

## DO ROOTS OF ROSE CUTTINGS SUFFER FROM OXYGEN DEFICIENCY DURING PROPAGATION IN ROCKWOOL?

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### Abstract

Rose cuttings used in soilless culture are mostly propagated in 6.5 cm high rockwool blocks. We questioned whether this was the optimal rooting medium, particularly with regards to oxygen availability. In a number of experiments it was shown that number of roots, root length and shoot growth of c.v. >Frisco= were increased when a pressure head of -6.5 cm was applied.

Compared to aerated water culture (8.5 mg O<sub>2</sub>/l), alcohol dehydrogenase (ADH) activity and porosity were increased in roots grown in rockwool blocks without pressure head (0 cm treatment). Similar effects were found in roots grown in a stagnant water culture. These effects in rockwool and stagnant water culture indicated oxygen stress, since similar (but more severe) effects were found by limiting the oxygen supply, resulting in a concentration of 1.3 mg O<sub>2</sub>/l in water culture.

When different pressure heads (+2, 0, -3.3, -6.5, -10 cm) were applied in rockwool, optimal rooting and shoot growth was achieved at -6.5 cm. The volume fractions of water and air in the whole blocks and in the the lower 2.75 cm part of rockwool blocks (where the actual root initiation and root growth takes place) were determined and calculated at the different pressure heads. From these results, the data on root ADH activity and on root/shoot growth, it was concluded that oxygen stress is minimal and rooting is optimal at a volumetric air content between 20 and 25 % in the lower 2.75 cm of the rockwool; this corresponds to a volumetric air content between 37 and 42% in the whole block.

Additional Index words: alcohol dehydrogenase, growing media, root porosity, volumetric air content

### 1. Introduction

Roots require oxygen for growth, ion uptake and maintenance processes. When the oxygen supply is insufficient to meet oxygen demand, a number of physiological responses may occur. One such response, decreased root growth, is regarded as an indication of oxygen-deficient conditions (e.g. Armstrong and Webb 1985, Soffer and Burger 1988). However, besides the fact that restricted root growth can result from a number of other environmental conditions, measurements on root growth and biomass in growing media can be tedious and inaccurate, particularly after the rooting period, when rooting densities increase. Therefore, in order to be able to relate physical parameters of

growing media with optimal growth, other parameters indicating oxygen stress are desirable. Previous research on chrysanthemum indicated that extractable root ADH activity could be such an indicator (Baas and Warmenhoven 1995). ADH activity was increased particularly during the first growth stages when the volumetric air contents in mineral media were below about 35%. The high air contents for optimal chrysanthemum growing raised the question whether other cut flower crops, such as rose would also benefit from an increase in volumetric air content in the growing medium. In 1995, in the Netherlands, the total production area of cut roses was more than 900 ha. Approximately 45% of the area was grown in soilless systems, with rockwool as the predominant growing medium (anonymous, 1996). The cuttings used for rockwool cultivation are mainly rooted in rockwool blocks which are placed on top of the rockwool slabs or alternatively in rockwool cylinders, placed in holes in the slabs. We tested whether the environmental conditions for adventitious rooting of these cuttings could be improved with regard to oxygen availability.

## 2. Materials and methods

Cuttings were made from flowering stems of cultivar >Frisco= from a local greenhouse. After harvesting, the stems were prewatered at 4°C. After 24 h, 3-5 single node cuttings were made from one stem, and were placed in a 10µM NAA ( $\alpha$ -naphthalene acetic acid) solution for 24 h before further use. Either water culture or commercial available rockwool blocks were used as rooting media. For water culture 48 litre tanks were used, on which 10 cuttings were placed in polystyrene holders. For rockwool propagation, commercially available blocks of 6.5 cm height and 7 cm width were used in which the cut surface was inserted to a depth of 2-3 cm from the bottom of the block.

