

# Ex-vitro establishment of *Phalaenopsis amabilis* seedlings in different substrates

Giorgini Augusto Venturieri\* and Elsa Aurora Mendoza de Arbieto

Laboratório de Evolução Aplicada, Centro de Ciências Agrárias, Departamento de Fitotecnia, Universidade Federal de Santa Catarina, Rod. Admar Gonzaga, 1346, 88034-001, Itacorubi, Florianópolis, Santa Catarina, Brazil. \*Author for correspondence. E-mail: giorgini@cca.ufsc.br

**ABSTRACT.** Eight substrates were evaluated for *ex-vitro* establishment of *Phalaenopsis amabilis* seedlings. They were: Fibráflor® (commercial substrate), Turfa Fértil FG2® (commercial substrate), vermiculite, coconut fiber from the pericarp, decomposed pine bark, organic compost, sphagnum and shredded xaxim, in the establishment of seedlings of *Phalaenopsis amabilis* L., in combination, with and without immersion of the seedlings, after they were removed from the growth flasks, in Manzate 800 at a dosage of 1 g L<sup>-1</sup>. The evaluated parameters were: the sum of the lengths of the two largest leaves, the sum of the lengths of the three largest roots and the percentage of surviving seedlings, evaluated at 190 days after planting. It was observed that there were significant differences between substrates, and sphagnum and shredded xaxim were the best in performance. The application of Manzate 800 showed interaction with substrates, but the isolated effect was not significant. Sphagnum is also an extractive product, but when it is impossible to obtain, an organic compost such as Turfa Fértil FG2® or Fibráflor® can be used, with the necessary application of Manzate 800.

**Keywords:** Mancozeb, acclimatization, ex-vitro, damping off.

**RESUMO. Aclimação “ex-vitro” de plântulas de *Phalaenopsis amabilis* em diferentes substratos.** Foram avaliados oito substratos na aclimação “ex-vitro” de mudas de *Phalaenopsis amabilis*. Eles foram: Fibráflor® (substrato comercial), Turfa Fértil FG2® (substrato comercial), vermiculita, casca de coco dilacerada, casca de pinheiro decomposta, composto orgânico, esfagno e xaxim desfibrado, para a aclimação de plântulas de *Phalaenopsis amabilis* L., em combinação com e sem imersão das plântulas, logo após a retirada dos frascos de crescimento, em Manzate 800 na dosagem de 1 g L<sup>-1</sup>. Os parâmetros avaliados foram: soma do comprimento das duas maiores folhas, a soma do comprimento das três maiores raízes e porcentagem de plântulas sobreviventes, avaliados aos 190 dias após o plantio. Observou-se que houve diferenças significativas entre substratos, sendo os de maior desempenho o esfagno e o xaxim desfibrado. A aplicação do Manzate 800 mostrou interação com os substratos, mas o efeito isolado não foi significativo. Na impossibilidade de ser obtido o esfagno, que também é um produto extrativo, poderiam ser usados: o composto orgânico, a Turfa Fértil FG2® ou o Fibráflor®, com a necessária aplicação do Manzate 800.

**Palavras-chave:** Mancozeb, aclimação, ex-vitro, podridão de mudas.

## Introduction

In Brazil, in the category of "pot flowers", orchids are in second position with regards to their planted area (8.3% of the total), below chrysanthemums (15.1% of the total), the most popular flowers of the category. Among orchids, *Phalaenopsis* and *Cymbidium* are the most commercialized. The commercial production of orchids, while still modest, is growing fast in terms of ornamental plants, having passed from 0.27% in 2002 to 0.7% in 2005. In 2005, US\$ 90,369.00 was spent on orchid sapling imports, corresponding to 14.63% of the importation for the

sector of flowers and ornamental plants (MÜLLER; VISCONTI, 2007).

