The correlation data presented in this paper support the idea that future harvests can in the short term be reliably estimated from existing or projected planting data. With this information, growers can make better decisions regarding the numbers and species of trees to produce and be able to choose an appropriate marketing approach. However, future research on the production and marketing of Christmas trees in southern states must address the issue of growing practices and the effect of selection of species on tree supply. Tree pricing and competition between retail tree outlets and choose-and-cut farms in urban areas will allow us to address the issue of Christmas tree market efficiency.

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Response of Periwinkle to Composted Sewage Sludge Used as a Soil Amendment[1]

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Abstract

An experiment was conducted in the greenhouse to investigate the effects of composted sewage sludge as a soil amendment on growth and mineral composition of 'Bright Eyes' periwinkle (*Catharanthus roseus* (L.) G. Dn.). Three desert soils (loamy sand, sandy loam, clay) were amended with two different composted sewage sludges (city and county) at rates of 0, 7.5, 15, 30 and 60% by volume. Plants were grown in the amended soils for four months. Composted city sludge had a positive effect on size, growth rate and number of flowers per plant in all three soils. Plants grown in soils amended with the county sludge grew poorly and developed an interveinal chlorosis. Tissue analysis suggested chlorosis was due to a calcium-induced manganese deficiency. Whole plant tissue Mn declined to as low as 23 mg/kg when the calcium in the soil extract exceeded 25 meq/liter.

Index Words: *Catharanthus roseus*, desert soils, tissue analysis, manganese

Significance to the Nursery Industry

The landscape/nursery industry has a growing need for high quality organic material for use as soil amendments, particularly in the desert southwest where soils are very low in organic matter. One promising source of organic matter is composted municipal sewage sludge. Cities across the country are seeking ways to dispose of their sewage sludge, with composting being one method that is both cost effective and environmentally sound. However, the commercial success of using composted sewage sludge as a soil amendment will depend on acceptance of the practice by horticultural and agricultural industries, which will, in turn, depend on positive experimental results.

In the present experiment, composted sewage sludge had contrasting effects on the growth and quality of periwinkle cultured in three different desert soils, depending on the source of the sludge. Because of the addition of large amounts of lime to the county sludge, an apparent calcium-induced manganese deficiency developed. This suggests that sludges with high lime content may not be appropriate for use as a soil amendment, at least with periwinkle. The greatest response to the sludge amendment was observed in the sandy soil, where water holding capacity increased dramatically. Based on flower production, a 30% composted sludge application rate of a low-lime sludge would be recommended.

Introduction

Municipalities across the country are increasingly faced with the challenge of sewage sludge disposal. Adding to this challenge, government agencies are closely regulating the disposal of such waste products to reduce the risk of environmental contamination, and actively promote the beneficial reuse of waste products whenever possible. As ex-

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isting sanitary landfills close and new landfills are opened at greater distances from waste treatment facilities, the use of composted sewage sludge in horticulture will become a more viable and attractive solution. In the arid southwestern United States, desert soils are typically low in organic matter (<1.0%) macro- and micronutrients, and water holding capacity, making plant production difficult. Amending these soils with organic matter generally improves soil physical properties and plant growth and vigor (14), providing that heavy metals, salts, toxic ions and pH are not excessive in the organic matter.

Several studies have demonstrated the value of composted sewage sludge to the nursery industry as a component of container soil mixes (4, 7, 11, 16). However, few studies have reported the complete tissue analysis of plants at different sludge application rates (1, 9, 11, 13) and, to our knowledge, only one has examined plant response to desert soils amended with sewage sludge (3). Therefore, an experiment was conducted to determine the growth and nutrition of periwinkle in response to soil type, source of composted sewage sludge, and sludge application rate.

Materials and Methods

The experiment was conducted from January 10 to May 10, 1989 in the research greenhouse located at the Southern Nevada Field Laboratory in Logandale, Nevada. The greenhouse temperature was maintained above 18°C (65°F) at all times. Photosynthetically active radiation (PAR) in the greenhouse was approximately 70% of ambient.

