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Ex vitro acclimatization of *Cattleya forbesii* and *Laelia purpurata* seedlings in a selection of substrates

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ABSTRACT. Four substrates were compared (peat, no. 2 gravel only, mixture of no. 2 gravel and peat at a 3:1 ratio, and xaxim) for use in *ex vitro* growth of *Cattleya forbesii* and *Laelia purpurata* - Orchidaceae. A substrate x species interaction was observed for the variables vigor and height, showing that each species has its own specificity for substrate. For *C. forbesii*, xaxim placed first in two of the four assessed parameters. Next came the gravel:peat substrates and peat in the second position for two parameters and in third place for two. In last place was gravel, which was in fourth place for all four parameters assessed. For *L. purpurata*, the substrates with the greatest number of parameters in first and second positions were gravel:peat and peat, both with two first positions, one second and one third. Next was xaxim, which had one second position, two third positions and one fourth. As the last came gravel, which had one second position and three fourth positions. Xaxim was the best substrate for *C. forbesii*, but could be replaced, with a minor reduction in performance, by the no. 2 gravel:peat mixture. For *L. purpurata*, the best substrate was no. 2 gravel:peat mixture. The low effectiveness of the no. 2 gravel substrate when compared with no. 2 gravel:peat provides evidence of the importance of organic matter for these orchids.

Keywords: *ex vitro*, damping-off, alternative substrates.

RESUMO. Aclimatização ex-vitro de mudas de *Cattleya forbesii* e *Laelia purpurata* em diferentes substratos. Quatro substratos (turfa, pedra brita nº 2, mistura de turfa: pedra brita nº 2 na proporção 1:3 e xaxim) foram testados sobre o crescimento *ex-vitro* de *Cattleya forbesii* e *Laelia purpurata* – Orquidácea. Foi observada interação significativa entre substrato x espécie para as variáveis vigor e altura, demonstrando que cada espécie tem sua especificidade para o substrato. Para a *C. forbesii*, o xaxim obteve duas primeiras posições em todos os quatro parâmetros avaliados. Em seguida, vieram os substratos turfa:pedra brita nº 2 e a turfa com dois parâmetros ocupando a segunda posição e dois, a terceira. Por último a pedra brita nº 2, que nos quatro parâmetros avaliados, ocupou sempre o último lugar. Para a *L. purpurata* os substratos com maior número de parâmetros nas primeira e segunda posições foram a turfa:pedra brita nº 2 e a turfa, ambos ocupando duas posições em primeiro lugar, uma em segundo e uma em terceiro. Em seguida, teve-se o xaxim, que ocupou uma posição em segundo lugar, dois em terceiro e um em quarto. Por último, teve-se a pedra brita nº 2, que ocupou uma posição em segundo lugar e três em quarto. Xaxim foi o melhor substrato para *C. forbesii*, mas que poderá ser substituído, com pequena desvantagem, pela mistura turfa:pedra brita nº 2. Para a *L. purpurata*, o melhor substrato foi a mistura turfa:pedra brita nº 2. Para *L. purpurata*, o melhor substrato foi a mistura turfa:pedra brita nº 2. A baixa eficiência do substrato de pedra brita nº 2 quando comparado com a turfa:pedra brita nº 2 evidencia a importância da matéria orgânica para estas orquídeas.

Palavras-chave: *ex-vitro*, podridão de mudas, substratos alternativos.

Introduction

Orchids are among the most sought-after ornamental plants by consumers, due to the longevity of their flowers and their exotic and sophisticated symbolism. In Brazil, orchids occupy the second largest total planted area within the category of 'flowering pot plants' (8.3% of the total), with only Chrysanthemums, which are the

best-selling flowers of all, occupying a larger area (15.1% of the total).

Brazilian commercial production of orchids, while still modest, is growing fast and already appears on the industry's export figures as a distinct item, having grown from 0.27% in 2002 to 0.7% in 2005. Importation of orchid seedlings, which are the basic raw material for commercial production,