According to Yu-Ping et al. (2004) and Chen and Chang (2004), *Phalaenopsis* germinates easily in culture media with a low concentration of salt, such as MS ½ (MURASHIGE; SKOOG, 1962) and Hyponex (NISHIMURA, 1982). However, published studies about ex-vitro acclimatization, considered as the most difficult phase for the culture of orchids (COLOMBO et al., 2005), are rare, and even though the knowledge exists, it is kept by producers as a “secret”. In this process, the used substrate is of crucial importance, but the correct

management of the shade and irrigation are also very important, and considered as an "art", that depends on the ability of the producer to obtain good survival indexes and growth rate. The precocity of *P. amabilis* L., the beauty of its flowers and its capacity to blossom many times during the year, even inside houses where usually there is a low light level, have made this species and its hybrids the most commercially produced orchid in the world. In Brazil, the most reputed substrate for the acclimatization of orchids is shredded xaxim, made from the adventitious roots of samambaiçu (*Sellowiana dicksonia* Hook.) (FARIA et al., 2001). Nevertheless, it is an extractive product from the Brazilian Atlantic Forest, and its exploitation is forbidden by law (National Council for the Environment resolution no 278/1 of May 24th, 2001) (LORENZI; SOUZA, 1996). In the present work, alternative substrates to xaxim are studied, with and without application of Manzate 800, in the acclimatization of *P. amabilis* L. seedlings.

### Material and methods

The effect of 8 different substrates was studied in combination with the disinfection and lack of disinfection of seedlings of *P. amabilis* L., just after their withdrawal from the in-vitro vials of growth. The used substrates were: Fibriflor<sup>®1</sup>, vermiculite, dilacerated coconut (*Coccus nucifera* L.) husk, decomposed pine bark from pine trees (from *Pinus elliottii* and *P. taeda*), organic compost (elaborated with wood shavings taken from the raising bays of experimental guinea pigs and rats, cut grasses and vegetable wastes, approximately 2:2:1, made on the decomposition patio of the Federal University of Santa Catarina - UFSC), sphagnum (dried plants of the genus *Sphagnum* extracted from the swamps of Joinville, Santa Catarina State), shredded xaxim (dilacerated pseudotrunk of *Dicksonia sellowiana* Hook.), Turfa Fértil FG2<sup>®2</sup> (composed of commercial substrate prepared with turf, perlite, coconut fiber and calcareous rock, and mineral fertilizer). The seedlings used had an average height of 2.8 cm (min. 2.1 max. 3.8 cm) in relation to the sum of the 2 largest leaves. Disinfection of the seedlings was carried out with Manzate 800, at the dosage of 1g L<sup>-1</sup>, for 1 minute of immersion. They were then planted in plastic trays, 24 hours after their withdrawal from the growth pots. The experiment was carried out at the Federal University of Santa Catarina, in Florianópolis, Santa Catarina State, on 9<sup>th</sup> December 2006. The evaluated

parameters were: the sum of the lengths of the two largest leaves - LL, the sum of the lengths of the three largest roots - RL, and the percentage of seedling survival - SS, evaluated at 190 days after planting. It was assumed that the variables LL and RL reflected the vegetative growth of the seedlings. For each treatment, a minimum of 18 and a maximum of 27 seedlings were planted in an entirely casualized form. The acclimatization was done in a greenhouse, covered with a milky plastic able to reduce 75% of the incident light. Seedlings were irrigated twice a week and were fertilized biweekly by aspersion of 1 g L<sup>-1</sup>, with Peters<sup>2</sup> 30-10-10 plus micronutrients (30% of Nitrogen with 2.06% in the ammoniac form, 3.31% as nitrate and 24.63% as urea, 10% of P<sub>2</sub>O<sub>5</sub>, 10% of K<sub>2</sub>O and Mg, B, Cu, Fe, Mn, Mo and Zn as a micronutrients).

The substrates were analyzed for water-holding capacity by volume - WHC v v<sup>-1</sup> and water-holding capacity by mass - WHC m m<sup>-1</sup>, dry density, and electrical conductivity - EC (measured in a suspension of 1:2 substrate:deionized water). All the parameters were evaluated according to MAPA (2007) and Embrapa (1997). EC was measured by TDSTestr 4 conductivity meter (Oakton Instruments. P.O. Box 5136, Vernon Hills, IL USA 60061).

