negative results; probably due to the reduced water-holding capacity although this was not measured in our study.

Our study showed that the mortality rate differed between substrates and was significantly higher in hydroton (expanded clay particles of ±3 mm). Flowering properties of plants grown in hydroton were also negatively affected and this substrate is considered to be not suitable for the cultivation of Disa plants. This is in agreement with literature, which states that clay should be avoided. When the pH of an acid peat (50%): sand (50%) mixture was increased, with addition of lime, the growth and flowering of plants were negatively affected, indicating that high pH substrates should also be avoided. Taking into account the acidic environments where Disas grow in nature it seems that Disa plants prefer an acidic substrate. It is important to note that substrate performance may be dependant on other factors and even though NZ-sphagnum produced good results in this study; other substrates should also be considered and tested. The growth of plants seemed to be affected by the stage of decomposition of the organic substrates and this opens the possibility that organic substances affected growth. Future research should provide for substrates to be analysed for physical as well as inorganic and organic substances. Choosing a substrate for cultivation purposes are subject to availability and cost, these factors should therefore also be considered. Guidelines are needed for new growers and thus future research has to include growing Disas in various substrates under different climatic conditions, using different cultivation methods.

According to available literature, an air temperature of 30°C can be tolerated, provided adequate humidity and free air movement is present, while the temperature should never fall below 0°C. Disas prefer a cool environment with a relatively high (50-70%) humidity. A cool root environment ranging from 10°C - 20°C seems to be desirable. In this study cooling of the root medium from 21.4°C to 16.8°C had a negative effect on plant mass and root length, while it had a positive effect on leaf area. However, cooling of the root medium could have been more effective in a conventional greenhouse because the plants were grown in a mechanically cooled glasshouse at relatively low temperatures (13 - 18°C). If Disas are to be commercialised as an ornamental crop, the question arises whether flowering can be manipulated. To consider this we have to investigate if flowering of Disa plants is sensitive to temperature, daylength or is simply the end result of a critical level of biomass accumulation.

Literature states that Disas are best grown with shading of 40 – 50%, but can also be grown in full sun provided they are given some shading during the hottest part of the day. In this study two levels of shading (56% and 69%) were compared but no significant differences on the growth of plants were found.

Water quality is very important where the cultivation of Disas are concerned and according to literature water containing high concentrations of soluble salts should be avoided, while water with a neutral or low pH is ideal. Evergreen terrestrials can be fed weekly, with due allowance made for the plant's seasonal cycle. Feeding should be supplied with a reduction during winter months, when plants are almost dormant. Nutrients given frequently in weak concentration are preferable to heavy dosages at long intervals, while a maximum concentration of 0.6 mS $cm^{-1}$ can be tolerated.

Results showed that Disa plants can tolerate an EC of 0.8 mS $cm^{-1}$. This is in contrast with literature stating that Disas prefer a lower EC value. An increase in nutrient solution concentration was found to lower the root:shoot ratio of plants and also reduced the production of tubers and daughter plants. These results are in agreement with literature, which states that a high EC nutrient solution may reduce the regrowth potential in the following season. The question still arises whether plants grown with a high EC (0.6 – 0.8 mS $cm^{-1}$) are superior to plants grown at a low

EC level (0.3 – 0.4 mS $cm^{-1}$), for regrowth potential can be neglected if it is economically viable to purchase new plants at the beginning of a new season.

In this study it was found that Disa plants prefer to be fertigated with a relatively high percentage of nitrogen in the form of $NH_4$. Most greenhouse crops are grown with 3 – 9% $NH_4$-N, while Disa plants grown with 40 – 60% of the total N supply in ammonium form produced the best growth before flowering in this study. Apart from the apparent beneficial $NH_4^+$-nutritional effect, the associated lower rhizosphere pH could also have contributed to improve growth. This was not measured in our study but should be considered as a parameter in future research.

Plants receiving drip irrigation accumulated more biomass compared to plants receiving 'Ebb-and-Flood' irrigation, but this difference was not big enough to compensate for the extra care needed for the drip system.

In this study Disa plants in different growth stages were compared and potential flowering plants of *D.kewensis* were more positively affected by foliar feeding than the vegetative reproducing plants. The regrowth of plants that received foliar sprays containing ammonium nitrate may also have been reduced. Although vegetative reproducing plants seems to be important in nature it is not needed for greenhouse production. Plants seemed to benefit more by the presence of $NH_4NO_3$ than urea, this agrees with literature where the use of $NH_4NO_3$ is advised as foliar feed. Clearer results could have been obtained in this study if plants were not sub-fertigated (0.37 mS $cm^{-1}$) and only received nutrients as foliar spray treatments.

Results indicate that requirements of plants differ between growth stages. Some cultivation practices also imply this, for instance, some growers lower the amount of nitrogen and adds more phosphorous during the flowering stage of Disa plants, while young plants are given more shade than mature plants. This opens the field of required research even more.

According to literature Disas are best repotted in the autumn, after flowering, when new plants are growing vigorously. Vegetative propagation can be done after stolons, tubers and new plantlets have formed at the base of the dying flowering stem. Tissue culture using certain meristematic tissue can also be used to replicate Disas vegetatively, while sowing of seeds on agar produces strong seedlings at a much

faster rate than sowing on sphagnum or peat. Cross-pollination by hand between species and subsequent propagation by seed is the only way to produce Disa hybrids.

Disa plants and propagules are becoming more readily available to would-be growers. Seed, pollen, flasks and plants are now routinely being traded and exchanged internationally. Many potential growers are discouraged by the bad reputation of Disas in cultivation and are unwilling to persist after initial losses.