totaled 128,611 units in 2000, reaching 280,333 in 2004. In 2005, US\$ 90,369.00 was spent on importation of orchid seedlings, accounting for 14.63% of the flower industry's imports for that year. In terms of area under cultivation dedicated to flowers and ornamental plants, the state of Santa Catarina is the second largest producer of ornamental plants (16.9% of the total) and, in 2005, it was the sixth largest in the national market in terms of sales to other Brazilian states, accounting for just 0.68%. The majority of its domestic interstate sales are sent to the states of São Paulo, Rio Grande do Sul and Paraná. In 2005, Santa Catarina state exported orchids worth US\$ 50,580.00 to foreign markets, representing 29.01% of the total value exported from Brazil, the majority (38.34%) of which were sent to Taiwan (MÜLLER; VISCONTI, 2007). Curiously, Taiwan figures among the largest producers of orchids in the world and is one of the countries that hold the most patents on commercial orchid varieties. Brazil is one of largest repositories of orchid genetics, which the law states cannot be removed from their natural habitat and commercialized. With adaptation and/or development of technologies for the mass propagation of Brazilian orchids, legal commercial exploitation could become a possibility.

This paper describes a study conducted with the species *Cattleya forbesii* and *Laelia purpurata* (Figure 1), which are considered to be 'fast growing' when compared with other orchids of the same genus. *C. forbesii* (Figure 1a), flowers from October to December, and produces flowers of around 11 cm in diameter, from green to pale brown in color. *L. purpurata* (Figure 1b), is the state flower of Santa Catarina (Governmental Decree no. 20,829 of December 13, 1983) and the symbol of its capital city Florianópolis (Municipal law no. 7,037, of May 9, 2006). It blossoms from September to December, producing racemes with 3 to 5 flowers of 7 to 10 cm in diameter, with a rare beauty, especially when in groups. From the 1920s to the 1940s, this species was heavily exploited, extracted from its habitat and exported via the city of Florianópolis. Studies of its DNA resulted in it being transferred to the genus *Sophranitis* (VAN DEN BERG; CHASE, 2000; VAN DEN BERG et al., 2000). More recently, with the addition of morphological data, it was once more reassigned to a newly designated genus, the *Hadrolaelia* (CHIRON; CASTRO NETO, 2002). In spite of its new botanical assignment, the old name was kept in this paper due the large use of the name *Laelia purpurata* in commerce and among orchid

growers. It is easily cultivated and has more than 25 groups of cultivars, selected by orchid hobbyists, on the basis of flower color, which can vary from white to dark purple, diversified patterns of venation, color patches, forms, lure effects, thickness of petals and sepals. The availability of preselected genotypes will facilitate its domestication and horticultural exploitation as a flowering pot plant.

The initial phase of the germination process of orchids has been fully mastered (ARDITTI, 1967; ARDITTI, 1982), but published papers on the *ex vitro* phase are rare. Notwithstanding, this knowledge does exist and is kept secret, being considered the most difficult part of orchid cultivation (COLOMBO et al., 2005). During this process, the substrate employed is crucial, but this alone is not enough; correct shade management and irrigation are of extreme importance as well.

In Brazil, the substrate with the best reputation for acclimatization and growth of orchids is xaxim, which is made from the chopped up pseudotrunks of a tree fern (*Dicksonia sellowiana*). It is available in the form of plates, shredded and as a powder (FARIA et al., 2001; LORENZI; SOUZA, 1996). However, since xaxim is the result of extraction from the Brazilian Atlantic Rain Forest, and its exploitation is forbidden by law (National Council for the Environment resolution nº. 278/1 of May 24, 2001), experimental studies comparing its efficiency with alternative substrates have been sought and published.

This paper reports on a study carried out in order to identify alternative substrates to xaxim that could be used for the establishment (*ex vitro* phase) of *C. forbesii* and *L. purpurata* seedlings. The effectiveness of these substrates was evaluated under application of Manzate 800 fungicide.



Figure 1. a) *Cattleya forbesii* cv. Oliva, b) *Laelia purpurata* cv. Tipo.

Material and methods

This research was conducted at the Laboratory of Applied Evolution at the Center of Biological Science at the Federal University of Santa Catarina, Florianópolis, Santa Catarina State Brazil, during the period from April 24 to September 31, 2004.

Two species of orchid were used: *Laelia purpurata* and *Cattleya forbesii*. They were established in four different substrates: a layer of xaxim shredded over a layer of no. 2 gravel, a layer of Fibraflor® (a commercial substrate produced by Florestal S.A., Araranguá, SC, Brazil, made from roughly shredded peat with the capacity to retain water - CWR of 90%) on top of a layer of no. 2 gravel; no. 2 gravel mixed with Fibraflor® peat at the ratio of 3:1; and gravel alone.