Two sewage sludges, composted by the aerated pile method (15) were provided by the Las Vegas Sanitation District (designated city sludge) and by the Clark County Sanitation District (designated county sludge). The city sludge was an anaerobically digested sludge to which alum was added at 160 mg/l during the clarifier step. The county sludge was a raw organic secondary sludge chemically conditioned to 20% lime and filter pressed. Wood chips were used as the bulking agent with both sludges to bring each to 40% solids. Each sludge was composted for thirty days and aged for an additional six months. Characteristics of these two sludges are presented in Table 1.

Three desert soils, a Jean loamy sand, a Calico sandy loam, and an Overton clay (Table 2), were first leached to reduce the level of soluble salts to a final EC_e of less than 3.3 dS m^{-1}. The soils were then amended with each sludge at rates of 0, 7.5, 15, 30 or 60 percent by volume. A fertilized control was included for comparative purposes. This treatment consisted of applying a granular 15N-6.5P-12.5K (15-15-15) commercial fertilizer to the soil surface at a rate of 0.6 g/pot preplant and again at six and twelve weeks.

Standard greenhouse pots, 15 cm (6 in) in diameter by 18 cm (7 in) deep, were filled with each of the thirty six soil/composted sludge treatments. Periwinkle (*Catharanthus roseus* (L.) G. Nd. 'Bright Eyes') was seeded at a rate of 20 seeds/pot. Plants were thinned to three uniform seedlings two weeks after germination. The experimental design was a 3 × 2 × 6 factorial with three replicates, arranged as a randomized block.

All pots were irrigated with tap water having an EC = 0.8 dS m^{-1} and containing (in meq/liter) 3.2 Ca^{2+}, 2.4 Mg^{2+}, 3.4 Na^{+}, 0.2 K^{+} and 3.5 Cl^{-}. Irrigations were scheduled based on tensiometers (Irrometer, Riverside CA) installed at a depth of 7.5 cm (3 in) in one pot of each soil/sludge treatment. Tensiometers were read three times per week and irrigation of all pots within a given treatment took place whenever the measured soil matric potential exceeded 0.02 MPa (20 centibars). Initially, 183 ml of tap water/pot (1 cm, 0.4 in depth) was applied at each irrigation; this was increased during the seventh week to 365 ml/pot (2 cm, 0.8 in depth). The redox potential was measured weekly in each pot at a depth of 7.5 cm (3 in) using platinum electrodes and a calomel electrode-portable pH/mV recorder (Orion Research ionanalyzer, model 399A, Cambridge, MA). Oxygen diffusion rates (ODR) were also recorded weekly at a depth of 7.5 cm (3 inch) in those pots equipped with the tensiometers, using platinum electrodes and an ODR ratemeter (Jensen Instruments, Tacoma, WA).

Plants were harvested at the end of the four month experiment by cutting at the soil line. Plant height was measured on a representative plant from each pot. The number of flowers and the maximum diameter of the flowers were also recorded for all plants. The three plants per pot were bulked and divided into the most recently expanded leaves

Table 1. Characteristics of the two composted sewage sludges used as an amendment in the experiment.

Soil Characteristic	Composted Sludges: City Sludge	Composted Sludges: County Sludge
EC_e(dS/m)	3.13	8.30
pH	6.93	7.04
Moisture content (%)	25.0	9.0
	% (dry weight)	
Nitrogen	1.57	1.27
Phosphorus	2.94	0.99
Potassium	0.18	0.10
Calcium	0.55	9.13[z]
Magnesium	0.12	0.48
Sodium	0.09	0.07
Sulfur	0.40	0.62
	mg kg^{-1} (dry weight)	
Iron	3290	2830
Aluminum	44770[y]	2790
Manganese	121	134
Copper	270	140
Zinc	680	410
Chromium	92	80
Lead	136	33
Nickel	13	16
Cadmium	3.1	1.1

[z]Due to addition of lime in sludge processing.
[y]Due to addition of alum in sludge processing.

Table 2. Characteristics of the three unamended desert soils used in the composted sludge application experiment.