The data were submitted to analysis of variance. The comparison of averages was made using the LSD test. The correlation between LL, RL, SS, dry density, WHC v v<sup>-1</sup>, WHC m m<sup>-1</sup> and EC was evaluated using Pearson's *r* (SOKAL; ROHLF, 1981). Data were analyzed with the aid of the Statistica software (STATSOFT, 1995).

### Results and discussion

For the three evaluated variables (LL, RL and SS), there was a significant effect for substrate (respectively  $p \approx 0.00$ ,  $p \approx 0.00$  and  $p \approx 0.01$  for DF = 7), but there was no significant effect for Manzate 800 (respectively  $p = 0.67$ ,  $p = 0.30$  and  $p = 0.19$  for DF = 1). However, there was also evidence of interaction between the substrate and the action of Manzate 800 (respectively  $p = 0.03$ ,  $p = 0.05$  and  $p = 0.04$  for DF = 7). The DFs for the errors of the variables LL, RL and SS were respectively 315, 315 and 362). In the most decomposed substrates such as Fibriflor<sup>®</sup>, Turfa Fértil FG2<sup>®</sup> and organic compost, Manzate 800 clearly favored LL. For the substrate shredded coconut husk, this effect was also positive, but not so evident. For the remaining substrates, Manzate

<sup>1</sup>Commercial substrate produced by Florestal. S.A., Araranguá, Santa Catarina State, Brazil.

<sup>2</sup>Commercial product from Scotts Company, Marysville, OH-USA.

800 had an inexpressive effect, but there were indications of a depressive effect for the vermiculite and the sphagnum. For this variable, the best treatment was shredded xaxim without the application of Manzate 800 (Table 1 - right). The RL was also favored by Manzate 800, clearly by the substrate Fibriflor<sup>®</sup>, but not significantly by Turfa Fértil FG2<sup>®</sup> and the organic compost (Table 1 - middle). Manzate 800, even without statistical significance, gave indications that it was harmful to the RL and shredded coconut husk, an inverse effect to the one observed for the variable LL. Also for the RL variable, in the remaining substrates, Manzate 800 did not provoke any significant effect. For this variable, the best treatment was shredded xaxim with the application of Manzate 800 (Table 1 - middle). The variable SS was favored by Manzate 800, clearly on organic compost and, without statistical significance, on the substrate Fibriflor<sup>®</sup> (Table 1 - right). Although without statistical significance, there was a clear indication that it was harmful to the substrates of shredded coconut husk and shredded xaxim. In the remaining substrates, the differences had no value in statistical terms. For the variable SS, the best treatment was sphagnum with or without the application of Manzate 800 (Table 1 - right). Manzate 800, in general, provoked an improvement in the most decomposed substrates (Table 1- right), an indication that the existing decomposer fungus present in these substrates were capable of attacking seedlings. Dithane M-45, whose active principle is the same as that of Manzate 800<sup>3</sup>, was used on seedlings transferred from aseptic conditions (in-vitro) to the greenhouse conditions (ex-vitro) and prevented the dumping-off in *Thapsia garganica*, a species considered hard to acclimatize due to the attack of fungus (MAKUNGA et al., 2003, 2005). However, orchids need to be infected by a symbiotic fungus called mycorrhizae to favor their nutrition. In the case of *Phalaenopsis*, which are of the *Rhizoctonia* genus (CHANG, 2008), such fungus infection can cause damping-off in innumerable species of vegetables. At this point a bizarre situation occurs: how to protect seedlings, recently removed from an aseptic condition, preventing them from being decomposed by the *Rhizoctonia*, but leaving them to be invaded by it. In orchids, the use of fungicide can favor or interfere with the establishment of mycorrhizae and affect the growth of the plants (BAYMAN et al., 2002). Manzate 800 has been widely used in the culture of

orchids in Brazil, but even so it does not have any indication by the manufacturer for this end. Its use during the acclimatization phase has been considered as a “key technique” to prevent the damping-off of seedlings in the ex-vitro phase, considered to be the most critical of the productive processes of orchids. Seidel Jr. and Venturieri (2011) used Manzate 800 in the ex-vitro phase of *Laelia purpurata* and *Cattleya forbesii*, applied biweekly during the entire experiment, at the dosage of 2 g L<sup>-1</sup> in 4 substrates, and all seedlings survived.