Xaxim was used as a control, since it is its non-availability that prompted the study. The peat was used because it has a high organic matter content, good aeration, and is easily acquired on the market. The gravel only substrate was used because it is constantly mentioned on orchid discussion sites as being a good substrate for both *Laelias* and *Cattleyas*. Despite these species being epiphytic orchids, they can also be found growing on rocks in their natural habitat. The mixture of no. 2 gravel:peat was used to prove or disprove the need for organic matter on rocks for these plants to develop. The substrates were analyzed for true density (weight of substrate mL⁻¹), apparent density (weight of substrate in a cylinder of 100 ml mL⁻¹ filled with enough absolute alcohol to complete the cylinder's) and porosity (100 [(true density-apparent density)/true density]) (adapted from EMBRAPA (1997) where a 100 mL cylinder was used instead of a Kopecky ring).

Seedlings of the 2 species being investigated came from the treatments that had provided the best results in germination tests of different *in vitro* culture media carried out at the Applied Evolution Laboratory. These were removed from their *in vitro* cultivation pots, washed to remove the remains of the culture media, pre-selected to assure sample uniformity, and planted in 25 x 40 x 7 cm plastic trays with drainage holes in the base, on April 24, 2004. Three trays of each of the four different substrates were tested for each species, each containing 10 seedlings, spaced out by 2.5 x 3 cm. In total, 24 trays and 240 seedlings were used. The experiment was laid out according to a completely randomized design.

As soon as they had been planted, all seedlings were sprayed with a mixture of foliar fertilizers - Ouro Verde (a commercial fertilizer with NPK content of 6-6-8 made by Empresa Paulista de Produtos Químicos - EPPQ, Santo André, São Paulo State, Brazil), plus Supa-Potássio (a commercial potassium and silicon supplement produced by Agrichem of Brazil, Ribeirão Preto, São Paulo State, Brazil), both applied at a concentration of 2 ml L⁻¹, and combined with Manzate 800

(a commercial fungicide whose active ingredient is Mancozeb, by Du Pont do Brasil S.A.) at a concentration of 1 g L⁻¹. Applications were repeated at 14-day intervals throughout the experiment.

The parameters evaluated were: the height of 5 seedlings, the length of their 3 longest roots, vigor (subjectively evaluated using the grades: 1 = weak, 2 = moderate, 3 = good to excellent), and fresh weight. The experiment was conducted in a greenhouse, covered with a milky plastic capable of blocking out 75% of the light incident upon it, at the Center of Biological Science, Federal University of Santa Catarina.

Analysis of variance was performed, a comparison between averages was made using the T test, and the correlation between variables was evaluated using Pearson's *r* (SOKAL; ROHLF, 1981). Data were analyzed with the aid of the Statistica software package (STATSOFT, 1995).

Results and discussion

It was observed that, when the substrate was taken as the independent factor, there were significant differences in the variables vigor, fresh weight and plant height, while there was no statistical difference between substrates for the variable root length. When the differences between the two species were analyzed, they were significant only for the variable fresh weight. An interaction between substrate and species was observed for the variables vigor and height, showing that each species has its own specificity for substrate (Table 1). Due to this, the following analysis will be carried out considering the species separately.

Table 1. *Cattleya forbesii* and *Laelia purpurata* seedlings in a selection of substrates. Summary of the analysis of variance for all variables.

Factors	Vigor	Fresh weigh	Height	Root lengths
Substrate	****	***	***	n.s
Species	n.s.	****	n.s.	n.s
Substrate by Species	****	n.s.	***	n.s.

Note: n.s = difference not significant at a 5% level of probability. *** = significant difference at a level inferior to 0.01% **** = significant difference at a level inferior to 0.001%.

Comparisons between each of the variables assessed for *C. forbesii* are shown in Table 2 with the means in descending order. Xaxim was in first position for two of the four parameters assessed. In second place came the gravel:peat substrates; peat was in second position for two parameters and in third position for two. In last place was gravel, which was in fourth place for all four parameters assessed. On statistical analysis, there were no significant differences between the substrates for the variable root lengths. For the variables fresh weight

and vigor, only the gravel substrate differed from the others, having lower means. For the variable height, three homogenous groups emerged, in descending order: first, Xaxim and gravel:peat, which did not differ significantly; second, gravel:peat and peat; and third, peat and gravel.