Chemical characteristic	Unamended Soils: Loamy sand	Unamended Soils: Sandy loam	Unamended Soils: Clay
Organic matter (%)	0.4	0.8	2.5
pH	8.0	8.4	7.9
CEC (meq/100 g)	8.4	10.3	27.5
Clay content (%)	12.0	16.0	46.0

and into lower leaves plus stems. All fresh tissue was then washed in distilled water, oven dried at 70°C (158°F) for 48 hrs, weighed and ground to pass a 40 mesh screen. Tissue samples were analyzed commercially for N (Kjeldahl), P (colorimetric), K, Ca, Mg, Na, Zn, Fe, Mn, Cu, B, Al, Ni, Mo, Cd, Cr, and Pb (atomic absorption). Saturated paste extracts of soil samples [cores from each pot taken to a depth of 15 cm (6 in)] were analyzed for pH and EC_e by standard methods (14), and Ca and Mg by titration (14). Water content at saturation was calculated as grams of water per gram of dry soil required to bring the soil paste to saturation (14).

All data were analyzed using ANOVA to test for statistical significance of main effects and interactions. When significance was indicated, treatment means were compared by the least significant difference method. A general linear models procedure (SAS) was used to contrast sludge treatments. The model included all three soils, and all five composted sludge levels for both sludges plus the fertilizer control.

Results and Discussion

Soil Conditions: Oxygen diffusion rate (ODR) measurements varied from week to week, but no significant trends were found with either composted sludge application rate or soil type. Average ODR values for all treatments exceeded the critical value of 0.20 $\mu g\ cm^{-2}\ sec^{-1}$ suggested by Stolzy and Letey (10), and did not decrease with increased application rate. Similarly, no significant trends were observed for soil redox potentials. Average redox potential (Eh_7) of all treatments was equal to or greater than +445 mV, indicating that oxygen in the soil was not limiting.

The pH of both sludges was near neutral, whereas all three soils had pH values greater than 7.9 (Table 1, 2). The general linear models procedure indicated that the addition of either composted sludge significantly decreased soil pH ($P = 0.01$). For example, addition of 60% composted county sludge to the clay soil reduced the pH from 8.3 to 7.4.

Soil salinity varied between soil types and increased with composted sludge application rate in all three soils (Table 3). The composted county sludge had a higher initial salt content than the composted city sludge (EC_e = 8.30 vs. 3.13 dS m^{-1}). By the end of the experiment, there was no significant difference in soil salinity between the city and county sludge treatments, suggesting that soluble salts were effectively leached from the county sludge treatments during the experiment. However, all composted sludge amended soils had higher EC_e's than the controls.

The water holding capacity increased linearly with composted sludge application rate for all three soils ($P = 0.05$, data not presented). The largest change in water holding capacity was observed in the loamy sand soil between the 30% and 60% county sludge treatments, where water holding capacity increased from 0.39 to 0.80 g water/g dry soil.

Growth: Growth, as measured by plant height, was affected by the two composted sludges (Table 4). In the case of the city sludge treatments, growth increased with addition of composted sludge, exceeding the fertilizer control at the 7.5% rate. By contrast, growth was either not affected or reduced by addition of composted county sludge. In the sandy loam soil, plant height in the composted city sludge treatments exceeded that in the composted county sludge treatments by as much as a factor of three.

Table 3. Soil salinity (EC_e) expressed as dS/m at five sludge application rates on three different soils, measured before and after the experiment, and pH of the soil extract measured after the experiment.

Soil type	Sludge rate (%)	ECe (dS/m) City Sludge before	City Sludge after	County Sludge before	County Sludge after	pH City sludge	pH County sludge
Loamy sand							
	0	1.57	1.81	1.57	1.81	7.73	7.73
	7.5	2.77	2.27	3.54	3.28	8.10	8.36
	15.0	5.02	3.56	5.46	2.62	7.96	8.24
	30.0	5.57	4.08	7.18	2.56	7.52	7.25
	60.0	5.91	3.65	12.78	3.93	7.49	7.60
Sandy loam							
	0	3.21	3.72	3.21	3.72	8.26	8.26
	7.5	2.91	4.13	4.87	4.25	7.79	7.82
	15.0	4.43	4.22	6.30	3.94	7.82	8.13
	30.0	6.01	5.19	7.65	6.22	7.80	7.92
	60.0	6.97	4.55	13.15	6.74	7.60	7.44
Clay							
	0	2.48	3.70	2.48	3.70	8.33	8.33
	7.5	3.15	4.71	5.14	4.05	8.21	8.21
	15.0	4.06	4.24	5.02	5.31	8.12	8.17
	30.0	6.75	4.37	6.84	5.29	7.68	7.80
	60.0	7.31	4.72	11.59	5.00	7.95	7.36
LSD (0.05)		*	1.55	*	1.55	0.22	0.22

*No LSD because single samples from the bulk soil mixes were analyzed.

