Increased Levels of Calcium in the Nutrient Solution Improves the Postharvest Life of Potted Roses

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ABSTRACT. Experiments were performed on miniature potted roses (*Rosa*) to investigate whether increasing the Ca concentration in the nutrient solution would increase the Ca concentration in the rose plant, resulting in better postharvest quality. The plants were watered during the whole production period with six different nutrient solutions with Ca at 1.1 to 4.4 mM and varying meq ratios between Ca and the cations. During the postharvest period, plant characteristics were recorded at days 0, 11, 14, 18, and 25. Increasing Ca in the nutrient solution significantly increased the Ca concentration of the plants. However, the NH_4 level (2% to 5% vs. 22% to 25%) also influenced the Ca concentration of the plants. Leaves had the highest Ca concentration, which was more than twice the level in flowers and buds, stems and roots. There were no differences in dry matter content between treatments in roots or tops at marketing stage. At the start of the postharvest treatment (day 0), plants from the highest Ca treatment had significantly fewer flowers than those in all of the other treatments due to delayed development. The number of good flowers increased from days 0 to 14 irrespective of treatment, while a further increase to day 18 was observed in plants from the three highest Ca treatments (low NH_4) due to a negative effect on flower longevity of low Ca and high NH_4 in the three low-Ca treatments. By day 25, the number of good flowers had decreased markedly in all treatments, but plants from the second-highest Ca treatment had significantly more good flowers than plants from the other treatments. Flower wilting was due to infection by grey mold (*Botrytis cinerea*) and a physiological condition that caused wilting that began at the petal edges with a dark discoloration. Infection with grey mold was negatively correlated with Ca concentration in the flowers. The physiological wilting was seen earlier in plants from the treatments with high NH_4 and is probably not related to Ca concentration in the flowers.

The postproduction quality of potted roses differs between growers with different growing conditions (Borch et al., 1995), but it is not clear how and to what extent different growing conditions influence the postharvest life of potted roses. The main causes of decreased postharvest life are wilted flowers, infection by grey mold (*Botrytis cinerea*), and yellowing of flower buds.

Potted roses are sensitive to ethylene and silver thiosulfate (STS), and other ethylene inhibitors have been shown to extend the postharvest life of potted roses (Serek, 1993; Serek et al., 1994; Tjosvold et al., 1994). Other results show that the cultivars respond differently to STS (Williams, personal communication) and that STS has the greatest effect on cultivars that already have a relatively good postharvest quality.

It is clear that Ca influences senescence, expressed as a loss of chlorophyll and proteins, increased membrane leakage, dissolution of middle lamella, and associated changes in the cell wall (Poovaiah, 1988). Shear (1975) lists more than 30 Ca-deficiency disorders that are often associated with meristematic or storage tissue, such as tipburn of lettuce (*Lactuca sativa*), blossom end rot of tomatoes (*Lycopersicon lycopersicum*) and peppers (*Capsicum annuum*), bitter pit of apples (*Malus*), and internal browning of brussels sprouts (*Brassica oleracea* var. *gemmifera*).

Calcium is also involved in ethylene synthesis, perhaps through its stabilizing effect on cell membranes, as the conversion of ACC to ethylene is mediated by an uncharacterized membrane-bound enzyme (Lurie et al., 1989). Studies with climacteric tissue such as apples has shown that treatment with concentrations of Ca >0.04 M inhibits ethylene production (Chevery et al., 1988; Lurie et al.,

1989; Song and Bangerth, 1993), while lower concentrations (<0.01 M) seemed to stimulate ethylene production (Chevery et al., 1988). In rose flowers, ethylene production was lower from plants given a higher Ca concentration in the nutrient solution (Volpin and Elad, 1991).

Calcium is considered to move preferentially in the xylem (Bangerth, 1979), and the Ca concentration in low-transpiring organs such as flowers, which are predominantly supplied via the phloem, is therefore low. High growth rates of low-transpiring organs increases the risk that the tissue concentration of Ca will fall below a critical level for cell wall stabilization and membrane integrity (Marschner, 1995). In the bracts of poinsettia (*Euphorbia pulcherrima*) with low stomatal density, Ca concentration was found to be considerably lower than in the leaves (Strømme et al., 1994). A correlation between Ca concentration of the bract edges and the incidence of bract necrosis was found in susceptible cultivars; this necrosis could be avoided by sprays of Ca to the bracts.