Rooting was carried out in a near air-tight tent made of transparent polyethylene foil at a height of about 1 m table on a 10 m<sup>2</sup> table situated in a 60 m<sup>2</sup> greenhouse. In the greenhouse a 15 table was used, over which. In the tent the relative humidity was maintained at 90% " 10% using high-pressured nozzles. After two weeks, the relative humidity was gradually decreased by ventilating the foil. CO<sub>2</sub> was provided at 700 ppm, and temperature setpoint was 25°C during the propagation period. Artificial lighting was provided by high pressure sodium lamps (Philips SON-T) at an intensity of about 260 µmol/(m<sup>2</sup>.s) during 20 h (4 a.m.-12 p.m.). If the natural light level outside the greenhouse was higher than 230 µmol/(m<sup>2</sup>.s) the lights were switched off.

### 2.1 Experiment 1

Experimental treatments were: aerated water culture, non-aerated (stagnant) water culture, rockwool blocks (referred to as rockwool 0 cm) and rockwool blocks stacked on top of other rockwool blocks (referred to as rockwool -6.5 cm). The rockwool blocks were saturated with the nutrient solution prior to use by submersion. After approximately two weeks the blocks were resaturated with fresh nutrient solution. The nutrient solution used had an EC of 1.1 mS/cm with the following composition (mM): NO<sub>3</sub><sup>-</sup>, 7.0; H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 0.5; SO<sub>4</sub><sup>2-</sup>, 1.55; NH<sub>4</sub><sup>+</sup>, 0.3; K<sup>+</sup>, 2.9; Ca<sup>2+</sup>, 2.5; Mg<sup>2+</sup>, 1.0. Concentrations of minor elements were (µM): Fe, 30; Mn, 5; Zn, 3.5; B, 25; Cu, 1.5;

Mo, 1. Nutrient solutions in the culture tanks were not refreshed during the propagation period. The pH was measured at least once a week and was kept between 5 and 6. There were four replicates per treatment, each replicate consisting of ten cuttings. The planting date was November 30 1995 (day 0).

At day 20, the number of primary roots and the length of the longest root per cutting were determined in all treatments. Shoot length and fresh weight were recorded. Major elements were analyzed in the dried material (48h at 70<sup>0</sup>C). Root porosity (v/v) was determined by a method based on pycnometry using vacuum-infiltration (van Noordwijk and Brouwer 1989).

## 2.2 Experiment 2

Experiment 2 was a replication of experiment 1. The planting date was January 12 1996 (day 0). At day 20, plants were harvested as in experiment 1. For determination of extractable root alcohol dehydrogenase (ADH) activity, two to four cm root tips were sampled, immediately frozen in liquid nitrogen and stored at -20<sup>0</sup>C. Root ADH activity (n=4) was determined as described before (Baas and Warmenhoven 1995), with the modification that 10% (w:w) PVPP was added to the extraction buffer in order to prevent loss of ADH activity.

## 2.3 Experiment 3

In this experiment the effect of oxygen availability was investigated by comparing a low oxygen treatment in water culture with aerated and non-aerated water culture and the rockwool 0 cm treatment. The planting date was March 13 1996 (day 0). The low oxygen treatment started at the stage of primary root formation on March 25 (day 12) and was achieved by supplying air and nitrogen gas in a ratio of 5%/95% (v/v) in a 48 l air-tight container at a rate of approximately 15 l/min. This resulted in an average air-saturation of the solution of 15% (1.3 mg O<sub>2</sub>/l) as measured by oxygen membrane electrodes (YSI). At day 22, plants were harvested as in exp. 1 and 2. There were 20 replicate plants per treatment. For determination of ADH activity and root porosity 4 replicate samples were taken.