Disas are quite susceptible to root-, stem- and crown rotting. *Cylindrocladium*, *Rhizoctonia* and *Cylindrocarpon* had been detected in infected plants. The best safeguard against fungal infection is constant vigilance and the regular use of fungal sprays. All fungicides and insecticides should be in wettable powder form as the cuticle of the leaves can be damaged by solvents. Bacterial Soft Rot (*Erwinia carotovora*) causes leaf spots and necrotic spots in stems and root stocks, which can destroy the whole plant. Growers have reported damage from red spider mite, thrips, greenfly, gallmidge fly, aphids and caterpillars, while snails and slugs seem to be a common problem.

The only disease encountered in our trial was crown rot and the organism involved was identified as *Cylindrocarpon* sp. Whether this organism is parasitic or saprophytic in our conditions is unknown. A mortality of 10% is considered normal by growers. In this study the mortality rate varied between substrates and reached a level as high as 81.3%. *D.kewensis*, plants irrigated with a high EC level as well as plants in the vegetative reproducing stage were more susceptible to *Cylindrocarpon* sp.

Literature states that the Disa species which hybridise with *Disa uniflora* can be grown using the same cultural practices. This study showed that *D.kewensis* and *D.unidiorosa* reacted differently to $NH_4$:$NO_3$ ratios, with *D.unidiorosa* producing the heaviest plants at 40% $NH_4$ while *D.kewensis* produced heavier plants at 60% $NH_4$. Differences between the hybrids are likely to be of genetic origin, mainly as part of adaptation to environmental conditions in their natural habitat. If the hybrids were to be used commercially, *D.unidiorosa* would be an ideal hybrid for cut flower production and *D.kewensis* would be more suitable as a pot plant, mostly because *D.unidiorosa* produced heavier and bigger plants in general.

Research showed that before Disa production can be commercialised; the different hybrids have to be classified as suitable for cut flower or pot plant production because the possibility exists that hybrids differ in terms of cultural requirements.

There are a few limiting factors influencing the cultivation of Disas, such as the high start-up costs involved and the sensitivity of this crop towards water quality. Our study did not investigate the effect of treatments on flower quality or quantity. Shade, for instance, may affect the colour intensity of the flowers. Future research needs to provide for this, because the flower is the product and is thus considered to be relatively more important than the growth of plants. Although our study seems to indicate a poor correlation between biomass accumulation and flower index; future research is needed to correlate the vegetative parameters measured in this study with flowering properties. However, flowers that were produced in this study were of excellent quality with a vase life of 4-6 weeks.

Little scientific information is available on Disa plants; therefore a lot of research is still needed before this ornamental crop can be commercially grown. Certain aspects of commercial production are already in place - a selection of hybrids is available from growers and ALBA labs (Pty). However, other factors still need to be established such as a stable market. A complete nutrient solution for Disas is also needed. Since these plants are shown to be more tolerant towards $NH_4$ than Cymbidiums, they may also differ regarding requirements for the other nutrients.

# ADDENDUM

## A. List of Disas considered to be threatened in Southern Africa

(Linder & Kurzweil, 1999)

Conservation status is indicated using the old IUCN Red Data categories, namely extinct, endangered, vulnerable, rare and indeterminate.

Extinct:
*Disa brevipetala*
*D. ecalcarata*
*D. forcipata*

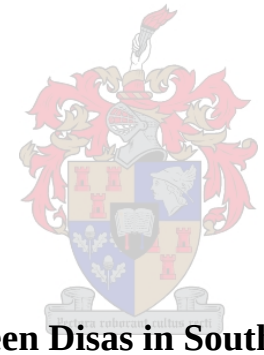
Endangered:
*D. hallackii*
*D. scullyi*
*D. maculomarronina*
*D. physodes*
*D. draconis*
*D. barbata*
*D. lugens* var. *nigrescens*
*D. procera*

Vulnerable:
*D. subtenuicornis*
*D. clavicornis*
*D. macrostachya*
*D. cochlearis*
*D. tenella* subsp. *tenella*
*D. arida*
*D. amoena*
*D. newdigateae*
*D. schlechteriana*
*D. spathulata* subsp. *tripartite*
*D. venusta*
*D. lugens* var. *lugens*
*D. alticola*

Rare:
*D. sankeyi*
*D. neglecta*
*D. introrsa*
*D. longifolia*
*D. cedarbergensis*
*D. ocellata*
*D. galpinii*
*D. tysonii*
*D. extinctoria*
*D. sabulosa*
*D. pygmaea*

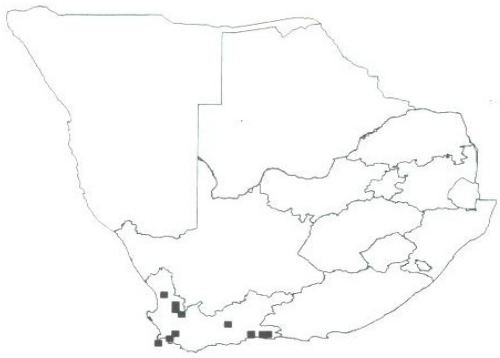
*Disa ovalifolia*
*D. tenuis*
*D. salteri*
*D. marlothii*
*D. brachyceras*
*D. cephalotes* subsp. *frigida*
*D. oreophila* subsp. *erecta*
*D. forficaria*
*D. intermedia*
*D. minor*
*D. bodkinii*
*D. begleyi*
*D. virginalis*

