

CUT ROSE QUALITY AS AFFECTED BY CALCIUM SUPPLY AND TRANSLOCATION

Baas, R.¹ and Marissen, N.²

Research Station for Floriculture and Greenhouse Vegetables (PBG)

Linnaeuslaan 2A

1431 JV

Aalsmeer

Netherlands

Tel: ++31/297/352525 Fax: ++31/297/352270 E-Mail: r.baas@pbga.agro.nl¹;
n.marissen@pbga.agro.nl²

Dik, A.

Research Station for Floriculture and Greenhouse Vegetables (PBG)

Kruisbroekweg 5

PO Box 8

2670 AA Naaldwijk

Netherlands

Tel: ++31 / 174/636700 Fax: ++31 / 174/636835 E-Mail: a.dik@pbgn.agro.nl

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Abstract

Possible relations between Ca and quality characteristics of cut roses (*Rosa hybrida*) were studied. In greenhouse experiments, factors presumably influencing the Ca flux in the xylem and/or the assimilate flux in the phloem were investigated with cut rose grown in a rockwool system. Previous work in 1996 had indicated that Ca in the buds was particularly influenced by the Ca concentration in the nutrient solution and by leaf removal. However, no effects on post-harvest parameters were found. In 1997, effects of Ca concentration and nutrient solution composition were studied in more detail in cut rose varieties First Red, Escada and Mercedes. It was found that compared to 4 and 7 mM Ca, 0.5 mM Ca decreased the number of stems and stem weight (length), particularly at higher K and Mg concentrations. Under Ca-deficient conditions, susceptibility to *Botrytis cinerea* was increased, necrosis and abscission of the older leaves occurred, and petal necrosis was slightly higher. Ca uptake and transport within rose is discussed.

1. Introduction

Calcium plays an important role in horticultural practice, due to its functions in plant metabolism (e.g. Palta 1996; Pooviah *et al.*, 1988). A very significant function of Ca is the strengthening of the cell walls and plant tissues, due to the binding of Ca to pectins present in the middle lamella. The degradation of pectins, ultimately leading to deterioration of tissues, is mediated by polygalacturonase activity, which is drastically inhibited by high calcium concentrations (Konno *et al.*, 1984)

Since Ca transport in the phloem is extremely low (Marschner 1995), Ca deficiency symptoms appear primarily in sinks for assimilates such as fruits. Low-transpiring tissues having low influx of Ca via xylem transport also can be sensitive to Ca deficiency. Examples of Ca deficiency in glasshouse horticulture are mostly known in vegetable crops, e.g. blossom-end-rot in tomato and sweet pepper, and tipburn in lettuce (Battey 1990). In floriculture, bract necrosis in *Poinsettia* (e.g. Woltz 1986) and spathe breakdown in *Anthurium* (Higaki 1980) can be the result of Ca deficiency.

In the Netherlands, various quality problems in cut roses can occur. For instance,

in some red varieties buds may show discoloration, malformation and necrosis (tip burn) of petals. Leaf problems include necrosis and abscission of particularly the leaflet leaves on the lower part of stems. In the post-harvest phase, flower blight or grey mould (infection with *Botrytis cinerea*) is a major threat for cut roses (Elad 1997).

The aim of this project was to investigate the possible role of Ca in the above described quality problems of cut roses. Previous work showed that particularly the Ca concentration in the nutrient solution, and leaf removal influenced Ca concentration in the buds, whereas B concentration and relative humidity had no or inconclusive effects on variety First Red (Baas, unpublished results). The present study describes the effects of Ca concentration in relation to other cations on Ca uptake and distribution, and the effects on production and quality of varieties First Red, Escada and Mercedes.

2. Materials and methods

2.1. Cultivation conditions

In a 150 m² greenhouse, 6 rolling benches (length 12 meter, width 1.2 m) were positioned. On each bench two gutters were fixed on which rockwool slabs (width 15 cm; height 7 cm) were placed. Nutrient solution was supplied with drippers 12-18 times per day during the experiment resulting in drain percentages of 75-95%. The drained nutrient solution was collected in a recirculation tank. The rockwool slabs were covered with plastic. Through holes in the plastic, rose cuttings propagated in rockwool blocks were planted in week 12 of 1997 at a density of 10 plants per m². Each experimental unit had a separate recirculation tank and consisted of two rows of 6 m, containing 60 plants.

Set-point temperature in the greenhouse was 18°C day/night during the experiment. CO₂ was supplied at 1000 PPM if no ventilation was performed. Assimilation lighting during 16 h.day⁻¹ was used from September until May if the outside radiation was less than 100 W/m².