## 2.4 Experiment 4

Four different pressure heads were created by placing saturated rockwool blocks on 0, -3.3, -6.5 or -10 cm pressure head using rockwool blocks of different heights. After about four hours the blocks were weighed. In a fifth treatment the lower 2 cm of the rockwool was continuously immersed in nutrient solution (treatment +2 cm). In order to avoid microclimatological differences, care was taken that the top of all the blocks were at the same height. The planting date was april 17 1996. On may 6 (day 18) the plants were harvested as before after the blocks had been weighed. Volumetric air content ( $\theta_a$ ) of the rockwool blocks was calculated as the difference from total porosity (calculated from bulk density as 98%) and volumetric water content. ADH activity in roots sampled at day 18 was determined (n=2-10).

## 2.5 Laboratory measurements

Similar rockwool blocks as used in the propagation experiments were divided in a top layer of 3.75 cm and a lower layer of 2.75 cm. The entire blocks (top on lower layer) were weighed before saturating them with water (n=9). After weighing, the top layer was removed, and the lower layer was weighed (pressure head 0 cm). The same procedure was repeated with pressure heads of -3, -6.5 and -10 cm. Using these data  $\theta_a$  was calculated for whole blocks ( $\theta_{a6.5}$ ) and for the lower 2.75 cm ( $\theta_{a2.75}$ ).

## 3. Results and Discussion

In both experiments 1 and 2, root length of primary roots in the rockwool 0 cm treatment were comparable to the non-aerated water culture treatment (table 1), and were lower than in the aerated water culture and the rockwool -6.5 cm treatments. This indicates that suboptimal conditions for root growth in the commercially used rockwool blocks (treatment rockwool 0cm) occur. In experiment 2, also number of primary roots, shoot length and shoot fresh weight in the 0 cm treatment were decreased compared to water culture aerated and rockwool -6.5 cm. Root ADH activity and root porosity were higher in the rockwool 0 cm treatment as well, indicating that oxygen stress occurred in the treatment, as both parameters show an increase under conditions of oxygen stress (Crawford and McMannon 1968, Pezeshki et al. 1993, Baas and Warmenhoven 1995).

Total N-concentrations in the leaves in both the non-aerated and the rockwool 0 cm treatments were decreased compared to aerated water culture (table 2). Ion uptake has frequently been shown to decrease under stagnant conditions (e.g. Atwell and Steer 1990). Alternatively, it may be reasoned that a low refreshment rate in the rockwool blocks was responsible for the decreased root and shoot growth. Indeed, in chrysanthemum cuttings the low nutrient refreshment rate probably caused local depletion of nutrients such as N resulting in decreased shoot growth rates (Buwalda and Kim 1994). However, it seems unlikely that the nutrient refreshment rate in the rockwool -6.5 cm treatment was better than in the rockwool 0 cm treatment, as nutrients were similarly applied during the propagation period, and no significant differences in N-concentrations were apparent (table 2).

Further evidence that oxygen deficiency can decrease root and shoot growth and increase root ADH activity comes from experiment 3 in which oxygen availability was decreased by using an air/nitrogen mixture to aerate the water culture. The occurrence of possible local depletion (oxygen, nutrients) or accumulation (e.g. ethylene, CO<sub>2</sub>) zones occurring around the roots under non-aerated water culture conditions is unlikely under these experimental conditions (Soffer and Burger 1988). The decreased oxygen concentration (on average 15% of the 100% air aerated treatment, i.e. 1.3 mg O<sub>2</sub>/l) reduced shoot and root growth and increased ADH activity to a large extent, higher than in the non-aerated water culture or the rockwool 0 cm treatment (table 3). In chrysanthemum and *Ficus*, adventitious rooting was already decreased when the oxygen concentration was lowered from 8 to 5 mg/l (Soffer and Burger 1988), suggesting that a less drastic oxygen concentration than applied in our experiment would have had similar

