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Effect of substrate, Fusarium, laboratory, root damage and pH on growth and die-back during propagation of gerbera tissue-culture plants.

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October 1989

Intern Verslag nr. 44

2220346

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Inhoud

1. INTRODUCTION

In the autumn of 1988 in horticultural practice problems arose with the die-back of gerbera plants on rockwool, especially when the rockwool was in gullies. Gullies were bought by growers on a large scale from the Brinkman company, without testing the growth. The NAK-S always found Fusarium in the dead plants. The growers blamed the propagation nurseries of the young plants, but on their turn these nurseries recieved the plants from the tissue culture laboratories.
The reason for the die-back remained unclear. Therefore we started an experiment, which is described here, to find out which factors influence the growth and death of young plants. Two other experiments with mature plants were started in Vleuten (Regional Research Station) and Aalsmeer (Research Station for Floriculture).
In our experiment we tested the following assumption. Plants from tissue culture are rooted in the last stage (the rooting stage) in agar, peat or rockwool. When rooted in agar some agar remains behind on the roots when the plants go to the propagation nurseries. In this agar it is possible that Fusarium starts growing. So, plants rooted in the laboratory in agar should be more affected than plants rooted in peat or rockwool. Another theory was that Fusarium may affect the plants when they are weak. On rockwool, plants are weaker than on peat (Jiffy), which perhaps has something to do with the rooting system, which is different in the two substrates. Therefore we tested the effects of substrate and Fusarium and their interactions. It was thought possible, that root damage could give rise to the dying of plants, because Fusarium could grow into the injury.
In rockwool the pH can rise above 7. Lowering the pH is difficult: NH_4 has little effect because uptake by roots is small and the possibilities of watering with solution with low pH are also restricted because transpiration is low. The pH is higher than what is accepted as desirable for plant growth. So, it was assumed that this high pH resulted in reduced growth and perhaps also dying of plants. In a second experiment also described here the influence of pH was investigated.

2. MATERIAL AND METHODS

Two seperate experiments were carried out in 1989 from 11 July - 6 September 1989 (8 weeks).

Experimental design
In experiment 1, 16 treatments of the 4 factors were carried out in randomized blocks with 3 replications. The factors were two substrates (peat and rockwool) with and without Fusarium, two different laboratories and with and without root damage. The treatments of the experiment are shown in table 2. Each experimental unit consisted of 35-49 plants.
Experiment 2. 16 Treatments of four factors were carried out with one replication. Low and high pH were investigated. The other three factors (substrate, laboratory, root damage) and experimental design were the same as in experiment 1.

Preparing the experiment
The experiment was conducted on benches. The benches were 1.4 m^2 in size and the dimensions of the substrates were 110x110x8 cm for peat and 110x110x10 cm for rockwool. 12 Benches were arranged in three rows. Two of four benches in each replication were filled with peat and on the other two benches rockwool slabs were placed. Each bench was filled with plants (cultivar Cora) from two different laboratories. The experimental area was lime-shaded on the outside of the glasshouse and completely covered from inside of the glasshouse with both 10 micron perforated polyethylene and netting sheets.

Substrate characteristics
Peat was 25% perlite + 75% white peat. In experiment 1, to the peat no PG-mix, and 3 kg/m^3 Dolokal was added. In experiment 2, no PG-mix, and 3 kg/m^3 Dolokal for low pH and 5 kg/m^3 Dolokal for high pH was added. Rockwool was Grodan No 632 FM. Fiber structure was vertical.

Fusarium inoculation
In each replication the plants, one bench of peat and rockwool, were inoculated with Fusarium after four days from planting. Three cm^3 Fusarium suspension of two isolates (F. oxysporum from NAK-S and no 80-266), which contain about one million spores in each cm^3, were poured to the stem of each plant. At the end of the experiment the substrates were analysed for Fusarium concentration.

Laboratory
Half of the plants from laboratory 1 were rooted in small rockwool cubes for planting in rockwool and the other half in small peat (ST400-AA16) cubes for planting in peat. Plants from laboratory 2 were rooted in agar.
Multiplication of all the plants was done on laboratory 2. Only in the last stage lab 2 delivered plants to lab 1 for the rooting stage.

Root damage
At 31 days after the planting, when plants rooted and started to grow fast, the roots of half of the plants were damaged with a knife.