2.2. Experimental design

The experiment was designed in a full-factorial design with 4 fertilisation treatments and 3 varieties as independent variables. The varieties used were First Red, Mercedes and Escada. There were two replicate greenhouses. The target concentrations in the fertilisation treatments were:

concentration (mM)	Treatment			
	Ca0.5+Na	Ca4	Ca7	Ca0.5+KMg
Ca	0.5	4	7	0.5
Na	13	6	0	0
K	5	5	5	12
Mg	1.8	1.8	1.8	4.8

Hence, Na in the Ca0.5+Na and Ca4 treatments and K and Mg in the Ca0.5+KMg treatment were used to compensate for the lower Ca concentrations compared to the Ca7 treatment.

Target concentrations for the other elements were equal in all treatments: NH₄⁺, <0.5 mM; NO₃⁻, 15 mM; H₂PO₄⁻, 1.5 mM; SO₄²⁻, 2.9 mM; B, 10 µM; Fe, 30 µM; Mn, 5 µM; Zn, 3 µM; Cu, 1 µM; Mo, 0.7 µM.

EC and pH of nutrient solution in the recirculation tanks were adjusted every week, and nutrient analysis was performed every two weeks. The amount of water and nutrients added to adjust to the target concentrations was recorded on a weekly basis. The experiment lasted until week 45 1997. Harvesting started in week 20. At harvest, number and weight of the stems were recorded.

2.3. Chemical analysis

Several times during the experiment Ca, K, Mg and Na concentrations in different plant parts were determined after drying (48 h at 70°C), grinding, and digesting. Plant parts consisted of petals, stems, or leaves of marketable stems. In week 45 the top and bottom three 5-7 leaflet leaves of a stem were sampled separately, and analysed on Ca, K, Mg and Na and B.

2.4. Post-harvest determinations

After harvest the roses were placed in a cold room at 5°C in water during two hours to recover from loss of turgor prior to post-harvest determinations.

2.4.1. Susceptibility to *Botrytis cinerea*

Flower buds including at least 10 cm pedicel were cut from the stems, and leaves and sepals were removed. The flowers were infected with conidia of *B. cinerea* (isolate BC16). This isolate was originally obtained from naturally infected rose flowers. Inoculation was carried out with conidia from 7-10 days old PDA cultures. The roses were placed horizontally under a Potter spray tower, and sprayed on all sides with 1 ml of a solution containing $5-9 \cdot 10^4$ conidia. Regularly, as a control for background infection, roses were sprayed with 1 ml of demineralized water.

After spraying, the pedicels of the roses were put in rectangular polythene boxes containing oasis saturated with demineralized water in boxes. After 24 h at 20°C at a r.h. >95%, the boxes were placed in a post-harvest chamber (20°C, r.h. 60%). Evaluation of susceptibility was done by estimating the area infected with spreading lesions at different days up to a maximum of 9 days, by viewing the flower from the bottom and estimating the percentage of diseased area in classes of 5%. Estimations were performed on 5-6 replicate flowers per plot. Afterwards, in the pooled flowers Ca, K, and Mg were analysed.

2.4.2. Vase-life, bud opening and petal necrosis

Six stems per plot were cut and individually placed in vases containing 1 litre demineralized water in a post-harvest room. Conditions in this room were 20°C, r.h 60% and light intensity of $1.5 \text{ W} \cdot \text{m}^{-2}$ during $12 \text{ h} \cdot \text{day}^{-1}$. Vase life was considered to be ended at the loss of turgor of the flowers. At that day, bud opening was visually estimated on a scale of 1-5 (1 = bud in early marketable stage; 5 = fully opened flower) and necrosis of the petals on a scale of 0-3 (percentage of the tips of the outer petals affected: 0=0%, 3=100%).

2.5. Statistical analysis

All data were subjected to analysis of variance (ANOVA). Data are presented as overall means from the fertiliser treatments and from the varieties. Significance of fertiliser treatment*variety interaction is given as follows: n.s = not significant; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Data from *B. cinerea* susceptibility testing were analysed using the standardised area under the disease progress curve (AUDPC), i.e. the calculated area under curve divided by the number of days the tests lasted (Campbell and Madden 1990).

3. Results

3.1. Nutrient concentrations and calculated nutrient uptake

Realised nutrient concentrations are given in Table 1. In general, target concentrations were reached well. The uptake of Ca in treatment Ca0.5+Na was higher than in treatment Ca 0.5+KMg, as is evident from the calculated Ca uptake (equals the loss, determined from the amount of added fertiliser divided by the transpiration).