Composted sludge application rate and cation exchange capacity (CEC) accounted for 74% of the variability in plant height in the city treatments ($P = 0.001$). In the county treatments, separation was by cation exchange capacity only (coeff. of determ. 0.52, $P = 0.01$), with application rate not improving the coefficient. The linear models procedure indicated that the city and county treatments were significantly different in their effect on plant height ($P = 0.001$). Additionally, the city treatments were also significantly different from both the plus-fertilizer or minus-fertilizer controls, whereas no differences were found with the county treatments.

Dry weights of periwinkle followed a similar pattern as plant height, increasing with composted city sludge application rate (Table 4). However, dry weight showed either a negative or no response to application rate of the composted county sludge. In the sandy loam soil, dry weights were up to twenty times greater in the city as opposed to the county treatments. Composted sludge application rate and CEC again accounted for a large portion of the variability in the dry weights with the city treatments (coeff. of determ. 0.59, $P = 0.001$), whereas CEC alone accounted for 64% of the variability in the county treatments ($P = 0.001$).

No significant correlations were found for any of the soils between dry weights and initial soil salinity, final soil salinity, or their average. Thus, although growth may have been reduced by soil salinity early in the experiment, treatment differences could not be attributed to the final soil salinity, consistent with the response of other bedding plants to salinity (2).

Flowers. The number of flowers at final harvest was strongly correlated with the dry weight of the plant material (flower number = $-0.97 + 2.42 \times$ dry weight, $r = 0.91$, $P = 0.001$). More flowers were produced with composted

Table 4. Plant height, dry weight, and total number of flowers per treatment for periwinkle grown in three desert soils amended with composted city or county sewage sludge. Values are means of 3 composite samples.

Sludge rate (%)	Soil type	Plant height (cm)	Dry weight (g/pot)	Number of flowers
		City Sludge		
	Loamy sand			
0		5.4	0.93	0
7.5		9.3	0.80	8
15.0		17.1	6.76	22
30.0		15.8	7.20	17
60.0		18.7	4.03	16
Fert.		8.2	0.30	0
	Sandy loam			
0		7.8	1.93	0
7.5		8.7	0.73	0
15.0		14.5	5.17	11
30.0		18.0	11.87	32
60.0		19.4	8.50	22
Fert.		8.0	1.80	3
	Clay			
0		11.4	3.13	0
7.5		15.7	10.70	22
15.0		18.4	6.00	26
30.0		23.1	14.33	33
60.0		21.9	14.20	25
Fert.		13.6	2.93	1
		County Sludge		
	Loamy sand			
0		5.4	0.93	0
7.5		10.1	1.20	4
15.0		7.8	2.07	0
30.0		6.8	1.37	0
60.0		10.1	1.00	0
Fert.		8.2	0.30	0
	Sandy loam			
0		7.8	1.93	0
7.5		8.9	2.20	2
15.0		8.6	2.07	10
30.0		7.1	1.47	0
60.0		6.5	0.37	0
Fert.		8.0	1.80	3
	Clay			
0		11.4	3.13	0
7.5		10.1	3.83	5
15.0		12.9	5.30	14
30.0		10.8	2.20	0
60.0		11.9	3.73	14
Fert.		13.6	2.93	1
LSD (0.05)		2.9	1.84	—

city sludge amendment, since plants grown in the city treatment were larger. No correlation was found between the number and size (diameter) of flowers, and only in the clay soil was there a significant positive correlation between plant size and flower size ($r = 0.82$, $P = 0.001$).

Tissue Analysis. Tissue analysis for both upper leaves and lower leaves plus stems is presented in Table 5. Whole shoot concentrations of N, Zn and Mn were also calculated based on a weighted average. ANOVA indicated that of all the elements tested, only N, Zn and Mn were significant with treatment. However, it should be noted that both Cd and Pb were below 1.0 and 5.0 mg/kg (ppm) respectively, reflecting the non-industrial origin of both sludges.