Indeterminate: *D. montana*

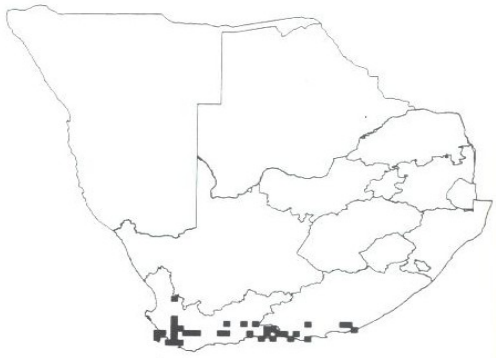


## B. Distribution of Evergreen Disas in South Africa

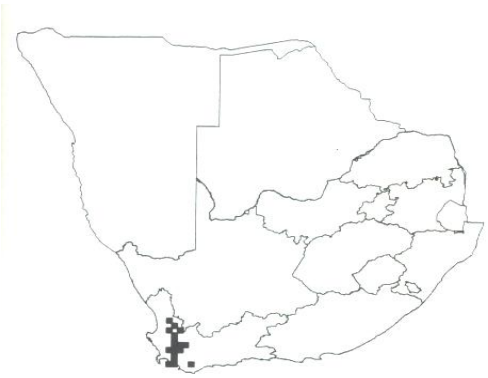
*Disa venosa* occurs in swampy areas in the Western Cape (Figure 4). *Disa racemosa* is widespread and often common in the Western and Southern Cape, generally in the mountains, where populations generally occur in swampy areas. *Disa caulescens* is occasionally found on streambanks, often in half shade, in the mountains of the Western Cape. *Disa tripetaloides* is widespread and locally common in the South-Western, Southern and Eastern Cape, Transkei and Natal, where populations occur on streambanks and on damp mountain slopes. *Disa uniflora* is common along streams and in seepages over cliffs in Western and South-Western Cape, growing in half to full shade or full sunlight *Disa aurata* is restricted to the mountains in the Swellendam area. *Disa cardinalis* is local along streams on the inland slopes of the Langeberg, where plants grow clustered in groups (Stewart *et al*., 1982).



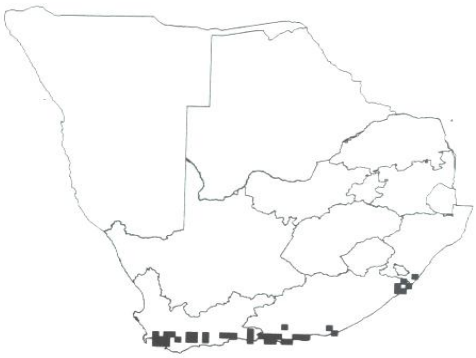
Map 249. *Disa venosa*



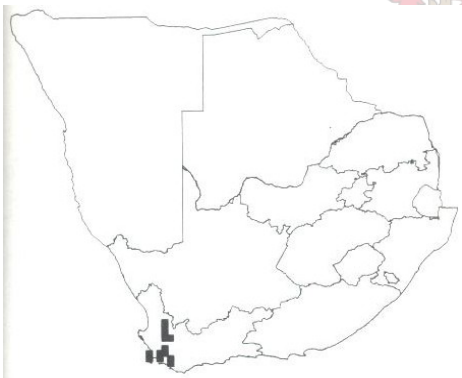
Map 250. *Disa racemosa*



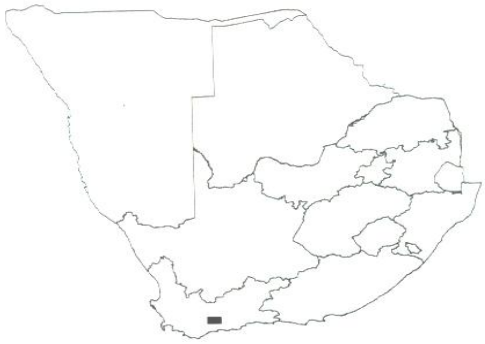
Map 251. *Disa caulescens*



Map 252. *Disa tripetaloides*



Map 248. *Disa uniflora*



Map 253. *Disa aurata*

Map 254. *Disa cardinalis*

**Figure 4** Distribution of the evergreen Disas in South Africa (Linder & Kurzweil, 1999)

## C. Potting procedure

(Vogelpoel, 1983)

The bottom of the 13 cm pot is first covered to a depth of 2-3 cm with polystyrene or stone fragments, then follow a 1 cm layer of coarse pebbles or stone chips of about 5 mm diameter; this in turn is followed by medium to coarse sand 1-2 mm in diameter, until the pot is filed to three-quarters of its depth (Figure 5). This sequence of particle sizes prevents the loss of the finer fractions through the large drainage holes in the pot. The plants can then be positioned and the roots suitably spread, after which the pot is filled with the same grade of sand. The pot is now immersed in water and the contents will settle into place. A 1 cm layer of pebbles about 5 mm in diameter is used to cover the sand, this reduces evaporation from the surface and discourages the growth of algae and moss. Live sphagnum moss provides an alternative to the pebbles; it creates humidity and is an excellent growing medium for Disa roots.

**Figure 5** Growing medium, as discussed in potting procedure (Vogelpoel, 1983).

## D. Sowing procedure

(Vogelpoel, 1983)

A 13 cm pot with ample holes for drainage is filled with clean riversand to a depth of about 4 cm, over this is placed a 2 cm layer of boiled sphagnum moss previously soaked in water (Figure 6). A thin layer of small polystyrene granules can be introduced at the sand/sphagnum interface to ensure good aeration. The prepared seedbed is watered from below, by standing the pot in shallow water for a short time. After allowing excess water to drain from the pot, sprinkle the seed sparsely over the surface; the pot is then covered with thin transparent plastic and kept in place by a rubber band. A few puncture holes in the cover will reduce condensation of moisture in the pot. The water level in the shallow container in which the pot stands must be kept just below the top of the sphagnum layer, otherwise the seedbed will be disturbed. The seedbed must never be allowed to dry out and the pot must not be left standing in stagnant water.