Table 2. *Cattleya forbesii* and *Laelia purpurata* seedlings in a selection of substrates. Summary of the analysis of variance for all variables within species. Averages followed by the same letter have no significant difference between them, at the level of 5%, according to the Tukey test.

Variable	Species			
	<i>C. forbesii</i>		<i>L. purpurata</i>	
	substrate	mean	Substrate	Mean
Vigor (grade)	Xaxim	2.9 a	gravel+peat	2.9 a
	peat	2.9 a	peat	2.9 a
	gravel+peat	2.5 a	Xaxim	2.8 a
	gravel	1.9 b	gravel	1.2 b
Fresh weigh (g)	Xaxim	1.1 a	Peat	1.4 a
	peat	0.8 a	gravel+peat	1.3a
	gravel+peat	0.8 a	Xaxim	1.3 a
	Gravel	0.4 b	gravel	0.5 b
Height (cm)	Xaxim	5.6 a	peat	5.8 a
	gravel+peat	4.8 ab	Xaxim	5.4 a
	peat	4.4 bc	gravel+peat	5.3 a
	gravel	3.7 c	gravel	3.5 b
Root length (cm)	Xaxim	14.9 a	gravel+peat	16.0 a
	gravel+peat	14.7 a	gravel	15.0 a
	peat	13.8 a	peat	13.1 a
	gravel	13.6 a	Xaxim	12.9 a

Comparisons between each of the variables assessed for *L. purpurata* are also shown in Table 2, also with means in decreasing order, in common with the data for *C. forbesii*. The substrates with the greatest number of parameters in first and second positions were gravel:peat and peat, both with 2 first positions, one second and one third. Next was Xaxim, which had one second position, two third positions and one fourth. As the last, came gravel, which had one second position and three fourth positions.

In order to provide an overview of all of the variables studied in conjunction, these were transformed into standard units (Z-scores) (SOKAL; ROHLF, 1981), where all averages per variable within each treatment were centralized on zero and then divided by its standard deviation, thereby transforming them into dimensionless units while preserving the proportional rank distance between them (Figures 2 and 3).

Xaxim was, on average, the highest ranked substrate for *C. forbesii*, but could be substituted by the gravel:peat mixture with a certain loss of performance, indicating that improvements could be achieved through the application of fertilizers such as those used by Nomura et al. (2008), who achieved improvements in almost all of the variables assessed in an experiment

using micropropagated cv. Nanicão (*Musa* spp. AAA) banana plantlets, grown in different substrates and fertilized with two different systems, plus controls, to the extent that the performance of 4 of the 5 substrates tested could be made equal, despite having been distinctly different when tested without fertilizer. There are also probiotic compounds, which can also improve the growth of plants in a range of different substrates, such as those used by Catunda et al. (2008) when acclimatizing pineapple plantlets on Plantmax substrate and a mixture of composted sugarcane bagasse and solid industrial sugar cane residue, where spraying with an analogue of brassinosteroid, BIOBRAS-16 at 0.1 mg L⁻¹, resulted in plants that produced 2.8 times more dry matter than the control cultivated in Plantmax substrate.

For the *L. purpurata* species, the substrates gravel and gravel:peat were the highest ranked, and similar in response for the variables height, fresh weight and vigor, but no. 2 gravel:peat, showed a better root length, an important variable for ornamental plant that usually have to be transported far and so, might be preferred. For this species, xaxim was the third ranked substrate, and clearly possible to be substituted.

Table 3. *Cattleya forbesii* and *Laelia purpurata* seedlings in a selection of substrates. Correlation between evaluated variables.

Evaluated variable for	Vigor	Fresh weigh	Height	Root length
<i>C. forbesii</i>				
Vigor	1.00	0.56***	0.51***	0.26 n.s.
Fresh weigh		1.00	0.69***	0.49***
Height			1.00	0.35***
Root length				1.00
Evaluated variable for	Vigor	Fresh weigh	Height	Root length
<i>L. purpurata</i>				
Vigor	1.00	0.64***	0.58***	0.07 n.s.
Fresh weigh		1.00	0.75***	0.40***
Height			1.00	0.20 n.s.
Root length				1.00

Note: n.s = difference not significant at a 5% level of probability. *** = significant difference at a level inferior to 0.01% **** = significant difference at a level inferior to 0.001%.