Nitrogen concentration in the tissue was positively correlated with application rate of the composted county sludge in all soils (for the 7.5% through 60% application rate, $r = 0.85$, $P = 0.01$ for the upper leaves and $r = 0.71$, $P = 0.01$ for the lower leaves plus stems) but not with the composted city sludge treatments. This response to the county treatments is likely due to the reduction in plant dry weight concentrating the N rather than increased N absorption. ANOVA revealed significant differences between the city and county application rates on tissue N in both the upper ($P = 0.01$) and lower ($P = 0.001$) leaves. Percent N in the upper leaves of plants grown with composted city sludge was approximately 1% higher than that in comparable leaves from county treatments. Upper leaf N was also approximately 1% higher than lower leaf plus stem N when ANOVA was performed without regard to sludge source.

Tissue concentrations of Mn and Zn were not significantly correlated with dry weight in any treatment. However, whole plant concentration of Zn was correlated with composted sludge application rate in both the city ($P = 0.01$) and county ($P = 0.05$) treatments. Zinc concentration in both upper leaves and lower leaves plus stems increased significantly with composted sludge application rate ($P = 0.05$).

Manganese was the only nutrient found to be consistently higher in the tissue of plants from the city treatments as compared to the county treatments, in spite of similar Mn concentrations in the two composted sludges. The concentration of Mn in the upper leaves was significantly affected by application rate ($P = 0.001$) and composted sludge source ($P = 0.001$), with means for the composted city and county sludge treatments of 123 and 59 mg kg^{-1}, respectively. Manganese concentration in the lower leaves plus stems was also affected by soil type ($P = 0.001$) and the interaction between soil type and composted sludge source ($P = 0.001$). Mean concentrations for Mn in the lower leaves plus stems were 101, 94 and 75 mg kg^{-1} for the clay, sandy loam and loamy sand soils, respectively. Sanchez et al. (8) reported that extractable Mn decreased with increasing peat content in a limed sandy loam soil, but was not affected by peat content in the unlimed control. It is possible that the present results were at least partially due to a similar interaction of lime and organic matter.

The Ca concentration in the county sludge was approximately 16 fold greater than that in the city sludge, due to the addition of lime to the county sludge during the dewatering process. Because of the similar nutrient analysis of the two sludges (Table 2), it is difficult to ascribe the different growth response to different nutrient contents, except possibly for Ca. Calcium in the soil extract increased linearly ($P = 0.001$) with composted sludge application rate. High concentrations of tissue Mn, averaging 175 mg kg^{-1}, were found at low concentrations of Ca in the soil extract, but decreased rapidly to a low of approximately 23 mg kg^{-1} with increasing soil Ca. This relationship is described by the equation: $Mn = 216 - 5.40(Ca) + 0.05(Ca)^2 - 0.0002(Ca)^3$ ($r = 0.83$, $P = 0.001$) where Mn is whole plant Mn (mg/kg) and Ca is soil extract Ca (meq/l). The possibility of a Ca induced Mn deficiency was also supported by the observation of interveinal chlorosis. Because there was a very strong correlation between Ca concentration in the soil extract and soil salinity ($r = 0.94$, $P = 0.001$), it was not possible to separate the effect of these two variables on tissue Mn. It was interesting to note that, for plants

Table 5. The effects of sludge application rate and soil type on elemental composition (dry weight basis) for both upper and lower leaf tissue. Values are means of 3 composite samples.