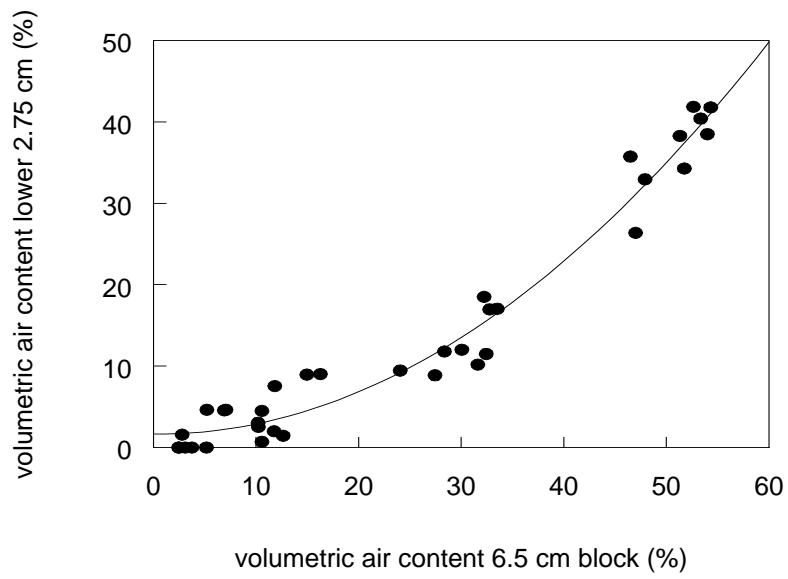
effects as the stagnant water culture or the rockwool 0 cm treatment.

Different pressure heads were compared to investigate the range for optimal rooting with regard to volumetric air content ( $\theta_a$ ) in experiment 4. Not only the -6.5 cm, but also the -3.3 cm and -10 cm showed a lower root ADH activity indicating less oxygen stress under these conditions (table 4). Optimal rooting defined as the number of primary roots formed, occurred at -6.5 cm.

When the  $\theta_a$  at day 0 and at day 18 are compared, a significant increase in the 0 cm and -3.3 treatments is apparent (table 5). Since this increase did not occur in the -6.5 and -10 cm treatments it is assumed that higher evaporation at the (moist) surface of the rockwool blocks in these treatments is responsible for this increase. The increase presumably occurred particularly during the last days of the propagation period when the relative humidity in the propagation tent was decreased. As root ADH activity can respond within a day to a change in oxygen availability (Warmenhoven, unpublished results), it is probably better to relate root ADH activity to the  $\theta_a$  at the time of harvest than to the  $\theta_a$  at the start of the experiments. This increase in  $\theta_a$  may explain the lower ADH in the rockwool 0 cm treatment compared to the non-aerated water culture treatment in experiments 2 and 3 (tables 1 and 3).