A striking effect is the higher NH_4 uptake in the Ca 0.5 treatments compared to the Ca4 and Ca7 treatments. This higher NH_4 addition was necessary to control the pH in the low Ca-treatments, probably due to the low cation uptake relative to anion uptake. Mg uptake was increased in the Ca0.5 treatments, indicating a Ca/Mg antagonism.

3.2. Production and leaf quality

Production per period (both number of stems, average stem weight and total weight) during the experiment is given in Table 2. Production in the Ca 0.5 treatments lagged behind treatments Ca4 and Ca7, which between themselves did not differ significantly. Treatment Ca0.5+KMg had a lower average stem weight than treatment Ca0.5+Na, which was related to a shorter stem length (no data available).

In treatment Ca0.5+KMg leaf discolouring (yellowing of part of the leaf) occurred first in the leaves of bent-down shoots of c.v. First Red. Later, chlorosis followed by leaf necrosis became apparent on leaves of upright stems, also in Escada; the symptoms started at the base of stems, and moved upwards as the stems matured. Eventually, at the marketable stage, abscission of the leaves occurred in c.v. First Red. In Mercedes, these quality symptoms of Ca deficiency were less distinct.

In First Red, and on average for all varieties, total weight production in treatment Ca0.5+KMg was significantly lower than in treatment Ca0.5+Na.

3.3. Transpiration and water use efficiency

Transpiration during the experiment fluctuated between 200 and 750 $\text{l}\cdot\text{m}^{-2}$ per period of four weeks. The water use efficiency was not different between the treatments, and was - apart from the first period - remarkably similar during the experiment, around 100 l/kg FW per period.

3.4. Post-harvest determinations

3.4.1. Chemical analysis

Chemical analysis of different plant parts is given in Tables 3 and 4. As was found the previous year, Ca concentrations in the petals were lowest; Ca concentrations in the leaves were highest. For K, this was not the case. Ca concentrations were lowest and K and Mg were highest in the Ca 0.5 treatments, particularly in the Ca0.5+KMg treatment. Na concentrations were very low and were only slightly higher in the stems of the Ca 0.5+Na treatment, confirming the efficient exclusion of Na by cut roses.

When a distinction was made between top and bottom 5-7 leaflet leaves of a stem, Ca concentrations in the top are approximately twice the concentration in the bottom leaves (Table 4). Ca concentrations in young (approximately 2-3 week old) shoots had the lowest Ca concentrations, around 60 mmol/kg DM in the Ca 0.5 treatment (data not shown). The lower Ca concentrations therefore coincided with the chlorosis/necrosis in the bottom leaves (see 3.2).

Although to a lesser extent Mg showed the same pattern of low concentrations in the bottom leaves. In contrast, K and B concentrations in the bottom leaves were higher than in the top leaves.

3.4.2. Susceptibility to *Botrytis cinerea*

Disease progress curves were made by estimating the affected (brown coloured) area with spreading lesions in time. The normalised areas under these disease curves were calculated, and were highest in the Ca0.5+KMg treatment (Table 5). Lowest susceptibility was found in the treatments with 4 or 7 mM Ca. A slightly negative relation between *Botrytis* susceptibility and Ca concentration in the petals (Fig. 1) was found for Mercedes ($r^2=0.58$). This negative relation was also found for First Red ($r^2=0.76$) and Escada ($r^2=0.51$). Differences in susceptibility to *B. cinerea* between the varieties were clear: Mercedes was less susceptible to *Botrytis* than First Red and Escada.

3.4.3. Vase life, bud opening and petal necrosis

Twice during the experiment post-harvest characteristics were determined (Table 6). In week 22 only significant variety differences were apparent: vase life and bud opening were lower, and necrosis of the petals in Escada was higher than in varieties First Red and Mercedes. In week 32 however, significant treatment differences were found: vase life in the Ca0.5+KMg was lower than in the Ca7 treatment, and more necrosis of the petals occurred in the Ca0.5 treatments.