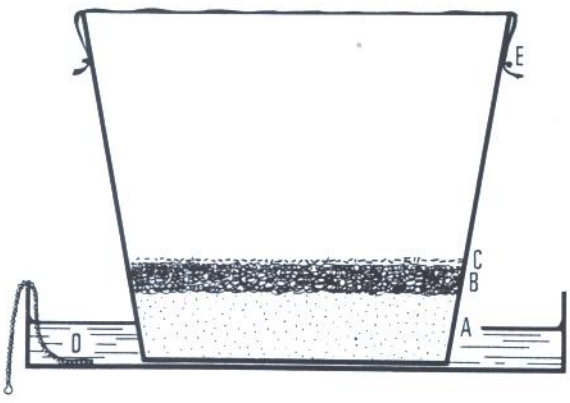



**Figure 6** Seedbed in a 13 cm pot. A. 4 cm clean river sand. B. 2cm layer of boiled sphagnum moss or peat moss. C. Seed on surface of moss. D. Water in shallow container being siphoned off by wick. E. "Gladwrap" cover held in place by rubber band (Vogelpoel, 1983).

## E. Pricking out

(Vogelpoel, 1983)

A 13 cm pot is filled to about three-quarters with coarse washed riversand after which a layer of sterilised peat is added (Figure 7). After dampening the medium, small clumps of approximately five seedlings are pricked out and settled in the new medium. It is advantageous to cover the pots with transparent plastic for a month or so, to ensure high humidity. The cover should be removed when growth is observed. Dilute feeding from below at weekly intervals is necessary, while feeding from above is preferred once the covers are off.

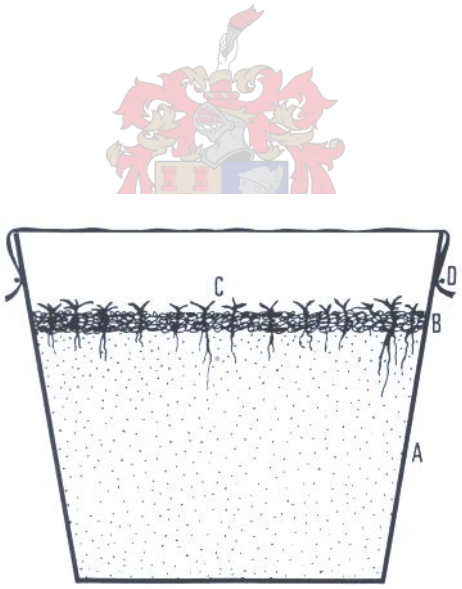



**Figure 7** Community pot: A. 13 cm pot filled to three-quarters with medium to coarse river sand. B. 1 cm layer of either living sphagnum moss or peat moss. C. Young seedlings. D. "Gladwrap" cover (Vogelpoel, 1983).

## F. Quarter strength modified Murashige and Skoog medium

(Wodrich, 1997)

| | Stock solution | Stock solution concentration |
|---|---|---|
| Solution A | $NH_4NO_3$ | 20.625 g/l |
| | $KNO_3$ | 23.75 g/l |
| Solution B | 13% Iron chelate (Kompel) | 2.15 g/l |
| | Or as alternative: | |
| | $Fe_2SO_4$-$7H_2O$ | 1.39 g/l |
| | $Na_2EDTA$-$2H_2O$ | 1.865 g/l |
| Solution C | $CaCl_2$-$2H_2O$ | 22 g/l |
| Solution D | $MgSO_4$-$7H_2O$ | 18.5 g/l |
| | $KH_2PO_4$ | 8.5 g/l |
| | $MnSO_4$-$H_2O$ | 3.38 g/l |
| | $ZnSO_4$-$7H_2O$ | 1.725 g/l |
| | $H_3BO_3$ | 1.24 g/l |
| | KI | 0.166 g/l |
| | $NaMoO_4$-$2H_2O$ | 0.05 g/l |
| | $CoCl_2$-$6H_2O$ | 0.005 g/l |
| | $CuSO_4$-$5H_2O$ | 0.005 g/l |
| Solution E | Nicotinic acid | 0.05 g/ 100 ml 95% ethyl alcohol |
| | Pyridoxine HCl | 0.05 g/ 100 ml 95% ethyl alcohol |
| | Thiamine HCl | 0.01 g/ 100 ml 95% ethyl alcohol |
| 0.08 g | Adenine sulphate | |
| 0.1 g | Myo-inositol | No stock |
| 2 g | Peptone | No stock |
| 20 g | Sucrose | No stock |
| 20 g | Banana pulp | No stock |
| 5 g | Agar | No stock |
| 1000 ml | Distilled water | |

(Adenine sulphate can be removed from the medium, but should be added for tissue culture.)

Make up stock solutions A, B, C, D and E. Stock solutions A, B, C and D can be stored in a cool, dry place or in a refrigerator. Stock solution E should be stored in a freezer.

**Making up the culture medium:**

Add 20 ml of stock solutions A and B, 5 ml of stock solution C and D and 1 ml of stock solution E to 800 ml distilled water. Add the sucrose, myo-inositol, adenine sulphate and peptone and top up the solution to 1000 ml. Adjust the pH to 5.8. Heat the medium, add the agar and stir until all solids are dissolved. Dispense into flasks and sterilise. The pH of the culture medium should lie between 5.2 and 5.4 after sterilisation and should be checked after the flasks have cooled.

The MS medium can be modified for stage one and stage two tissue culture of the evergreen Disas by removing the banana pulp from the medium and adding the following:

| Cytokinin | Stock solution concentration |
|---|---|
| 6 Benzylaminopurine | 0.02 g/ 100ml 95% ethyl alcohol |

(In stage one 2.5 ml of stock solution should be added per litre of culture medium.
In stage two 1 ml of stock solution should be added per litre of culture medium.)