Calcium uptake is considered to be passive. Interactions with other cations strongly influence Ca uptake, and cation antagonism by K, Mg, and NH_4 can substantially depress Ca uptake (Kirkby, 1979).

The objective of this work was to determine whether an increased Ca concentration in the nutrient solution could increase the Ca concentration in miniature potted roses, resulting in an improved postharvest quality.

Materials and Methods

PLANT MATERIALS AND GROWING CONDITIONS. Cuttings of the potted rose variety 'Poulprima' (Purple Parade) were received from a commercial grower in week 14, 1995. The growing medium was a commercial product, Seramis, consisting of kiln-dried clay particles, of which 70% was 2 to 4 mm with a bulk density of 390

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Table 1. Concentrations (mM) of major nutrients in the six nutrient solutions used in the experiment.

Nutrient	Treatment					
	1	2	3	4	5	6
	Ca/cation meq ratio					
	0.15	0.24	0.32	0.32	0.44	0.53
NO ₃ -N	10.2	10.1	10.1	12.2	11.9	11.8
NH ₄ -N	2.9	3.2	3.3	0.2	0.7	0.4
P	1.0	1.0	1.0	1.0	1.0	1.0
K	7.3	6.8	4.9	7.3	5.9	4.9
Ca	1.1	2.1	2.5	2.5	3.5	4.4
Mg	1.3	1.3	0.9	1.4	0.8	0.9
Na	0.4	0.5	0.5	0.5	0.4	0.5
Cl	0.7	1.0	1.0	0.9	0.8	1.0
S	1.6	2.3	1.4	0.5	0.5	0.7

g·L⁻¹. The medium has a low cation exchange capacity (5 meq/100 g of substrate) and a very low nutrient concentration. Five cuttings were inserted in a 10-cm pot and after rooting were reduced to four plants per pot.

The plants were watered during the whole production period with six different nutrient solutions (in the following referred to as treatments 1–6) with varying Ca concentration and meq ratios between Ca and the total amount of cations (Table 1). The nutrient solutions were made using rainwater or a mixture of rainwater and borehole water. The bicarbonate level was titrated with acid down to a level of 50 ppm. In the nutrient solution the electrical conductivity (EC) was almost the same (1.6–1.8) and pH was 5.4–5.6 for all treatments. The total amount of nitrogen varied only slightly between the solutions (12.2–13.3 mM). To achieve this when the amount of Ca(NO₃) was low in the low-Ca treatments (1–3), NH₄NO₃ was the supplementary nitrogen source. To separate the effects of Ca and NH₄, two of the solutions had the same Ca/cation meq ratio (0.32), but a high and low concentration of NH₄NO₃ (treatments 3 and 4). In treatment 4, which had a low NH₄NO₃ content, KNO₃ was used as a nitrogen source. Micronutrients were added to all six solutions in the same concentration (mM): Fe, 0.02; Mn, 0.01; B, 0.02; Cu, 0.002; Zn, 0.005; and Mo, 0.0006.

Treatments were replicated four times. Each plot consisted of a 0.3-m² ebb-and-flood bench unit connected to a 25-L container from which the nutrient solution was recirculated. The plants were subirrigated 1–2 times a day and pH and EC were measured twice a week in the solution after an irrigation. As it is not possible to measure pH and EC in Seramis as it is in peat, it was assumed that by frequent irrigation the pH and EC of the rootzone would be the same as in the recirculated solution. The solution in all plots was changed if the pH was <4.5 or >6.5 in the recirculated solution from one of the plots. The pH in the solution was rising with low NH₄ content and falling with high NH₄ content. The solution was changed seven times during the experiment.

The plants were grown according to commercial practice for potted roses: 3 weeks after start of propagation, plants were cut back to 30 mm in height (first cutting back) and 3 weeks later cut back again (second cutting back). The plants were spaced twice to a final spacing of 30 plants/m². Minimum air temperature was 24 °C during propagation, 21 °C between the first and second cut back, and 19 °C from a week after the second cut back. Supplementary light (90 μmol·m⁻²·s⁻¹) was given 20 h a day.