Physical characteristics of evaluated substrates are presented in Figure 1 for WHC v v<sup>-1</sup>.

For the variable WHC m m<sup>-1</sup> substrates did not show such discrepant values as those observed for WHC v v<sup>-1</sup> because the latter is strongly influenced by the variation in dry density observed among substrates (Figure 1). A high EC was observed for the dilacerated coconut husk substrate, attributed, in this case to the high concentration of potash, usually present in this material (THAMPAN, 1993).

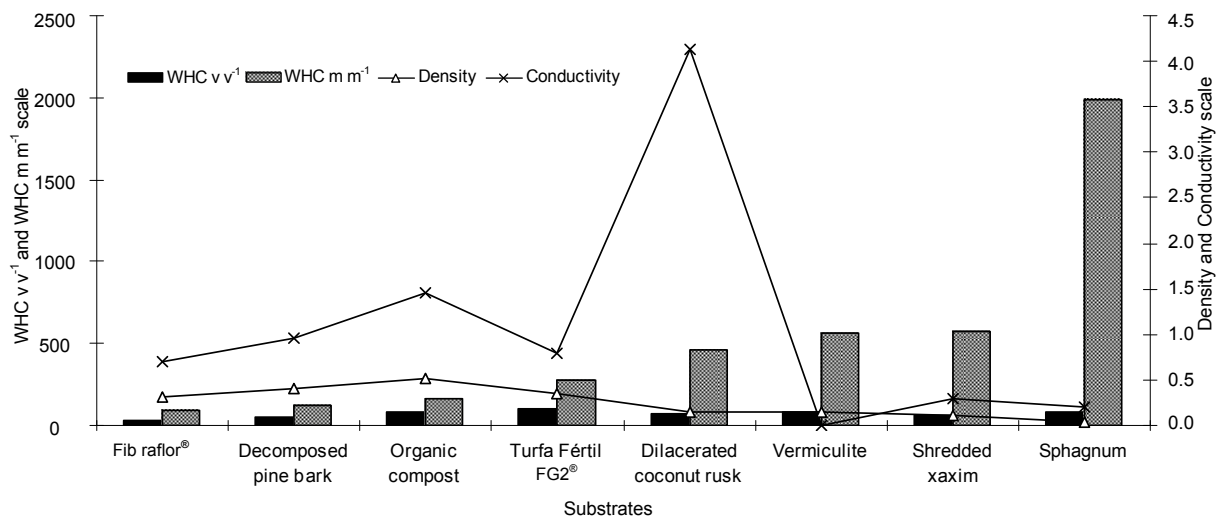
The highest correlation was observed between the parameters for the sum of the two largest leaves (cm) and the sum of the three largest roots (cm), both reflecting vegetative growth. The sum of the three largest roots was significantly correlated to WHC m m<sup>-1</sup>, and negatively correlated to dry density, in other words, substrates with higher porosity were able to retain more water by unit of mass and were more beneficial to *P. amabilis* root development. The negative correlation between WHC m m<sup>-1</sup> and dry density reinforced such tendency. Nevertheless, without statistical significance, it is interesting to note that EC, that is an indirect measure of the salt concentration of the substrates, had a negative influence on the variables for the sum of two largest leaves and the sum of the three largest roots and also on seedling survival, indicating that the use of chemical fertilizers in the substrates or the use of substrates that naturally have salts, such as dilacerated coconut husk, could be harmful to the plants (Table 2). There was no significant association between the percentage of seedling survival and the sum of the lengths of the two largest leaves, the three largest roots, or even any physical properties of the evaluated substrates, but nevertheless a discrete correlation between WHC m m<sup>-1</sup> and the percentage of seedling survival ( $r = 0.47$ ,  $p = 0.06$ ) was observed.