## G. Total radiance as measured, outside the glasshouse, for the time period Jun'03 – Jul'04 at Stellenbosch

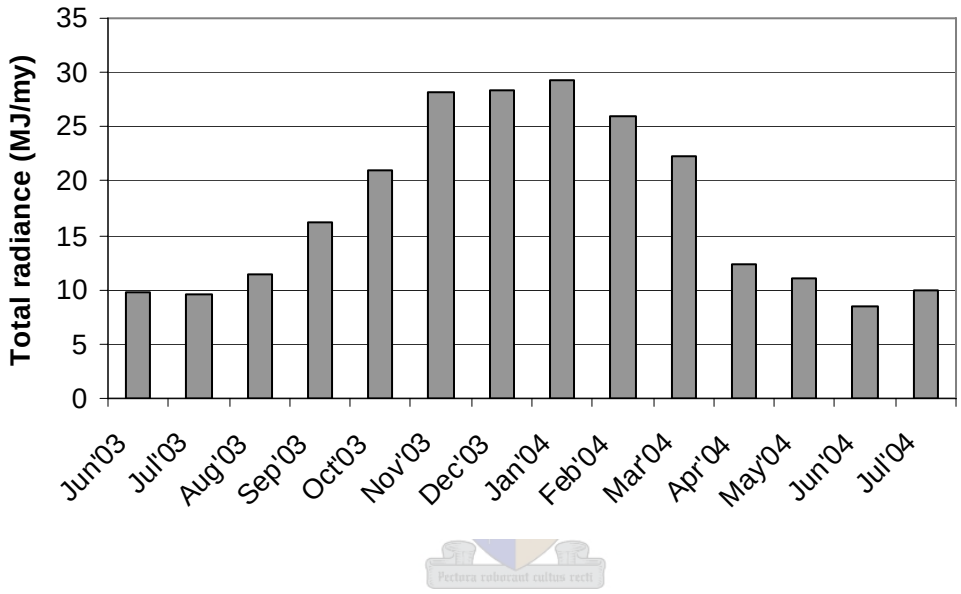



**Figure 8** Total outside radiance measured between June 2003 and July 2004. Plants in the 56% and 69% shade treatments were subjected to 44% and 31% of the total radiance, respectively. In this study plantlets were transplanted on 17/06/2003 and harvested on 08/03/2004, after 266 days of growth.

## H. Cooling of the root medium temperature

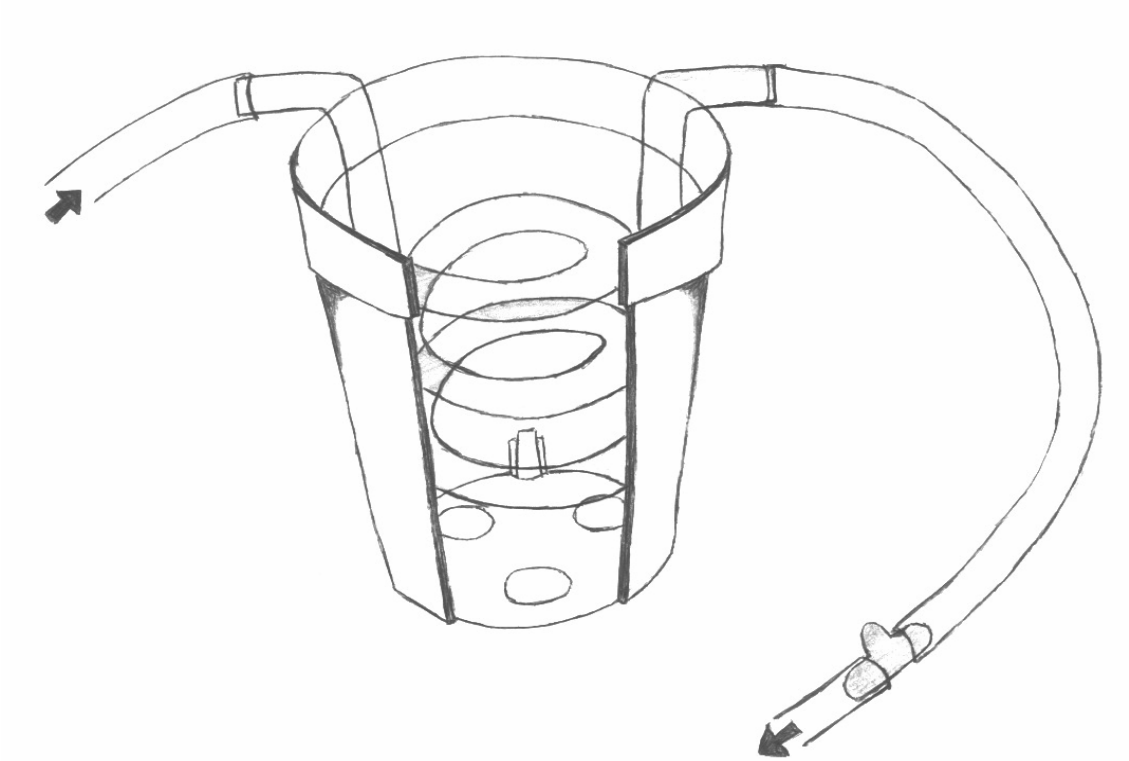


**Figure 9** A diagram showing the technique used to lower the root medium temperature. A glass spiral was placed inside each pot, connected with plastic tubing to each other and then to a water cooler that circulated water through the system.