PLANT ANALYSIS. When the plants were cut back the first time, plant material from all four plots per treatment was analyzed as one sample for major and minor elements (total N, P, K, Mg, Ca, Na,

Cl, S, Fe, Mn, B, Cu, and Zn). After the second cutting back, plant material from one plot per treatment was analyzed for major and minor elements and from four plots for Ca. In week 25, when plants were spaced the second time, the fresh and dry masses of tops and roots from four plants from each plot were recorded, and the top from one plot was analyzed for major and minor elements. At marketing stage, in week 26, fresh and dry masses of stems, leaves, flowers and buds, and roots were recorded, and the same plant parts were analyzed for Ca. Plant analyses were conducted at an authorized Danish laboratory using the dry-ash method.

POSTHARVEST TREATMENT AND PLANT MEASUREMENTS. At marketing stage, three plants per plot (twelve per treatment) with three to five open flowers were subjected to simulated transport for 4 d [16 °C, 90% relative humidity (RH) in darkness] and subsequently moved to a post production evaluation room (20 ± 0.2 °C, 60% RH, 8 μmol·m⁻²·s⁻¹, 12-h day) and subirrigated with a weak nutrient solution of 1.5 mS·cm⁻².

Keeping-quality value, total number of flowers (including fresh, damaged, and wilted flowers), number of damaged and wilted flowers, and number of plants with grey mold were measured at days 0, 11, 14, 18, and 25. On days 14, 18, and 25, the number of yellow leaves (which were then removed) and the number of flowers with wilting petal edges were recorded.

Keeping-quality values (1–5) are a subjective assessment based on the overall appearance of a plant. At day 0, all plants were given a keeping-quality value of 5, indicating no damaged or dead flowers and no other negative parameters. A keeping-quality value of 3 represented a plant that was no longer a perfect product, but still had an acceptable quality for the consumer. A plant with a keeping-quality value of 1 had unacceptable quality, suggesting that the plant had no value to the consumer. Day 11 results indicated plant quality after the plants had been transported but remained marketable, while day 18 results indicated the consumer satisfaction of the plant. The cause of death was recorded.

A plant was considered dead (no ornamental value for the consumer) if

- on day 11 it had more than one wilted flower or >50% yellow leaves on the bottom third part of the plant or had one shoot with more than three dead consecutive nodes due to grey mold, or
- on days 14, 18, and 25 it had >30% wilted flowers or >50% yellow or wilted flower buds or 100% yellow leaves on the bottom of the plant or had one shoot with more than three dead consecutive nodes due to grey mold.

SURVIVAL ANALYSIS AND STATISTICAL ANALYSIS. The postharvest measurements have been analyzed by means of survival analysis

methods (Kalbfleisch and Prentice, 1980). For each treatment and cause of death (first occurrence of wilted flowers, yellow leaves, etc.), a survival function expresses the probability of surviving as a function of time after transport simulation; i.e., the value at a given time point is the probability of surviving a period of that length after transport simulation. Treatments are compared by statistical comparison of the survival functions.

Calcium uptake was analyzed by SAS's GLM procedure (SAS Institute, Cary, N.C.) and keeping-quality value, percent wilted flowers, and number of good flowers were analyzed using SAS's ANOVA procedure. The percentage of plants with grey mold in relation to Ca concentration in dry matter of flowers and buds was analyzed using SAS's GENMOD procedure.

Results

PLANT GROWTH. There were no significant differences between treatments on the dry mass of the buds and flowers, leaves, stems, or on the total dry mass of the top of the plant at the end of production. At the second spacing (2 weeks before sale) treatment 5 had a significantly higher dry mass of the top compared to treatments 1, 2, 3 and 6. All the other treatments were not significantly different (data not shown). There was no significant difference on root dry mass at the time of second spacing or at the end of production.

CALCIUM UPTAKE. The Ca concentration in the different plant parts analyzed at the end of the production was significantly increased by increased Ca/cation meq ratio in the nutrient solution (Fig. 1 a–d). For leaves and stems, no interaction between the Ca/cation meq ratio and the NH_4 level in the nutrient solution was evident. The Ca concentration in flowers and buds was slightly higher when the NH_4 concentration in the nutrient solution was high ($p = 0.06$), but in the roots it was slightly lower ($p = 0.15$).