For a better overview of the studied variables, they were transformed into standard units (“Z” score) (SOKAL; ROHLF, 1981) for the expression of proportional rank distances among the variables for each treatment (Figure 2).

<sup>3</sup>Commercial product of Du Pont do Brasil S.A., containing Mancozeb.

**Table 1.** The evaluated parameters: averages of the length sum of two largest leaves - LL, sum of three largest roots - RL, and seedling survival as percentage - SS, of *Phalaenopsis amabilis* L., by substrate, at 190 days after planting. Averages indicated by the same letter show no statistical difference by the LSD test ( $\alpha = 0.05$ , SQD = 3.19 and DF = 315). Treatments are ordered by parameter averages respectively.

Treatments		Variables		Treatments		Variables		Treatments		Variables	
Substrates	Manzate 800 (g L <sup>-1</sup> )	The sum of two largest leaves (cm)	Substrate	Manzate 800 (g L <sup>-1</sup> )	The sum of three largest roots (cm)	Substrate	Manzate 800 (g L <sup>-1</sup> )	Seedling survival (%)			
Decomposed pine bark	1	4.56 (a)	Fibraflor®	0	10.91 (a)	Organic compost	0	55.56 (a)			
Decomposed pine bark	0	4.88 (a, b)	Decomposed pine bark	1	13.27 (a,b)	Fibraflor®	0	77.78 (b)			
Fibraflor®	0	5.21 (a,b)	Decomposed pine bark	0	13.66 (a,b,c)	Dilacerated coconut husk	1	77.78 (b)			
Dilacerated coconut husk	0	5.30 (a,b)	Dilacerated coconut husk	1	14.16 (a,b,c)	Shredded xaxim	1	83.33 (b,c)			
Turfa Fértil FG2®	0	5.32 (a,b)	Organic compost	0	14.30 (a,b,c,d)	Dilacerated coconut husk	0	83.33 (b,c)			
Organic compost	0	5.33 (a,b,c)	Vermiculite	1	16.26 (b,c,d,e)	Turfa Fértil FG2®	0	85.19 (b,c)			
Vermiculite	1	5.40 (a,b)	Fibraflor®	1	16.28 (b,c,d,e)	Turfa Fértil FG2®	1	85.19 (b,c)			
Dilacerated coconut husk	1	5.61 (b,c)	Turfa Fértil FG2®	0	16.63 (b,c,d,e)	Vermiculite	1	88.89 (b,c)			
Vermiculite	0	5.76 (b,c)	Vermiculite	0	18.06 (c,d,e,f)	Fibraflor®	1	88.89 (b,c)			
Turfa Fértil FG2®	1	6.53 (c,d)	Organic compost	1	19.61 (d,e,f,g)	Decomposed pine bark	0	92.59 (b,c)			
Fibraflor®	1	6.58 (c,d,e)	Dilacerated coconut husk	0	19.88 (d,e,f,g)	Shredded xaxim	0	92.59 (b,c)			
Sphagnum	1	6.59 (c,d,e)	Turfa Fértil FG2®	1	20.66 (e,f,g)	Vermiculite	0	92.59 (b,c)			
Organic compost	1	6.91 (d,e)	Sphagnum	1	22.37 (f,g)	Organic compost	1	94.44 (b,c)			
Sphagnum	0	7.14 (d,e)	Sphagnum	0	22.52 (g)	Decomposed pine bark	1	96.30 (c)			
Shredded xaxim	1	7.41 (d,e)	Shredded xaxim	0	23.62 (g)	Sphagnum	1	100.00 (c)			
Shredded xaxim	0	7.65 (e)	Shredded xaxim	1	24.53 (g)	Sphagnum	0	100.00 (c)			



**Figure 1.** Physical characteristics of evaluated substrates in the present work. Substrates are ordered crescently by WHCm m<sup>-1</sup> averages.