		City Sludge					
		Nitrogen %		Zinc mg/kg		Manganese mg/kg	
Sludge rate	**Soil type**	**Upper**	**Lower**	**Upper**	**Lower**	**Upper**	**Lower**
	Loamy sand						
0		4.16	2.56	*	*	*	*
7.5		2.52	0.72	57	57	161	128
15.0		2.14	2.31	41	46	134	153
30.0		1.08	1.01	35	39	78	74
60.0		4.02	2.73	83	101	105	75
Fert.		4.91	2.98	*	*	*	*
	Sandy Loam						
0		4.44	2.76	27	29	172	189
7.5		3.69	2.75	53	58	173	164
15.0		1.33	1.96	48	50	162	142
30.0		2.18	0.67	33	35	124	98
60.0		3.59	3.02	67	78	95	93
Fert.		3.89	1.95	33	32	154	109
	Clay						
0		3.15	2.17	38	45	164	177
7.5		1.79	1.90	30	33	104	101
15.0		5.13	3.28	79	101	93	94
30.0		2.37	1.98	56	71	130	105
60.0		4.14	3.23	86	93	99	101
Fert.		5.16	3.36	56	71	131	140
		County Sludge					
	Loamy sand						
0		4.16	2.56	*	*	*	*
7.5		1.84	1.18	44	53	76	64
15.0		1.84	1.01	80	76	41	31
30.0		2.37	1.21	68	79	70	57
60.0		3.50	1.62	75	89	31	20
Fert.		4.90	2.98	*	*	*	*
	Sandy Loam						
0		4.44	2.76	27	29	172	189
7.5		1.29	0.98	44	46	36	42
15.0		1.43	1.39	72	80	32	19
30.0		1.32	0.82	54	53	35	31
60.0		2.43	1.42	48	51	39	27
Fert.		4.90	1.95	33	32	154	109
	Clay						
0		3.15	2.17	38	45	164	177
7.5		1.37	1.09	62	78	172	191
15.0		1.63	1.27	62	69	73	80
30.0		1.80	1.42	92	131	31	27
60.0		3.31	1.99	56	71	30	25
Fert.		5.16	3.36	56	71	130	140
LSD (0.05)		2.84	1.75	51	73	69	30

*Indicates insufficient tissue for analysis.

grown in the loamy sand soil, there was a significant multiple correlation between whole plant Ca (increased), whole plant Mn (decreased) and the number of flowers produced (decreased), accounting for 90% of the variability in flower production (No. flowers = 61.7 − 38.4(Ca) + 0.11(Mn); coefficient of determination 0.90, $P = 0.01$; tissue Ca is expressed as % and tissue Mn as mg kg^{-1}).

Decreased availability of micronutrients such as Fe, Zn and Mn in the soil are often attributed to increasing soil pH (12). In the present study, the decrease in soil pH with composted sludge application might be expected to increase micronutrient availability. However, it appears that the opposite occurred. When soil extract Ca and pH, and composted sludge application rate were regressed against whole plant Mn, using a backward stepwise procedure with variables removed when $P > 0.05$, the pH and application rate variables were both eliminated from the multiple regression. Calcium accounted for 48% ($P = 0.001$) of the variability in whole plant Mn. Thus, the decrease in tissue Mn was apparently not the result of high soil pH reducing soil Mn availability. Rather, these findings suggest that the increased Ca at the lower pH values was inducing a Mn deficiency, possibly due to competition between the two divalent cations during the absorption process. A similar competition has been reported by Kannan (5) and Maas (6).

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Effects of Growing Media and Aerial Environments on Acclimatization of *In Vitro*-Grown Miniature Rose Plantlets[1]

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Abstract

Initial acclimatization of *in vitro*-rooted plantlets of miniature rose (*Rosa chinensis* var. *minima* 'Red Ace') in high humidity and continuous light (most similar to the *in vitro* environment) increased plant growth (33% greater root area and 34% greater shoot area) relative to plants acclimatized under intermittent mist during early *ex vitro* growth stages, but later transfer to standard greenhouse conditions caused a temporary lag in continued growth. Direct transfer from *in vitro* culture conditions to a greenhouse mist bench inhibited growth during acclimatization, but permitted more rapid growth rate (28 and 30% more shoot and root area, respectively) during transition to the greenhouse growing environment. Direct transplant of *in vitro* rooted microcuttings to a growing medium containing soil resulted in high survival rate and circumvented the need for an interim potting medium, whereas transplant to Jiffy-9 pellets resulted in the highest plant losses.

Index words: greenhouse production, image analysis, micropropagation, *Rosa chinensis* var. *minima* 'Red Ace', 'Red Ace' miniature rose, tissue culture

Significance to the Nursery Industry

Procedures used by individual growers to acclimatize miniature roses produced *in vitro* to greenhouse conditions vary from use of high humidity tents or fog chambers to intermittent mist. During the transition from *in vitro* culture conditions to the *ex vitro* growing environment, any abrupt changes in the production environment will exert significant influence on the growth and quality of the final product. While miniature rose plantlets acclimatized in a low light, high humidity chamber exhibited superior overall survival and growth during the initial acclimatization process, plantlets transferred directly from *in vitro* culture to a greenhouse

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