## I. Differences between *D.kewensis* and *D.unidiorosa*

**Disa kewensis** (top half of page)

**Disa unidiorosa** (bottom half of page)

## J. Growth of the evergreen Disa hybrids used in this study

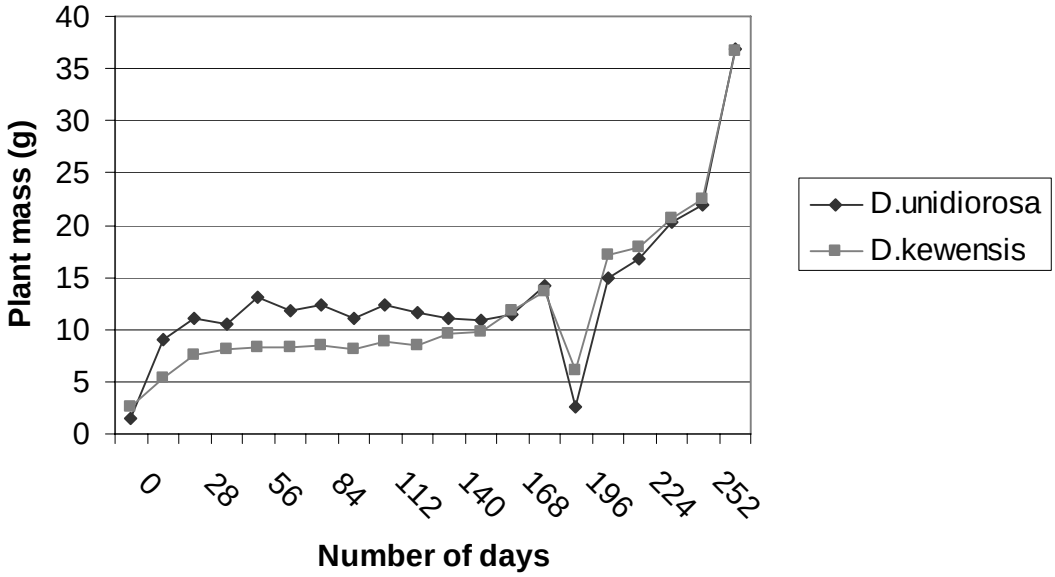



**Figure 10** The growth of *D.kewensis* daughter plants and *D.unidiorosa* plantlets between 17/06/2003 and 02/04/2004, showing the exponential increase in plant mass at the end of the measured growth period (when plants approach their flowering stage). Wilted plants may have caused the reduction in plant mass near 196 days, since the compressor of the glasshouse stopped functioning and had to be replaced. It was found not to have a permanent negative effect on plant mass and the exponential increase in plant mass continued.

## K. Preliminary trial conducted at Elsenburg

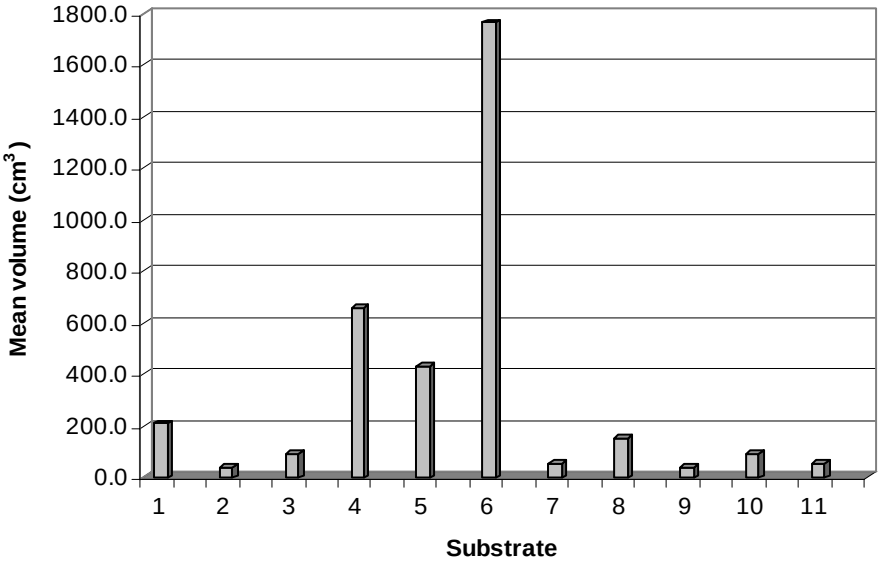



**Figure 11** The effect of different substrates on the mean volume of plants (1 = Canadian peat:sand; 2 = Sawdust; 3 = Cocopeat:sand; 4 = NZ-sphagnum:styrofoam; 5 = Chilli sphagnum:styrofoam; 6 = Green moss:sand; 7 = Sawdust:sand; 8 = Sawdust:styrofoam; 9 = Grape seed; 10 = Sand; 11 = Hydroton).

A formula to obtain the volume of a cone shape object [$V = (d \div 2)^2 \times \pi \times (h \div 2)$] was used to calculate the mean volume of plants; where V = volume of plant, d = diameter of plant and h = height of plant (A cone shape represents the volume of space a plant occupies, including air).