Watering and nutrition
Benches were well wetted with nutrient solution before planting and at the bottom of the benches a level of about three cm drainage water was maintained during the first 15 days to obtain high water content. The plants were hand watered once a week with nutrient solution recommended for gerbera.

Monitoring pH and EC
The pH and EC of the substrates were determined periodically. The pH was monitored by irrigation with different levels of pH and by adding NH_4 and potassium carbonate to the nutrient solution. For high pH always 0 mmol/l NH_4 and for low pH and exp-1 up to 3 mmol/l NH_4 was added. The nutrient solutions given were prepared at pH 5,0 - 5,5 for low and 5,5 - 6,0 for high pH. The EC was 1,0 - 2,0 $mS.cm^{-1}$ according to the measured values in the benches. The measurements concerning the mean value of pH and EC of the exp-1 and exp-2 are presented in table 1 and 7.

Temperature and humidity
No artificial heating and cooling was applied. The set point of ventilation was 26°C. After planting the separate benches were covered with polyethylene for 5 days to keep high humidity. Spray irrigations were given under the benches every hour during the day between 8 - 20 h and every 2 hours during the night. The dry and wet bulb temperatures and humidity were measured on a bench under the plastic foil, and in the glasshouse. The mean value of each hour was printed out. Temperature ranged between 16 - 20°C during the night. A few nights the temperatures decreased below 15°C. Several days the temperature increased over 35°C and humidity decreased under 40% during shiny days afternoon. The recorded minimum temperature was 12.5°C and maximum was 38.3°C. The first 5 days humidity under the plastic tunnel on the benches was 98-100%.

Lighting
From 28 July onwards artifically lighting was done with high pressure sodium lamps between 8 - 20 h during the day and 12 - 4 h during the night.

Evaluation
At the end of the experiments the dead plants were recorded of each plot. The remain ing plants fresh weight of the shoot were measured and visually evaluated and classified in four grades.
The grades are: grade-1: healthy, marketable plant, big
grade-2: healthy, marketable plant, small
grade-3: weak or nearly dead plant
grade-4: dead plant.
Fresh weight of the shoot were classified:
class-A: ≥ 5 g
class-B: 2.50 - 4.99 g
class-C: 0.01 - 2.49 g
class-D: 0 g (dead)
Irrespective the grading, bushy plants were also calculated. A bushy plant is a plant which contain more than one dwarf young plant.

3. RESULTS

3.1. Experiment 1

Table 1 shows the results of EC and pH measurements.

Table 1. The average pH and EC values of exp. 1.

Substrate	EC ($mS.cm^{-1}$) and pH	Week no. in 1989 29	30	31	32	33	34	35
Peat	EC	1.57	1.47	1.25	1.20	1.10	1.21	1.20
	pH	5.87	6.21	6.42	6.58	6.16	6.43	6.32
Rockwool	EC	1.80	1.70	1.60	1.69	1.82	1.77	1.80
	pH	5.77	6.15	5.80	5.20	5.35	5.65	6.00

Table 2 and 3 show the evaluation of the treatments and factors on mean fresh weight of the shoot (dead plants are excluded), the classification, the grading, and the fraction bushy plants.

Table 2. Effects of treatments on studied subjects (for explanation of class and grade, see table 3).

Experimental factors				Treat-	Mean	Class(%)				Grade (%)				Bushy
Subst	Fusa-rium	Lab no	Root damage	ment no.	fresh weight (g)	A	B	C	D	1	2	3	4	plants (%)
Peat	(-)	1	(-)	1	5.89	51.6	28.6	13.5	6.3	47.6	20.6	25.4	6.4	31
			(+)	2	5.64	45.2	29.4	18.3	7.1	44.4	24.6	23.8	7.2	38
		2	(-)	3	5.26	46.3	42.2	11.6	0.0	47.6	32.0	20.4	0.0	31
			(+)	4	5.10	46.3	46.3	7.5	0.0	44.9	36.1	19.0	0.0	34
	(+)	1	(-)	5	6.28	53.5	20.8	16.0	9.7	53.5	14.9	21.9	9.7	27
			(+)	6	5.49	38.4	27.3	11.6	22.7	36.5	19.8	21.0	22.7	26
		2	(-)	7	5.64	51.7	34.7	11.6	2.0	47.6	24.5	25.9	2.0	28
			(+)	8	5.52	47.6	32.7	13.6	6.1	44.2	26.5	23.2	6.1	33
Rock-wool	(-)	1	(-)	9	5.64	42.7	23.4	13.5	20.4	44.2	17.7	18.4	19.7	26
			(+)	10	3.92	18.4	32.0	19.0	30.6	21.1	24.5	23.8	30.6	40
		2	(-)	11	5.57	44.2	35.4	12.2	8.2	43.5	29.3	19.0	8.2	30
			(+)	12	5.16	44.9	33.3	9.5	12.2	42.2	25.9	17.7	12.2	38
	(+)	1	(-)	13	4.94	26.5	25.9	10.2	37.4	27.2	22.5	14.6	36.7	29
			(+)	14	4.31	19.0	27.9	17.0	36.1	19.7	19.7	24.5	36.1	34
		2	(-)	15	5.76	55.1	28.6	8.8	7.5	56.5	23.8	12.2	7.5	18
			(+)	16	5.24	50.3	33.3	12.2	4.1	53.7	27.9	14.3	4.1	26