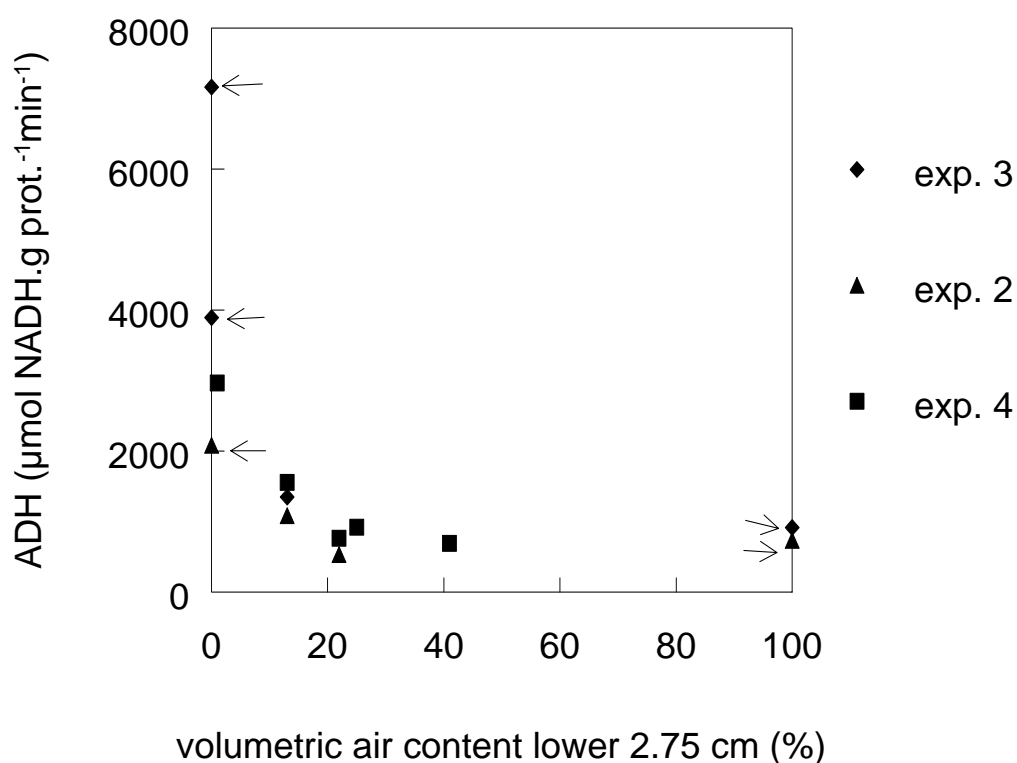
The correlation between  $\theta_a$  of the growing medium and adventitious rooting of woody ornamentals can sometimes be poor (Tilt and Bilderback 1987). This can be due to several factors. One reason may be that the  $\theta_a$  determined by laboratory measurements can differ from the actual  $\theta_a$  during the rooting period as a result of shrinking/settling of the medium. Considering the propagation period of around three weeks and the stability of rockwool this factor is not likely for rose cuttings propagated in rockwool. Another reason may be the fact that the volume used in the determination of the  $\theta_a$  can exceed the actual rooted volume. For rose cuttings in rockwool this is the case, as rooting starts at the cutting surface, which is situated in the lower half of the rockwool block. Therefore, in laboratory measurements, the relation between the  $\theta_a$  of the lower 2.75 cm of the rockwool block ( $\theta_{a\ 2.75}$  the zone in which the rooting of rose cuttings actually takes place) and the  $\theta_a$  of the whole block ( $\theta_{a\ 6.5}$ ) was determined. This relation could be fitted to a second-order polynomial as:

$$\theta_{a\ 2.75} = 1.67 + 0.0134*\theta_{a\ 6.5} + 0.0136*(\theta_{a\ 6.5})^2 \quad r^2=0.96 \text{ (fig.1)}$$



From this function, the  $\theta_{a,2.75}$  was calculated from the results of experiment 4 (table 5). Using these data, ADH activity as determined in the different experiments was plotted against  $\theta_{a,2.75}$  (fig. 2). In fig. 2 the data of the non-aerated water culture treatment (experiment 2 and 3) and the 15% air-saturation (experiment 3) were plotted on 0% air content, and the aerated water culture treatments were plotted as 100% air content. Considering that ADH indicates the oxygen stress at the day of determination, the conclusion from figure 2 is that ADH activity increases below a  $\theta_{a,2.75}$  of 20%. According to equation (1) this would correspond with a  $\theta_{a,6.5}$  of 37%.

This minimal  $\theta_a$  for optimal rose propagation is high in comparison to data given for rooting of roses, Poinsettia, Hydrangea and Azalea in rockwool and peat blocks (Gislerød 1983a, 1983b). Perhaps the difference between a  $\theta_a$  determined in the laboratory and the (higher) actual air content during the propagation phase (table 5) may account for this discrepancy. However, the results are in accordance with recent results which indicate the need for similarly high  $\theta_a$  values desirable for optimal growth of chrysanthemum cuttings, provided that the availability of water and/or nutrients are non-limiting (Baas and Warmenhoven 1995, Buwalda et al. 1995, Verhagen 1993, Warmenhoven 1995).



### Acknowledgements

We gratefully acknowledge Mary Warmenhoven for performing the root porosity measurements, and dr. Fokke Buwalda and dr. Hendrik-Jan van Telgen for their constructive comments on an earlier version of the manuscript.