**Table 2.** *Phalaenopsis amabilis* seedlings in a selection of substrates. Correlation between evaluated variables. Values in bold are significant at a 5% level of probability (N= 16).

Evaluated variables for <i>P. amabilis</i>	Manzate 800 (g L <sup>-1</sup> )	Sum of two largest leaves (cm)	Sum of three largest roots (cm)	Seedling survival (%)	Water-holding capacity (v v <sup>-1</sup> )	Water-holding capacity (m m <sup>-1</sup> )	Conductivity (mS cm <sup>-1</sup> )	Dry density (m v <sup>-1</sup> )
Manzate 800 (g L <sup>-1</sup> )	1.00							
Sum of two largest leaves (cm)	0.20	1.00						
Sum of three largest roots (cm)	0.12	0.85	1.00					
Seedling survival (%)	0.21	0.30	0.40	1.00				
Water-holding capacity (v v <sup>-1</sup> )	0.00	0.20	0.46	0.01	1.00			
Water-holding capacity (m m <sup>-1</sup> )	0.00	0.44	0.58	0.47	0.36	1.00		
Conductivity (mS cm <sup>-1</sup> )	0.00	-0.32	-0.23	-0.38	-0.06	-0.25	1.00	
Dry density (m v <sup>-1</sup> )	0.00	-0.41	-0.56	-0.39	-0.13	-0.73	0.08	1.00

The substrates that congregated the best positioned variables were shredded xaxim and sphagnum, both with and without the application of

Manzate 800. But such substrates are extractive products and sphagnum is starting to become as scarce as xaxim, so perspectives for studies of

alternative sources already tend to increase (LORENZI; SOUZA, 1996, HIROYUKI et al., 2004).

Although the use of sphagnum, as an isolated component or in mixture, for the acclimatization of seedlings or cloned explants of *Phalaenopsis* is commonly prescribed in manuals for orchid hobbyists (as in CAMPOS (2000) and for professional orchid growers (as in ANTHURA (2005)), there are few experimental studies supporting this prescription. Hiroyuki et al. (2004), searching for alternatives to sphagnum, cultivated *Phalaenopsis* hybrids in 4 different substrates - rockwool, pine bark (*Cryptomeria* sp.), coconut husk and peat moss (decomposed sphagnum) - evaluated the water retention and lixiviation of Nitrogen in the form of nitrate. They showed that: a) substrates were variable regarding their capacity to retain water, peat moss retaining the most and pine bark retaining the least, and b) the best substrate was peat moss and was suggested as an alternative to the coconut husk. The authors considered that deficiency in water retention could be balanced by the increase in irrigation frequency, and that Nitrogen lixiviation, in the form of nitrate, could be balanced by the irrigation length. Ichihashi (1998) evaluated the capacity of water retention in 5 different substrates: sphagnum with and without vermiculite, coconut husk chips, bark chips and rockwool.

The water retention capacity and aeration of these substrates was evaluated, in compacted and not compacted forms. The water absorption in the sphagnum reached its maximum after the second irrigation (irrigations carried out at hourly intervals, with 500 mL, in substrates placed in n. 3 plastics pots). The capacity of water retention in sphagnum decreased when vermiculite was added. Sphagnum was the substrate with the highest aeration capacity.

Compactness drastically diminished the aeration in sphagnum. The author considered that coconut husk chips and bark chips were the substrates for which aeration decayed less after compaction and that they could be used for the cultivation of *Phalaenopsis* under automatic and frequent irrigations.

Faced with the impossibility of obtaining sphagnum, organic compost can be used as a substitute followed by the commercial products Turfa Fértil FG2® and Fibraflor®, with the necessary application of Manzate 800.

Organic compost is a substrate made of organic wastes, so it is in agreement with a good self-sustainability policy. Turfa Fértil FG2® and Fibraflor® are substrates that although they are derived from extractive sources, they are still widely available in the States of southern Brazil, and up to the moment they are without restriction on exploitation.