Table 3. Effects of experimental factors on the studied subjects.

Experimental factors	Mean fresh weight (g)	Class (%)				Grade (%)				Bushy plants (%)
		A	B	C	D	1	2	3	4	
Peat	5.60	47.6	32.7	12.9	6.8	45.8	24.9	22.6	6.8	31.1
Rockwool	5.07	37.7	30.0	12.8	19.6	38.8	23.9	17.7	19.6	30.5
Fusarium (-)	5.27	42.4	33.8	13.1	10.6	42.2	26.3	20.9	10.6	33.8
Fusarium (+)	5.40	42.8	28.9	12.6	15.7	42.4	22.5	19.6	15.7	27.8
Lab-1	5.26	36.9	26.9	14.9	21.3	36.8	20.5	21.5	21.3	31.6
Lab-2	5.41	48.3	35.8	10.9	5.0	47.8	28.2	19.0	5.0	29.9
Root dam (-)	5.62	46.5	29.9	12.2	11.4	46.0	23.2	19.6	11.3	27.8
Root dam (+)	5.05	38.8	32.8	13.6	14.9	38.6	25.6	20.9	14.9	33.7
Mean	5.34	42.6	31.3	12.9	13.2	42.3	24.4	20.3	13.2	30.8

Class-A = ≥ 5 g
Class-B = 2.50 - 4.49 g
Class-C = 0.01 - 2.49 g
Class-D = Dead plants

Grade-1 and 2 marketable plants
Grade-3 unmarketable plants
Grade-4 dead plants

Substrate

There were some significant effects of substrate on the classification and grade, as well as some significant interactions between substrate and lab (table 4). There were no significant interactions between substrate and Fusarium and root damage. Peat gave less dead plants than rockwool, 6.8 and 19.6% respectively. In combination with lab 2 peat gave only 2% dead plants. Peat gave 45.8% grade 1 and rockwool 38.8%. Although this difference was not significant, the interaction between substrate and lab was significant. Rockwool combined with lab 1 gave only 28.1% grade 1 and with lab 2 49.5%. Substrate had no significant effect on grade 2 and 3.

Laboratory

The laboratory had significant effects on fraction class A, B, C and D and on grade 1 and 2 (table 4).

Table 4. Effects of substrates, laboratories and sub x lab interaction (class and grade see table 3, N.S. = not significant; *, ** and *** significant at $p < 0.05$; < 0.01 and < 0.001).

Experimental factors	Grade-1 (%)			Grade-2 (%)			Class-A (%)			Class-B (%)		
	lab-1	lab-2	mean	lab-1	lab-2	mean	lab-1	lab-2	mean	lab-1	lab-2	mean
Peat	45.5	46.1	45.8	20.9	29.8	24.9	47.2	48.0	47.6	26.5	38.9	32.7
Rockwool	28.1	49.5	38.8	21.1	26.7	23.9	26.7	48.6	37.7	27.3	32.7	30.0
Mean	36.8	47.8	42.3	20.5	28.2	24.4	36.9	48.3	42.6	26.9	35.8	31.3
p sub		0.151	N.S.		0.740	N.S.		0.084	N.S.		0.340	N.S.
lab		0.024	*		0.002	**		0.007	**		<0.001	***
sub x lab		0.031	*					0.011	*		0.135	N.S.