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Table 1- Determined parameters at day 20 in experiments 1 and 2. Significance symbols derived from analysis of variance: \*\*\* P<0.001, \*\* P<0.01, \* P<0.05, n.s. not significant. Abbreviations: w.c.= water culture, r.w.= rockwool, n.d. = not determined. LSD= least significant difference at P=0.05.

	length longest root (cm)	number primary roots	length shoot (cm)	FW shoot ( $\mu\text{mol}/$ (g)	ADH (g prot.min))	root porosity (%)
<i>Experiment 1</i>						
w.c. aerated	4.4	19.2	8.8	0.79	n.d.	6.0
w.c. non-aerated	3.0	14.6	9.0	0.70	n.d.	n.d.
r.w. 0 cm	3.0	17.9	7.6	0.56	n.d.	7.4
r.w. -6.5 cm	4.0	18.7	11.2	0.86	n.d.	4.4
significance	***	*	***	**		***
LSD	0.6	3.7	2.4	0.24		1.0
<i>Experiment 2</i>						
w.c. aerated	4.0	20.8	9.4	1.06	737	4.3
w.c. non-aerated	2.8	14.7	6.8	0.70	2083	9.1
r.w. 0 cm	3.1	15.6	6.2	0.53	1085	7.4
r.w. -6.5 cm	4.0	19.9	10.0	1.00	536	5.8
significance	***	**	**	**	***	**
LSD	0.5	3.1	1.9	0.27	295	2.1

Table 2 - Mineral concentrations ( $\mu\text{mol}/\text{kg}$  dry matter) in shoot material at day 20 in experiments 1 and 2

	total N	P	K	Ca	Mg
w.c. aerated	1459	89	469	91	68
w.c. non-aerated	1103	78	397	78	63
r.w. 0 cm	1207	88	452	89	74
r.w. -6.5 cm	1362	86	529	109	77
significance	*	n.s.	*	**	*
LSD	244		76	12	7

Table 3 - Morphological parameters and root ADH activity at day 22 in experiment 3.

	length longest root (cm)	number primary roots	length shoot shoot (cm)	FW shoot (μmol NADH/ (g (g protein.min))	ADH
w.c. aerated	4.1	n.d.	12.8	1.54	914
w.c. non-aerated	3.9	n.d.	12.6	1.48	3896
w.c. 15% air-saturation	2.6	n.d.	7.8	0.66	7164
r.w. 0 cm	n.d.	n.d.	10.3	1.07	1350
significance	***		***	***	***
LSD	0.5		1.4	0.18	812

Table 4- Morphological parameters and root ADH activity at day 18 in experiment 4.

	length longest root (cm)	number primary roots	length shoot shoot (cm)	FW shoot (μmol NADH/ (g (g protein.min))	ADH
r.w. +2 cm	0.3	1.1	4.6	0.26	2967
r.w. 0 cm	2.4	8.3	8.6	0.64	1557
r.w. -3.3 cm	2.7	7.6	11.0	0.74	922
r.w. -6.5 cm	3.4	10.4	12.1	0.95	762
r.w. -10 cm	3.2	7.5	10.9	0.79	689
significance	***	***	***	***	***
LSD	0.7	2.7	2.8	0.27	377

Table 5- Volumetric air content of whole rockwool blocks ( $\theta_{a6.5}$ ) at different pressure heads determined at beginning (day 0) and end (day 18) of experiment 4, and calculated vol. air content of the lower 2.75 cm ( $\theta_{a2.75}$ ) using the equation derived from figure 1.

	$\theta_{a6.5}$ day 0	$\theta_{a6.5}$ day 18	$\theta_{a2.75}$ day 0	$\theta_{a2.75}$ day 18
r.w. +2 cm	n.d.	4	n.d.	1
r.w. 0 cm	8	29	2	13
r.w. -3.3 cm	22	42	8	25
r.w. -6.5 cm	39	39	22	22
r.w. -10 cm	55	54	43	41