Overall, leaves had the highest Ca concentration with 1.15% of dry matter. Stems and flowers (including buds) had 0.50% and

0.49% Ca respectively, while roots contained 0.38% Ca.

NITROGEN UPTAKE. Data on uptake of nutrients other than Ca is weak, as only one sample per treatment was analyzed at three different times during production. However, there seems to be no effect of ammonium on N uptake in any of the three analyses (data not shown). The concentration of N in percentage of dry matter varied from 2.6 to 4. The highest values for all treatments were found in the youngest plant material.

POSTHARVEST PERFORMANCE. At the start of the postharvest treatment (day 0), the highest Ca treatment (treatment 6) had significantly fewer flowers than all the other treatments due to a later development (Fig. 2a). The number of good flowers per plant increased from days 0 to 14 in all treatments, while in plants from treatment 4–6 (low NH_4), a further increase to day 18 was observed. At day 25, the number of good flowers had decreased markedly in all treatments, but plants from treatment 5 had significantly more good flowers than plants from the other treatments.

The percentage of wilted flowers was significantly different between treatments on days 14 and 18 (Fig. 2b). At day 14, the lowest Ca treatment (1) had significantly more wilted flowers than treatments 3–6. At day 18, plants from the three lowest Ca treatments with high NH_4 (1–3) had significantly more wilted

Fig. 1. Calcium concentration as percent of dry matter in different plant parts at the end of production; (●) low NH_4 , (○) high NH_4 . Each symbol on the line represents a treatment. The six treatments—0.15–0.53 Ca/cation meq ratio—are described in Table 1. Treatments 3 and 4 have the same Ca/cation meq ratio but 25% and 2% NH_4 , respectively. Bars indicate 5% LSD between treatments. *Significant at $p = 0.05$.

(A) Flowers and buds: $p = 0.001$; $R^2 = 0.78$; low NH_4 , $y = 0.823x + 0.256$; high NH_4 , $y = 0.823x + 0.165$.

(B) Leaves: $p = 0.0001$; $R^2 = 0.91$; $y = 2.27x + 0.415$.

(C) Stems: $p = 0.001$; $R^2 = 0.89$; $y = 0.968x + 0.182$.

(D) Roots: $p = 0.001$; $R^2 = 0.79$; low NH_4 , $y = 1.0x + 0.11$; high NH_4 , $y = 0.57x + 0.11$.

Fig. 2. Development of flowers during postharvest. Postharvest treatment included simulated transport for four days [16 °C, 90% relative humidity (RH) in darkness] and subsequently plants were moved to a post production evaluation room (20 ± 0.2 °C, 60% RH, 8 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 12-h day). Treatments 1–6 are described in Table 1; n = 12 plants. Bars indicate 5% LSD for each day. ****Significant at $p = 0.05$, 0.01, or 0.001. (A) Number of good flowers. (B) Percent wilted flowers.

flowers than plants from the three highest Ca treatments with low NH_4 . The NH_4 concentration had a negative effect, as the 0.32 Ca/cation meq ratio treatment with high NH_4 had significantly more wilted flowers than the same treatment with low NH_4 .

Some of the flower wilting commenced with a shriveling of the petal edges followed by a discoloration and wilting of the whole flower. Other flowers wilted due to grey mold. The probability of dying due to wilting of flowers in treatments 1–3 and treatments 4–6, respectively, can not be separated statistically, but the survival function of the two groups differ significantly (Fig. 3a).

Yellow leaves appeared at the base or half way up the stems and would easily abscise. The yellowing typically started from both ends of the leaf, leaving a transverse green band. It first appeared in treatments with high NH_4 (day 11), but did not appear in treatments with low NH_4 until day 25. The total number of yellow leaves removed from the plants varied from 0.2 to 8; the greatest number of yellow leaves occurred in treatments with high NH_4 (data not shown).

The probability for a plant to develop the first yellow leaves showed that treatments 1–3 and treatments 4–6 can not be separated statistically, but the two groups differ significantly from each other (Fig. 3b). The survival analysis show that the probability of a plant not having developed yellow leaves on days 14, 18, and 25 is, respectively, $\approx 25\%$, 10%, and 5% as an average of treatments 1–3 and 100%, 100%, and 80% as an average of treatments 4–6.