For seedlings of both species, the worst results for almost all of the variables analyzed were for the no. 2 gravel substrate. Nevertheless, it was observed that, on no. 2 gravel substrate, *L. purpurata* had better root development than *C. forbesii*. In other words, *L. purpurata* demonstrated a greater capacity than *C. forbesii* to explore nutrients from a low fertility substrate. The low effectiveness of the no. 2 gravel substrate when compared with no. 2 gravel:peat (Figures 2 and 3) provides evidence of the importance of organic matter for these orchids. One point worth noting is that the highest values for *L. purpurata* root length were observed in the substrates no. 2 gravel and no. 2 gravel:peat. In the other substrates, visibly more compact, the values for this

variable were considerably lower, providing evidence that *L. purpurata* has a better root development on substrates that provide greater aeration (Figure 4).

It is difficult to evaluate many variables simultaneously when some are better in one substrate and worse in another, while others are similar. In order to facilitate this decision, correlations between the variables were evaluated, considering that those that were better correlated would lead decision making in the same direction, while variables with lower correlation coefficients can be assumed to be 'key' variables for decision making. For both species it was the variable Root length that had the least correlation with the others (Table 3). Therefore, this variable is well-suited to determine that gravel:peat is a better substrate than peat, both for *C. forbesii* and for *L. purpurata*.

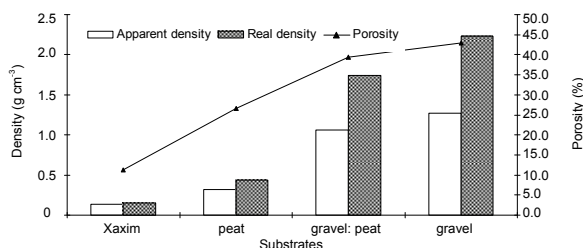


Figure 2. Physical characteristics of the substrates used in this experiment. Substrates are plotted by means in ascending order.

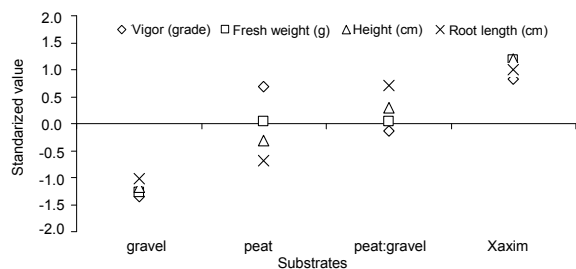


Figure 3. Comparison of different substrates for the acclimatization of seedlings of *C. forbesii* using only the statistically different variables: vigor, fresh weight and height transformed into standard values.

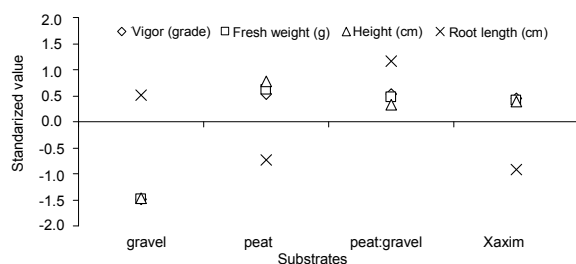


Figure 4. Comparison of different substrates for the acclimatization of seedlings of *L. purpurata* using only the statistically different variables: vigor, fresh weight and height transformed into standard values

Faria et al. (2001) studied nine different substrates for the acclimatization of *Oncidium buerfi* Lindl. seedlings, obtained by *in vitro* germination, and for the growth of *Maxillaria picta* Hook cuttings obtained by rhizome division. For the first species, the best substrates were: XAXIM in cubes and vermiculite. For the second, the best substrates were shredded Xaxim, Xaxim in cubes, and vermiculite:carbonized rice husks in a 1:1 mixture. Moraes et al. (2002) had the best results acclimatizing *in vitro* produced *Dendrobium nobile* Lindl seedlings when they used shredded Xaxim, with a survival rate of 95.2%. However, since the majority of the other variables they analyzed indicated there was no statistical difference between Xaxim and mixtures of vermiculite:Plantmax (a commercial substrate made by Eucatex Indústria e Comércio SA, São Paulo, São Paulo State, Brazil) in a 2:1 ratio, or between Xaxim and Plantmax:granulated charcoal:expanded polystyrene, at a 1:1:1 ratio, they considered these mixtures to be possible alternative substrates to Xaxim. Meneguete et al. (2004) studied the vegetative propagation of offshoots and cuttings obtained by rhizome division, of *Epidendrum ibaguense* Lindl on 3 different substrates, and concluded that the mixture of sand:Plantmax (at a 1:1 ratio) was better than shredded Xaxim for both kinds of propagules, and so considered them to be possible alternatives to Xaxim. Taking the results of the present study together with those of the references cited above, it is clear that Xaxim, either pure or as a component of mixtures, is usually among the best tested treatments; nevertheless, is not invariably the best substrate in a generalized manner. There are species that grow acceptably well in others or prefer further substrates than Xaxim, as observed here for *L. purpurata*, for which the highest ranked substrate was peat.