4. Discussion

From previous experiments in 1996, it was concluded that the experimental factors relative humidity, B concentration, and rootstock had little effect on the Ca concentration in the petals. Increased Ca concentration in the nutrient solution, and leaf removal did result in higher Ca concentrations, probably either by increasing the Ca flux in the xylem or by reducing the phloem flux. In these experiments, no effects were found on post-harvest parameters. In 1997, the variety Mercedes was used along the varieties First Red and Escada, and the method for studying *B. cinerea* susceptibility was changed: instead of counting lesions after inoculation, the progress of lesion spreading was estimated. The results show a clear effect of Ca treatments on *B. cinerea* susceptibility (Table 5). Susceptibility -determined as standardised AUDPC - correlated negatively with Ca in the petals (Fig. 1), which agrees with results from pot roses (Rasmussen Starkey and Pedersen 1997) and cut rose (Volpin and Elad 1991). Particularly in the Ca0.5+KMg treatment susceptibility was increased, which coincided with the lower Ca concentrations in the petals (Table 3), as well as in the other plant parts. Apparently, the presence of K and/or Mg in the Ca0.5+KMg treatment was responsible for a lower Ca uptake compared to the presence of Na in the Ca0.5+Na treatment. Although literature on antagonism of K, Mg and NH₄ on Ca uptake in crops is abundant (e.g. Kirkby 1979, Bar-Tal *et al.*, 1996) to our knowledge no information on antagonism is available for cut roses.

Concentrations less than 250 mmol/kg DM are considered liable to be Ca-deficient for fully grown 5-leaflet leaves (top leaves) in the Netherlands (De Kreij *et al.*, 1992). A level of 250 mmol Ca/kg DM (10 PPM) has been given as a minimum for adequate rose growth, for a range of growing conditions (White 1985, Ganmore-Neumann and Davidov 1993). Our results indicate that this guide value can be considered correct, although significant variety differences may exist.

Besides increased *Botrytis* susceptibility, quality parameters affected by calcium deficiency were leaf necrosis of the bottom leaves of mature stems, reduced stem length, shortened vase life, and increased necrosis of the petals (Table 6). These symptoms partly agree with results mentioned in literature. According to Asen and Tukey (1953), Ca deficiency in roses grown on glass beads was dependent on B supply, and resulted in bad root growth. The first and second 5-leaflet leaves were very small and distorted with curling and chlorosis at the tips of the leaflets, plus an abortion of the meristem which caused many short shoots to develop near the terminal. Older leaves became dull-grey and bent down. In our experiments the symptoms of the young leaflet leaves did not appear. With respect to the flower, a recent study on 'Royalty' roses in water culture indicated that 'black tip', described as sunken, black or brown areas on the tips of flower petals after harvesting, was related to extremely low Ca concentrations (15 mmol/kg DM) in the petals (Evans and Dodge 1997). Although in our study these low concentrations were never reached in the petals, a slight increase in necrosis of the petals was also found in the low Ca treatments in week 32 (Table 5).

The visual deficiency symptoms of leaves at lower regions of the stems corresponded with the low Ca concentrations of these leaves (Table 4). The lower stomatal conductivity of these leaves compared to that of top leaves that were fully expanded may be responsible for these results (data not shown). Since Ca is assumed to be transported primarily in the xylem, the accumulated transpiration of the top leaves should be higher than the transpiration of the lower leaves to account for the higher Ca

concentrations. This is remarkable, since the total period of transpiration of top leaves obviously is lower than that of bottom leaves. Presumably, transpiration increases during shoot growth, resulting in the above differences in Ca-accumulation. Following this theory, it is therefore likely that Ca deficiency resulting in visual leaf deficiencies starts in a stage that a new shoot is formed and growing/expanding. In this period, the new shoot is primarily phloem fed, resulting in low Ca concentrations. Young shoots indeed showed low Ca concentrations of around 60 mmol/kg DM. It can be hypothesised that if at this stage Ca import is too low compared to the phloem import, due to either low Ca uptake and/or low Ca translocation, or high phloem production, Ca deficiency may arise. These conditions may occur under high irradiation and/or low transpiration. Since visible Ca deficiency symptoms arise only in later stages (ca. 4-5 weeks later) of shoot development, the climatic or nutritional conditions responsible for the development of Ca deficiency can be blurred.

However, in contrast to Ca, B concentrations were higher in top leaves than in lower leaves (Table 4). Since B - as Ca - is also considered to be predominantly transported in the xylem, Ca and/or B distribution is more complex than anticipated. Detailed studies on transpiration and nutrient concentrations during shoot development may gain more insight in this matter.

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Tables

1. Realised average EC, pH and nutrient concentrations in the recirculation tanks during the experiment. Data are averaged over the varieties (n=78). ¹Loss of nutrients, determined by amount of fertiliser added, divided by the transpiration.