The first plants with grey mold were observed on day 11 after the start of the postharvest treatment. There was a tendency ($p = 0.07$) on day 11 for increased grey mold infection if the Ca concentration in flowers and buds was low. By days 14 and 18, there was a significant effect of the Ca concentration (Fig. 4).

A survival analysis of the probability of a plant's developing grey mold (Fig. 3c) showed that treatments 1–4 and treatments 5–6, respectively, were not statistically different. The sensitivity to grey mold is therefore clearly a Ca and not an NH_4 effect and supports the correlation between Ca concentration of the flowers and the incidence of grey mold (Fig. 4).

The two highest Ca treatments (5 and 6) kept the maximum keeping-quality value longest. Conversely the keeping-quality value of the lowest Ca treatment (1) decreased fastest (Fig. 5). The keeping-quality value at days 14, 18, and 25 was strongly influenced by the nutrient solutions. On day 14, only the two highest Ca treatments still achieved the maximum value of 5, which was significantly higher than all the other treatments. At day 18, only the two highest Ca treatments, 5 and 6, were given a keeping-quality value of >3 (still acceptable for the consumer). The keeping-quality value of the two lowest Ca treatments (1 and 2) was not significantly different from treatment 3 but were significantly lower than the same Ca treatment with low NH_4 (treatment 4). At day 25, treatment 5 had a significantly higher keeping-quality value than the other treatments, but at that time all plants had lost their ornamental value.

Fig. 3. Survival analysis ($p < 0.0001$). For each treatment and cause of death (first occurrence of wilted flowers, yellow leaves etc.) a survival function expresses the probability of surviving as a function of time after the start of transport simulation, i.e. the value at a given time point is the probability of surviving a period of that length after transport simulation. (A and B) Treatments 1–3 (■), 4–6 (●). (C) Treatments 1–4 (■), 5–6 (●). Open symbols and dotted lines indicate 95% confidence limits. (A) The cause of death is the first occurrence of wilted flowers. (B) The cause of death is the first occurrence of yellow leaves. (C) The cause of death is the development of grey mold.

Fig. 4. Percentage of plants with grey mold on day 14 and 18 of the postharvest period. For description of postharvest treatment see Fig. 2. (□) Observed values and (—) an estimated line: $y = e((2.88 - 6.98x)/3) \times 100$ for day 14; $p = 0.03$. (○) Observed values and (---) an estimated line: $y = e((3.02 - 6.69x)/3) \times 100$ for day 18; $p = 0.01$. Data were analyzed and the lines estimated by a poisson regression with a log transformation using the GENMOD procedure.

Discussion

The Ca concentration of the plant was influenced by increasing the Ca concentration in the nutrient solution. The correlation was linear, which has also been shown by Voogt (1988) in tomatoes.

It has been generally established that using NH_4 as the N source compared to NO_3 has a negative influence on Ca uptake (Kirkby, 1979). This has been confirmed in roses (Woodson and Boodley, 1982). Our results showed that, at flowering, only the Ca concentration in the roots was reduced at the high (22% to 25%) NH_4 concentration. Wilcox et al. (1973) showed that, at a 1 NH_4 : 1 NO_3 ratio, Ca concentration of tomatoes and corn (*Zea mays*) was reduced with a more pronounced effect in the roots. Errebhi and Wilcox (1990) found in one experiment with tomatoes that a NH_4 level of 18% caused a reduction in Ca concentration in the roots but not in the shoots compared to 0% or 9% NH_4 . When the experiment was repeated, 18% NH_4 reduced Ca uptake in the shoots and roots. This suggests that, at relatively low NH_4 concentrations, other factors may influence the interaction between NH_4 and Ca uptake.

For the same Ca level in the nutrient solution, the higher NH_4 level (and subsequently lower K) resulted in a higher Ca concentration in the flowers. This is supported by results of Strømme et al. (1994), who showed that K concentration depressed Ca concentration in the bracts of poinsettia more than NH_4 , resulting in a higher incidence of bract necrosis.