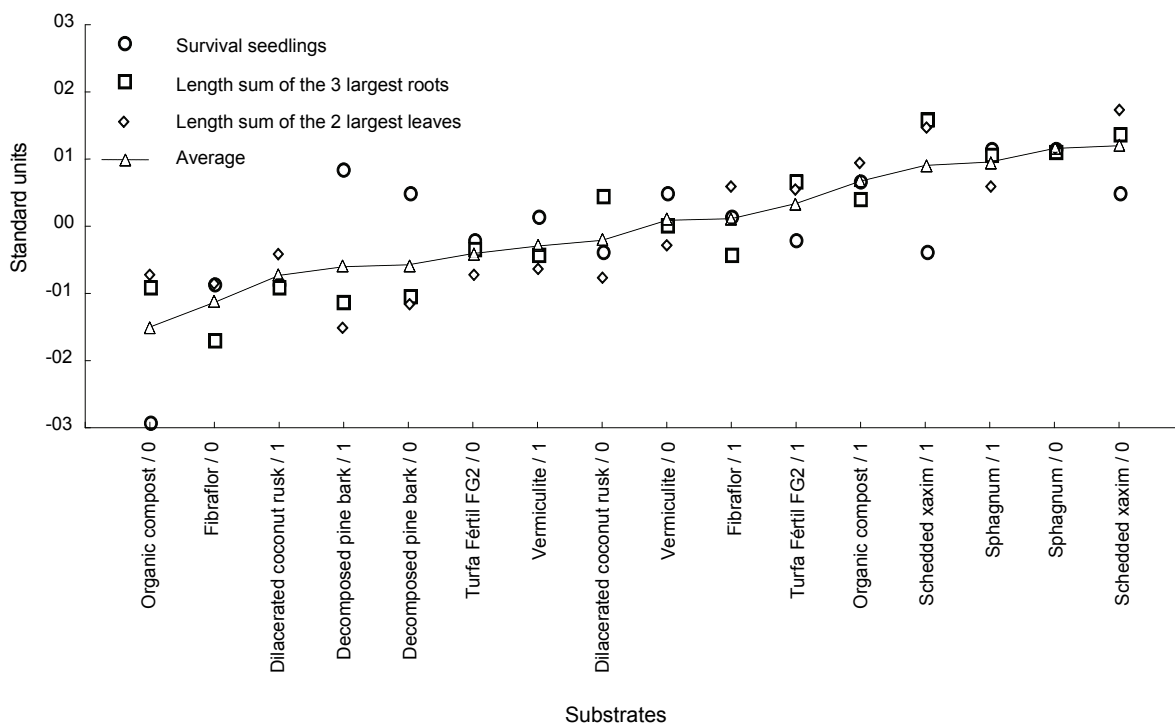


Figure 2. Comparison of different substrates/applications of Manzate 800 (g L<sup>-1</sup>) in the acclimatization of *Phalaenopsis amabilis* L. seedlings, using studied variables (LL, RL and SS) in standard units ("Z" score). Substrates are ordered by the average of all variables by treatment.

It is important to stress that these are conclusions for the conditions under which the experiment was conducted and that there are many references mentioning the fact that the efficiency of the substrates is influenced by the system of irrigation and fertilization (ARAUJO et al., 2007; COLOMBO et al., 2005; HIROYUKI et al., 2004; ICHIHASHI, 1998; WANG, 1996; WANG; GREGG, 1994; WANG; KNOW, 2002). Improvements are expected with studies that consider increases in irrigation frequency, the addition of components able to retain more water, and the application of foliar fertilizers.

## Conclusion

The substrates that brought together the best positions of the evaluated variables were: shredded xaxim and sphagnum, both with and without the application of Manzate 800. Sphagnum can be considered an alternative substrate to xaxim. When sphagnum cannot be obtained, organic compost should be used followed by Turfa Fértil FG2® and Fibraflor®, all with the necessary application of Manzate 800.

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