treatment	Ca0.5+Na		Ca 4		Ca 7		Ca 0.5+KMg	
	reali- sation	loss ¹	reali- sation	loss ¹	reali- sation	loss ¹	reali- sation	loss ¹
Ca (mM)	0.45	0.27	3.4	1.1	6.3	1.5	0.54	0.14
Na (mM)	12.9	0.5	6.7	0.1	1.2	0.1	1.3	0.1
K (mM)	4.2	2.0	4.0	2.0	4.0	2.1	10.5	3.2
Mg (mM)	1.6	0.5	1.7	0.3	1.7	0.3	4.4	0.8
NH ₄ (mM)	0.3	1.9	0.3	1.2	0.3	1.0	0.3	1.9
NO ₃ (mM)	13.9	5.0	13.6	4.9	13.9	5.3	14.3	5.9
H ₂ PO ₄ (mM)	1.2	0.6	1.1	0.7	1.1	0.8	1.2	0.6
SO ₄ ² (mM)	3.0	0.2	2.9	0.2	2.9	0.3	2.9	0.3
B (μM)	4	12	3	10	3	11	4	13
Fe (μM)	35	9	30	12	29	14	33	12
Mn (μM)	2	4	2	4	3	4	3	5
Zn (μM)	3	2	3	2	3	2	3	2
Cu (μM)	1	0.3	1	0.3	1	0.3	1	0.3
EC (mS/cm)	2.4	0.6	2.3	0.6	2.3	0.7	2.5	0.7
pH	5.2		5.3		5.2		5.3	

2. Average stem weight and production data of the harvest period week20-45 1997 of the varieties and totalled per plot. Each plot consisted of 60 plants.

production parameter	total no/plot	stem weight (g FW)	total wt./plot (kg FW)
treatment			
Ca 0.5+Na	900	36.4	29.7
Ca 4	1070	35.3	34.1
Ca 7	1041	34.9	33.3
Ca 0.5+ KMg	900	30.7	26.3
LSD	66	1.6	1.9

3. Chemical analysis (mmol/kg DM) of plant parts week 32 1997.

plant parts	petals				stem				leaves			
	Ca	Na	K	Mg	Ca	Na	K	Mg	Ca	Na	K	Mg
treatment												
Ca 0.5+Na	30	2	664	94	59	14	417	94	186	4	667	207
Ca 4	35	3	618	89	83	4	376	78	432	4	598	145
Ca 7	38	1	614	86	104	1	380	69	476	5	585	128
Ca 0.5+KMg	23	2	806	104	39	1	600	115	109	5	856	214
LSD	3	-	37	4	23	8	39	10	25	-	49	12
variety												
First Red	25	2	676	90	66	4	468	88	324	5	678	182
Escada	34	2	642	85	76	7	513	97	344	5	694	190
Mercedes	35	2	710	105	72	4	350	81	235	4	657	148
LSD	2	-	32	3	-	-	34	8	22	-	-	11
signific. treatm.*var.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	***	n.s.	n.s.	n.s.

4. Chemical analysis (mmol/kg DM, B in $\mu\text{mol/kg DM}$) of leaves of harvestable stems in week 45 1997.

plant parts	top 3 leaflet leaves					lowest 3 leaflet leaves				
element	Ca	Na	K	Mg	B	Ca	Na	K	Mg	B
treatment										
Ca 0.5+Na	243	2	642	223	6	102	6	918	132	10
Ca 7	625	6	500	137	6	303	4	745	110	9
LSD	19	2	39	9	-	25	2	49	18	-

5. Analysis of standardized Area Under Disease Progress Curve (AUDPC, in %-days) data of inoculated flowers of Mercedes and First Red and Escada.

variety	Mercedes week 25,33,43	First Red week 38	Escada week 38
treatment			
Ca 0.5+Na	22.5	28.8	39.1
Ca 4	17.4	25.6	40.5
Ca 7	12.2	24.3	39.0
Ca 0.5+KMg	28.7	42.9	51.0
LSD	9.2	8.6	8.6

6. Post-harvest determinations week 22 and week 32 1997.

post-harvest parameter	vase life (days)		bud opening (scale 1-5)		necrosis petals (scale 1-3)	
	week 22	week 32	week 22	week 32	week 22	week 32
treatment						
Ca 0.5+Na	9.8	15.1	3.8	4.2	1.5	1.9
Ca 4	-	16.0	-	4.0	-	1.4
Ca 7	11.4	16.8	4.1	4.1	1.1	1.5
Ca 0.5+KMg	10.7	14.3	4.0	3.9	1.4	1.9
LSD	-	1.7	-	0.2	-	0.4
variety						
First Red	11.8	14.9	4.2	4.2	1.3	2.0
Escada	8.3	15.0	3.5	3.9	1.7	1.7
Mercedes	11.8	16.7	4.3	4.1	1.0	1.2
LSD	1.9	1.5	0.4	0.2	0.6	0.3
signific. treatm.*var.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Figures

1. Figure Relation between Ca in the flower and infection (standardized Area Under Disease Progress Curve) of *B. cinerea*.