## L. ANOVA's for data used in this study

### 1. Chapter 2

| | | Plant mass | Plant diameter | Plant length | Stem diameter | Root diameter | $Mass_{motherplant}$ | $Mass_{new\ plants}$ |
|---|---|---|---|---|---|---|---|---|
| **Source** | **DF** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** |
| Cultivar | 1 | 0.0933 | 0.2042 | <.0001 | 0.6804 | 0.0666 | 0.0256 | 0.0044 |
| Shade | 1 | 0.8831 | 0.4147 | 0.7473 | 0.5251 | 0.3222 | 0.8210 | 0.9992 |
| Cult*Shade | 1 | 0.3741 | 0.7643 | 0.3923 | 0.7980 | 0.7853 | 0.8254 | 0.3292 |
| $NH_4NO_3$ | 3 | <.0001 | <.0001 | 0.0409 | 0.0019 | <.0001 | 0.0025 | 0.0083 |
| Cult*$NH_4NO_3$ | 3 | 0.0138 | 0.4119 | 0.4176 | 0.1610 | 0.3609 | 0.7519 | 0.0133 |
| Shade*$NH_4NO_3$ | 3 | 0.2730 | 0.8923 | 0.3261 | 0.6284 | 0.0436 | 0.3638 | 0.3306 |
| Cult*Shade*$NH_4NO_3$ | 3 | 0.8072 | 0.3434 | 0.8821 | 0.6453 | 0.6509 | 0.9519 | 0.7324 |
| Temperature | 1 | 0.0954 | 0.1160 | 0.3549 | 0.7031 | 0.6259 | 0.8020 | 0.0842 |
| Cult*Temp | 1 | 0.6425 | 0.3459 | 0.3174 | 0.2584 | 0.1373 | 0.5510 | 0.4200 |
| Shade*Temp | 1 | 0.0717 | 0.1128 | 0.2619 | 0.0466 | 0.2995 | 0.1142 | 0.4520 |
| Cult*Shade*Temp | 1 | 0.4802 | 0.3129 | 0.0311 | 0.2621 | 0.3805 | 0.0495 | 0.5556 |
| $NH_4NO_3$*Temp | 3 | 0.6436 | 0.3005 | 0.2605 | 0.3686 | 0.2890 | 0.1162 | 0.1362 |
| Cult*$NH_4NO_3$*Temp | 3 | 0.5364 | 0.1263 | 0.6396 | 0.7800 | 0.5161 | 0.8490 | 0.7924 |
| Shade*$NH_4NO_3$*Temp | 3 | 0.0849 | 0.1304 | 0.7281 | 0.4923 | 0.1005 | 0.3870 | 0.0309 |
| Cult*Shade*$NH_4NO_3$*Temp | 3 | 0.9639 | 0.2145 | 0.1396 | 0.2359 | 0.3180 | 0.0443 | 0.3154 |

| | | Tuber mass | Biomass accumulation | Total root length | Root:Shoot ratio$_{new\ plants}$ | Tuber shape | Root growth | Leaf length |
|---|---|---|---|---|---|---|---|---|
| **Source** | **DF** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** |
| Cultivar | 1 | <.0001 | 0.1698 | <.0001 | 0.0003 | 0.0072 | 0.0024 | 0.0109 |
| Shade | 1 | 0.8179 | 0.9670 | 0.7657 | 0.2132 | 0.1516 | 0.9229 | 0.2594 |
| Cult*Shade | 1 | 0.4743 | 0.3518 | 0.6924 | 0.3350 | 0.7507 | 0.2296 | 0.8763 |
| $NH_4NO_3$ | 3 | 0.0063 | 0.0008 | 0.0642 | <.0001 | 0.0018 | 0.1196 | 0.0002 |
| Cult*$NH_4NO_3$ | 3 | 0.0578 | 0.1004 | 0.4514 | 0.0024 | 0.0002 | 0.9166 | 0.0567 |
| Shade*$NH_4NO_3$ | 3 | 0.9419 | 0.3510 | 0.2567 | 0.2284 | 0.3127 | 0.8056 | 0.1059 |
| Cult*Shade*$NH_4NO_3$ | 3 | 0.6737 | 0.9134 | 0.9456 | 0.0873 | 0.5566 | 0.6841 | 0.5726 |
| Temperature | 1 | 0.5780 | 0.0549 | 0.0444 | 0.1749 | 0.9752 | 0.0244 | <.0001 |
| Cult*Temp | 1 | 0.0577 | 0.1306 | 0.3941 | 0.0969 | 0.0250 | 0.6467 | 0.2437 |
| Shade*Temp | 1 | 0.4366 | 0.0899 | 0.1168 | 0.2398 | 0.3966 | 0.2598 | 0.9498 |
| Cult*Shade*Temp | 1 | 0.9014 | 0.6945 | 0.0699 | 0.8413 | 0.0481 | 0.2815 | 0.0129 |
| $NH_4NO_3$*Temp | 3 | 0.3442 | 0.2384 | 0.1254 | 0.1536 | 0.0432 | 0.6580 | 0.7682 |
| Cult*$NH_4NO_3$*Temp | 3 | 0.3731 | 0.3422 | 0.6764 | 0.1009 | 0.0123 | 0.8512 | 0.0158 |
| Shade*$NH_4NO_3$*Temp | 3 | 0.1580 | 0.0941 | 0.9053 | 0.6073 | 0.8262 | 0.9926 | 0.0732 |
| Cult*Shade*$NH_4NO_3$*Temp | 3 | 0.6181 | 0.9781 | 0.0090 | 0.8933 | 0.6128 | 0.0979 | 0.0279 |