Generally, leaves contained the most Ca, while the Ca concentration in stems, roots, and flowers and buds was only half the leaf concentration. This agrees with the knowledge that Ca is moving upwards in the xylem, especially to plant parts with a high transpiration rate (Kirkby 1979, 1984).

The postharvest performance of a potted rose depends on the development of new flowers and the longevity of the flowers, i.e., expressed as the number of good flowers. The composition of nutrient solution in this experiment had an effect on the number of good flowers, due to more flowers developing, especially in the beginning of the period, and to fewer flowers wilting.

The wilting of the flowers was due to infection with grey mold and to a physiological condition, which caused wilting to begin at the petal edges as a dark discoloration. The physiological wilting

was seen earlier in the treatments with high NH_4 and is probably not related to the Ca concentration in the flowers. The negative effect of NH_4 could be due to the decrease in pH during cultivation, despite regular adjustment. This may have caused root damage directly, or it could be due to a lower carbohydrate reserve in NH_4 -treated plants, leading to an earlier depletion of resources during postharvest. Raab and Terry (1995) and others have shown that rapid NH_4 assimilation into glutamine consumes carbon skeletons, which leads to a depletion of foliar starch, sucrose and maltose. The survival analysis on the probability of a plant's being dead (expressed as wilted flowers) also shows that, on day 18, the six treatments can be divided into two groups, where the probability of a plant's still being alive on day 18 is $\approx 90\%$ for treatment with low NH_4 and $\approx 30\%$ for treatment with high NH_4 .

With respect to the appearance of yellow leaves, the treatments appear to be divided into two groups (1–3 and 4–6). Due to the treatments chosen, it is difficult to identify whether this is a Ca or an NH_4 effect. The reason for this is that the high NH_4 treatments also had a low Ca concentration in the solution and in the leaves, and the two treatments with the same Ca concentration in the solution but high or low NH_4 differed in the number of yellow leaves but not in Ca concentration in the leaves. In general there was no correlation between the Ca concentration in the leaves and the appearance of yellow leaves and, therefore, it is probably an effect of NH_4 . Chlorosis and loss of chlorophyll have been reported in connection with NH_4 toxicity in tomatoes (Puritch and Barker, 1967) and in *Sinapis alba* (Mehrer and Mohr, 1989). In both cases, NH_4 or nitrate were used as the sole source of nitrogen and not in different ratios as in this experiment (2% to 25% NH_4). In chrysanthemum (*Dendranthema grandiflorum* hybrid) a 50 NH_4 : 50 NO_3 ratio also reduced chlorophyll concentration compared to 100% NO_3 (Krizek et al., 1987).

At the start of the postharvest period, there were no visible signs of grey mold. This supports the results of Elad (1988), who found that latent infections occur at harvest but do not develop until flowers mature, especially under humid conditions. The development of grey mold during postharvest was negatively correlated to the Ca concentration of the flowers; plants with the lowest Ca concentration were the most infected with grey mold. Increasing Ca in the nutrient solution reduced grey mold on beans (*Phaseolus*) and tomatoes (Elad and Volpin, 1993), cut roses (Volpin and Elad,

Fig. 5. Keeping-quality value during postharvest. Keeping-quality values (1–5) are subjective assessments based on the overall appearance of a plant. At day 0 all plants were given a keeping-quality value of 5, indicating no damaged or dead flowers and no other negative parameters. For description of postharvest treatment see Fig. 2. Treatments 1–6 are described in Table 1; $n = 12$ plants. Bars indicate 5% LSD for each day.

1991), and kohlrabi (*Brassica oleracea* var. *gongylodes*) (Schwab et al., 1993) as well as *Penicillium* and *Glomerella* on apples during storage (Conway et al., 1993) and bacterial wilt in tomatoes (Yamazaki and Hoshina, 1995). Calcium has also been shown to inhibit pectic enzymes, e.g., polygalacturonase, which makes it more difficult for the pathogen to degrade the pectic substances in the middle lamellae (Conway et al., 1993; Volpin and Elad, 1991).

The results from this experiment clearly show that increased Ca/cation meq ratio in the nutrient solution increased the Ca concentration in the plant and subsequently resulted in better postharvest quality. Both NH_4 and Ca affect the survival of plants, and further investigations are required to elucidate interactions between NH_4 and Ca.

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