Experimental factors	Class-C (%)			Class-D (%)			Fresh weight (g)		
	lab-1	lab-2	mean	lab-1	lab-2	mean	lab-1	lab-2	mean
Peat	14.8	11.1	12.9	11.5	2.0	6.8	5.827	5.382	5.604
Rockwool	14.9	10.7	12.8	31.1	8.0	19.6	4.702	5.430	5.066
Mean	14.9	10.9	12.9	21.3	5.0	13.2	5.265	5.406	5.335
p sub		0.957	N.S.		0.005	**		0.225	N.S.
lab		0.010	**		<0.001	***		0.449	N.S.
sub x lab		0.879	N.S.		0.011	*		0.004	**

Lab 2 was better than lab 1: lab 2 had more (47.8%), marketable plants in grade 1 than lab 1 (36.8%). Also lab 2 had less (5%) dead plants than lab 1 (21.3%). There were significant interactions with substrate, mentioned before.

Fusarium

Analysis of water samples from rockwool and peat samples for Fusarium showed, that there were colonies in both inoculated and no-inoculated treatments, but the concentration was higher in oculated than in no-oculated (Table 5). Later on, it was recognised that the basinwater contained Fusarium. So, the Fusarium in the no-inoculated was probably coming from the water.

Table 5. Fusarium colonies in the substrates, at the end of the trial.

Treatment	Fusarium colonies	
	Rockwool number per cm^3 solution	Peat number per gram peat
- Fus.	175	6
+ Fus.	1780	863

Fusarium had no significant effect on mean weight, class and grade. There were no significant interactions with other factors. There was only a significant ($p = 0.049$) effect on fraction bushy plants: without Fus. 33.8% and with Fus. 27.8%.

Root damage

Root damage had no significant effect on class and grade. There were only significant effects on fresh weight and fraction bushy plants (table 6).

Table 6. Effects of root damage on fresh weight and bushy plants.

Fresh Weight (g)		Bushy plants (%)	
Without root damage	With root damage	Without root damage	With root damage
5.623	5.048	27.8	33.7
$p = 0.023$*		$p = 0.037$*	

With root damage mean fresh weight and fraction bushy plants were lower, respectively higher than without root damage. There were no significant interactions with other factors.

3.2. Experiment 2

The values for the low pH ranged between 5.45 - 6.17 and for the high pH between 6.41 - 7.10 (Table 7).

Table 7. The average pH and EC values of Experiment 2.

Treatment	EC ($mS.cm^{-1}$) and pH	Week no in 1989					
		29	30	32	33	34	35
Low	EC	1.80	1.50	1.42	1.40	1.50	1.35
	pH	5.86	6.17	6.00	5.72	5.45	6.00
High	EC	1.60	1.60	1.40	1.35	1.30	1.35
	pH	6.41	6.57	6.62	6.80	7.10	6.97

Some chlorotic symptoms appeared in the high pH plots, although substrate analysis showed optimum level for nutrient elements, especially Fe and Mn. There were no significant effects on grading (Table 8).

Table 8. Effect of pH on grade of plants.

pH	Grade			
	1	2	3	4
Low	41.1	23.5	22.2	11.6
High	67.7	19.9	9.2	6.2

High pH was found to be a little better for grade 1 than low pH, but not significantly.

4. DISCUSSION

A lot of plants died especially in the first weeks of the experiment. The reason for dying has not been determined. The question, therefore remains which disease caused the die-back? The hypothesis that Fusarium would attack weak plants and entered the plant through injuries by root damage was not confirmed. On the other hand the difference between peat and rockwool was confirmed. On peat die-back was less than on rockwool. Perhaps this has something to do with a stronger root system in peat and lower waterconcentration in peat than in rockwool.
Laboratory 2 was better than lab 1, although plants from lab 2 were bare rooted and from lab 1 plants were rooted in rockwool or peat. It was expected that bare rooted plants were worse, but the opposite turned out to be the case. The explanation is that lab 2 did the dividing in the tissue culture. When plants started the rooting stage in the laboratory, plants were weak. There had to be a dividing to get stronger plants, but because of the desired planting date for our experiment this was omitted. Rooting in agar (lab 2) in a protected climate gave a stronger plant than rooting in peat or rockwool (lab 1). When planting the plants in our experiments it could be seen that plants from lab 1 were smaller than from lab 2. It is evident that the stage of the tissue-culture is very important for propagation.
When small plants are planted in peat they gave better results than on rockwool (interaction between lab and substrate). This is in agreement with horticultural practice where small plants are planted in Jiffy-pots and big plants in rockwool.
High air humidity is important for the propagation of young gerbera plants from tissue culture. Therefore in these experiments the benches were covered with plastic tunnels during the first five days. In the tunnel air humidity was 99-100%. The question is, if this was perhaps too high. At high humidity most diseases grow faster. Also calcium-uptake by the plant is reduced due to low transpiration. In some leaves symptoms occurred which were similar to symptoms occurring in cucumber leaves with calcium deficiency. The leaf margins stopped growing whereas the inside of the leaves continued growing, which resulted in bulbous leaves.
On the other hand a lot of plants showed deformed, hard leaves. The cause of this phenomenon has not been determined. Perhaps it has someting to do with cytokinine used in the tissue-culture or with low or high air humidity or with variation in humidity. These deformed hard leaves occur frequently on propagation nurseries and give rise to a lower growth rate of the plants. This phenomenon needs further study, in which the influence of humidity and calcium spraying should be determined.