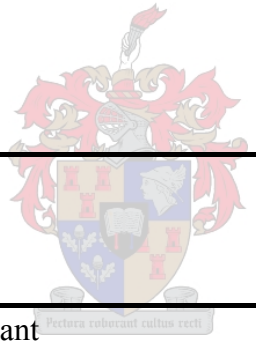
| | | Leaves removed | Single leaf area | Plant leaf area |
|---|---|---|---|---|
| **Source** | **DF** | **Pr > F** | **Pr > F** | **Pr > F** |
| Cultivar | 1 | <.0001 | 0.0842 | 0.0470 |
| Shade | 1 | 0.0580 | 0.9779 | 0.3725 |
| Cult*Shade | 1 | 0.5842 | 0.8184 | 0.5000 |
| $NH_4NO_3$ | 3 | 0.0070 | 0.0001 | <.0001 |
| Cult*$NH_4NO_3$ | 3 | 0.0675 | 0.0564 | 0.0597 |
| Shade*$NH_4NO_3$ | 3 | 0.0339 | 0.5917 | 0.8282 |
| Cult*Shade*$NH_4NO_3$ | 3 | 0.4845 | 0.5014 | 0.6519 |
| Temperature | 1 | 0.7635 | 0.0005 | 0.0002 |
| Cult*Temp | 1 | 0.5986 | 0.6549 | 0.7188 |
| Shade*Temp | 1 | 0.1460 | 0.3198 | 0.0458 |
| Cult*Shade*Temp | 1 | 0.7201 | 0.0757 | 0.0091 |
| $NH_4NO_3$*Temp | 3 | 0.4885 | 0.9620 | 0.3026 |
| Cult*$NH_4NO_3$*Temp | 3 | 0.8843 | 0.1073 | 0.1233 |
| Shade*$NH_4NO_3$*Temp | 3 | 0.6780 | 0.2474 | 0.0384 |
| Cult*Shade*$NH_4NO_3$*Temp | 3 | 0.2783 | 0.3148 | 0.1309 |

**2. Chapter 3**

| Source | DF | Total plant mass Pr > F | Plant length Pr > F | Stem diameter Pr > F | Root diameter Pr > F | $Mass_{motherplant}$ Pr > F | $Mass_{new\ plants}$ Pr > F | Tuber mass Pr > F |
|---|---|---|---|---|---|---|---|---|
| Cultivar | 1 | 0.0318 | 0.0060 | 0.3940 | 0.0063 | 0.3927 | 0.1309 | 0.0094 |
| Irrigation | 1 | 0.1431 | 0.1618 | 0.2705 | 0.5013 | 0.0867 | 0.8464 | 0.5521 |
| Cult*Irrigation | 1 | 0.3512 | 0.7839 | 0.3249 | 0.1064 | 0.3277 | 0.9995 | 0.8373 |
| EC | 3 | 0.2219 | 0.3887 | 0.0017 | 0.0152 | 0.0550 | 0.2100 | 0.6423 |
| Cult*EC | 3 | 0.6566 | 0.8887 | 0.7039 | 0.0209 | 0.6600 | 0.1758 | 0.5137 |
| Irrigation*EC | 3 | 0.7902 | 0.7728 | 0.5418 | 0.1916 | 0.2213 | 0.0308 | 0.2164 |
| Cult*Irrigation*EC | 3 | 0.6965 | 0.3709 | 0.5400 | 0.1643 | 0.7624 | 0.6146 | 0.2453 |

| | | Total root length | Root:Shoot ratio$_{motherplant}$ | Root:Shoot ratio$_{new\ plants}$ | Biomass accumulation | Leaves removed | Single leaf area | Plant leaf area |
|---|---|---|---|---|---|---|---|---|
| **Source** | **DF** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** |
| Cultivar | 1 | 0.0086 | 0.1695 | 0.3347 | 0.0829 | <.0001 | 0.1779 | 0.8269 |
| Irrigation | 1 | 0.0989 | 0.2569 | 0.7614 | 0.0114 | 0.4028 | 0.2531 | 0.0804 |
| Cult*Irrigation | 1 | 0.7524 | 0.7404 | 0.9026 | 0.3003 | 0.1473 | 0.4088 | 0.1841 |
| EC | 3 | 0.1560 | 0.0447 | 0.0337 | 0.2289 | 0.0005 | 0.0042 | 0.0125 |
| Cult*EC | 3 | 0.8885 | 0.4044 | 0.4982 | 0.5994 | 0.7022 | 0.3945 | 0.7290 |
| Irrigation*EC | 3 | 0.1537 | 0.2888 | 0.4310 | 0.3086 | 0.3107 | 0.2564 | 0.9579 |
| Cult*Irrigation*EC | 3 | 0.9185 | 0.4337 | 0.8609 | 0.6683 | 0.3697 | 0.1176 | 0.3310 |

| | | Total leaf loss |
|---|---|---|
| **Source** | **DF** | **Pr > F** |
| Cultivar | 1 | <.0001 |
| Irrigation | 1 | 0.0091 |
| Cult*Irrigation | 1 | <.0001 |
| EC | 3 | <.0001 |
| Cult*EC | 3 | <.0001 |
| Irrigation*EC | 3 | <.0001 |
| Cult*Irrigation*EC | 3 | <.0001 |



**3. Chapter 4.1**

| | | Single leaf area | Plant leaf area | Plant mass | Root:Shoot ratio | Increase in leaf number | Leaves removed |
|---|---|---|---|---|---|---|---|
| **Source** | **DF** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** |
| Growth stage | 2 | 0.0024 | <.0001 | <.0001 | 0.0293 | <.0001 | 0.0002 |
| Foliar feed | 3 | 0.4123 | 0.0066 | 0.0224 | 0.0294 | <.0001 | 0.0858 |
| Growth stage*Foliar feed | 6 | 0.1767 | 0.0920 | 0.2865 | 0.3607 | 0.1864 | 0.2662 |

**4. Chapter 4.2**

| | | Biomass accumulation | Root index |
|---|---|---|---|
| **Source** | **DF** | **Pr > F** | **Pr > F** |
| Cultivar | 1 | 0.1570 | 0.5330 |
| Substrate | 7 | 0.0407 | 0.6472 |
| Cultivar*Substrate | 4 | 0.4919 | 0.3027 |

**5. Chapter 4.3**

| | | Biomass Accumulation | Mortality rate | Flower stem mass | Flower index |
|---|---|---|---|---|---|
| **Source** | **DF** | **Pr > F** | **Pr > F** | **Pr > F** | **Pr > F** |
| Substrate | 7 | <.0001 | <.0001 | 0.0712 | 0.0010 |