Araujo et al. (2007) studied the growth of seedlings of a hybrid of *Cattleya* (*C. loddigesii* cultivar Alba x *C. loddigesii* cultivar Atibaia), previously acclimatized, for 6 months in Xaxim dust, on 4 different substrates (no. 0 gravel, carbonized rice husks, shredded Xaxim and piaçava palm [*Attalea funifera* Mart.] fibers), combined with 3 systems of fertilization plus a control. These authors observed that the effectiveness of the fertilizer is a function of the substrate used. The best results were achieved using the commercial NPK fertilizer Biofert Plus 8-9-9 (produced by Biokits Indústria e Comércio Ltda., Contagem, Minas Gerais State, Brazil) on the substrates carbonized rice husks and piaçava palm fiber. Colombo et al. (2005), studied the outcome seedling survival in the acclimatization of the hybrid

Cattleya Chocolate Drop X (*C. guttata* Lindl. x *Laelia tenebrosa* Lindl.) under 2 systems of irrigation (manual and by intermittent sprinkling). They achieved the best results with intermittent irrigation on the substrates coconut dust (*Coccus nucifera* L.) and shredded Xaxim, both with 98% survival. With the manual irrigation system, coconut dust had the highest survival rate (98%), and sphagnum exhibited the worst performance with both systems of irrigation, with rates of 72% for the manual system and 90% for the intermittent system. Yamakami et al. (2006) studied the growth of seedlings of the hybrid *C. labiata* Lindl. x *C. forbesii* Lindl. on seven substrates. Xaxim and coconut fiber were considered the best substrates and so coconut fiber could also be a possible substitute for Xaxim. So, there are several references to the fact that the effectiveness of substrates is influenced by the fertilization and irrigation systems used (HIROYUKI et al., 2004; ICHIHASHI, 1998; WANG, 1995; WANG; GREGG, 1994; WANG; KNOW, 2002). It is, therefore, possible that the effectiveness of alternative substrates that have not exhibited such good results as those observed with xaxim could be improved with studies that evaluate variations in frequency of irrigation, the addition of components to improve aeration and the application of fertilizers.

Makunga et al. (2003, 2005) used Dithane M-45, which has the same active ingredient as Manzate 800, on seedlings transferred from aseptic conditions (*in vitro*) to lathhouse conditions (*ex vitro*) and observed that it prevented damping-off in *Thapsia garganica*, which is a species considered hard to acclimatize due to fungal attacks. *Rhizoctonia* fungi are usually symbiotic with orchids, in a mycorrhizal relationship which aids their nutrient uptake. Nevertheless, they may also cause damping-off of orchid seedlings and countless species of vegetable (RASMUSSEN, 2002). This creates an odd challenge, to protect seedlings that have recently been removed from aseptic conditions, preventing them from being decomposed by *Rhizoctonia*, but allowing them to be invaded by it. With orchids, the use of fungicide can favor or hinder the establishment of mycorrhizae and impact on the growth of the plants (BAYMAN et al., 2002). Manzate 800 has been widely used in the cultivation of orchids in Brazil, even though this is not one of its manufacturer's recommended uses. Its application during the acclimatization phase has come to be regarded as a 'key technique' for preventing damping-off of seedlings in the *ex vitro* phase (its effectiveness was also confirmed in our laboratory - unpublished data), which is considered to be the most critical phase in the process of orchid

production. In this study, no deaths were observed due to damping-off, which was attributed to the protection provided by the Manzate 800.

Conclusion

Xaxim was the best substrate for *C. forbesii*, but could be replaced, with a minor reduction in performance, by the no. 2 gravel:peat mixture. For *L. purpurata*, the best substrate was no. 2 gravel:peat mixture. The low effectiveness of the no. 2 gravel substrate when compared with no. 2 gravel:peat provides evidence of the importance of organic matter for these orchids.

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