5. CONCLUSIONS

- On peat less plants died than on rockwool; 6.8% compared to 19.6%.
- The size of the plant from tissue culture had a strong significant effect on the dying of plants; from weak plants 21.3% and from strong plants 5% of the plants died.
- A small plant planted on peat gave a considerably better result in comparison with a small plant on rockwool (interaction between lab and substrate).
- Fusarium and root damage had no significant effect on fraction in different classes, grades (including dead plants), neither interactions with each other or with substrate or lab.
- More than 30% of the plants were bushy. Root damage gave more bushy plants.
- pH has no effect on grading or die-back of plants.

6. SAMENVATTING

Tussen 11 juli en 6 september 1989 (8 weken) werden twee proeven gedaan met opkweek van gerbera, cultivar Cora. De planten waren afkomstig uit weefselkweek. Laboratorium 2 had de (laatste) bewortelingsfase uitgevoerd in agar. Dit lab had vlak voor de bewortingsfase ook planten ter beschikking gesteld aan lab 1, die de plantjes bewortelde in veen en steenwol. Het vermoeden bestond dat bij beworteling in agar, agar aan de wortels zou blijven "kleven" en later een goede voedingsbodem zou zijn voor Fusarium. De plantjes uit de weefselkweek werden opgekweekt in veen en steenwol. Er waren aanwijzingen, dat bij opkweek in veen een sterkere plant, misschien door een ander, sterker wortelstelsel, ontstond dan bij opkweek in steenwol. Verder werd de helft van de planten geïnoculeerd met Fusarium en bij de helft van de planten werden de wortels doorgesneden. Fusarium zou dan door de wonden van de wortels de plant kunnen infecteren. In een tweede proef werd lage pH vergeleken met hoge pH.
Fusarium en wortelbeschadiging hadden geen betrouwbare invloed op het aantal "verkoopbare" en dode planten. Ook waren er geen interacties. Wortelbeschadiging gaf wel meer bossige planten.
Laboratorium en substraat hadden zeer betrouwbare effecten. Veen was beter dan steenwol: bij veen 6,8% en bij steenwol 19,6% dode planten. Dit resultaat komt overeen met de ervaring van opkweekbedrijven. Beworteling in agar (lab 2) was beter dan beworteling in veen en steenwol: 47,8% in de klasse grote verkoopbare plant ten opzichte van 36,8%, en 5% tegenover 21,3% dode planten. Voor het effect in de proef is wel een verklaring. De planten waren vlak voor de bewortelingsfase zwak. Eigenlijk hadden ze nog een delingsfase moeten ondergaan, maar daar was geen tijd meer voor. Tijdens de beworteling in agar in een gunstig microklimaat werden de planten weer wat sterker dan tijdens de beworteling in veen en steenwol.
Het blijkt, dat weefselkweekfase grote invloed heeft op de opkweek. Er waren ook betrouwbare interacties tussen lab en substraat. Een kleine plant op veen doet het verhoudingsgewijs beter dan een kleine plant op steenwol, d.w.z. minder uitval.
Er waren veel (gemiddeld 30,8%) bossige planten. Cytokinine werkte dus nog door tijdens de opkweek.
Bijna alle planten hadden harde misvormde blaadjes en sommige hadden bolvormige blaadjes. Het beeld komt sterk overeen met bolblad van komkommer, wat Ca-gebrek is en veroorzaakt wordt door te hoge luchtvochtigheid.