Influence of Calcium Nutrition on Susceptibility of Rose Flowers to Botrytis Blight

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ABSTRACT

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Cut rose flowers were incubated in solutions of $CaCl_2$ or $CaSO_4$ for 2 days and then incubated under gray mold-conducive conditions. Severity of gray mold symptoms was reduced 60% by the treatments. Results varied with calcium concentration (1–50 mM) and rose cultivar. $Ca(NO_3)_2$ was added to the nutrient solutions of rose plants grown in plots in commercial greenhouses. Other nutrients in the fertilizer solutions were constant in the different treatments. The calcium content of stems, leaves, and flowers was increased by calcium treatment in the greenhouse. Calcium at a concentration of 3.5 mM reduced the severity of postharvest Botrytis blight in naturally infected flowers by 55%, and by 30% when flowers were further inoculated before incubation under humidity conditions. Ethylene production in flowers with high calcium content was decreased by 50–95%. Increasing the potassium concentration in the fertilizer solution negated the ability of calcium to reduce Botrytis blight susceptibility of flowers, probably due to competition for cation uptake. Calcium presence in liquid medium decreased the ability of *Botrytis cinerea* to utilize pectate and to produce polygalacturonases by up to 100%.

Additional keywords: *Rosa hybrida*, soilless medium.

Botrytis blight, caused by *Botrytis cinerea* Pers.:Fr., is a widespread problem of roses (*Rosa hybrida* L.) grown in greenhouses. The pathogen mainly attacks flowers but also causes necrotic lesions on renewal canes (epicormic shoots). Infection induces lesions on petals; however, latent infections occur that are not visible at harvest but develop under humid conditions or as flowers mature (5). The most severe economic damage occurs when disease develops on flowers in storage or in transit (4,9). Fungicides are recommended for control of the disease on roses (9), but the efficacy of fungicidal treatments is limited. Generally, roses are harvested in the bud stage, but infection may occur between sprays on flowers that have opened slightly. Also, populations of the pathogen that are resistant to dicarboximide, benzimidazole, and diethofencarb fungicides have developed (12,13). One method of partially controlling the disease on roses is to lower greenhouse humidity by ventilation and heating (17).

Increased calcium content in plant tissues inhibits development of some diseases. Fertilizing with calcium reduced Fusarium wilt of tomato (3) and muskmelon (25), and also the susceptibility of bean hypocotyls to *Rhizoctonia solani* (2) and of soybean to *Colletotrichum dematium* (18). Liming of low calcic soils caused a reduction of tomato gray mold incidence (26). The effect of calcium on disease was related to the inhibition of polygalacturonase and other pectic enzymes (3) and, thereby, a decrease in the enzymatic degradability of pectic substances in the middle lamellae (2). Calcium deficiencies are involved in physiological disorders, such as bitter pit of apple and tip burn of lettuce (1),

and calcium may delay senescence in leaves of corn and *Rumex* sp. (20). The objective of our study was to determine the effect of calcium on the susceptibility of rose flowers to *B. cinerea*.

MATERIALS AND METHODS

Pathogen isolation, maintenance, and inoculation. *B. cinerea* was isolated from naturally infected rose flowers and maintained at 20 C on potato-dextrose agar (PDA, Difco Laboratories, Detroit, MI). Inoculation was carried out with conidia from 14-day-old PDA cultures. Conidia were washed three times by centrifugation (1,000 rpm), suspended in tap water, and adjusted to a concentration of 10^3 conidia per milliliter. The suspension was atomized onto rose flowers. Stems with inoculated flowers were placed in plastic jars containing distilled water, and the flowers were enclosed in a polyethylene bag to maintain high humidity and, thereby, facilitate disease development. Disease severity was recorded on a scale of 0–5: 0 = healthy flower and 5 = flowers decayed and completely covered with fungus. All experiments were incubated at 20 ± 2 C.

Postharvest treatment of cut rose flowers with calcium. Stems of cut roses (cultivars Mercedes and Jaguar) were placed in 300-ml plastic jars containing 100-ml solutions of 3 mM $CaSO_4$ or $CaCl_2$. Flowers were incubated for 48 h at 20 ± 2 C in an illuminated growth chamber (300 μmol photon m^{-2} s^{-1}) at 60–70% relative humidity before inoculation with a conidial suspension of *B. cinerea*.

Cut rose flowers (cultivars Golden Times and Mercedes) were treated, as mentioned above, with solutions of 1–50 mM $CaCl_2$. Experiments were arranged in completely randomized designs, with six replicates; experiments were repeated at least twice. Results are presented from a representative experiment.

Rose plants fertilized with calcium. Rose cultivars Golden Times or Mercedes were grown in the greenhouse on volcanic gravel or rock wool under commercial conditions (nine plants per square meter). Pest and disease management was according to recommended procedures for rose crops in the region, except that no fungicides were sprayed for control of *Botrytis*. Plants were watered daily by drip irrigation (30–50 L/m^2) with fertilizer solutions (Table 1). After 6 wk of treatment in the greenhouse, rose flowers (experiment 1, Table 1) of export quality were picked from Golden Times and divided into two groups. One group of 10 flowers was inoculated with a conidial suspension of *B. cinerea* as described previously, whereas the second was subdivided, and 10 flowers were stored for 2 days at 4, 10, or 20 C before inoculation with *B. cinerea*.

TABLE 1. Concentration (mM) of ions in nutrient solutions used for fertilization of two cultivars of rose plants in commercial greenhouse experiments

	Experiment[x]						
	1 cv. Golden Times		2 cv. Mercedes			3 cv. Mercedes	
Compound	a	b	a	b	c	a	b
Ca^{++}	2.5	5.0	1.2	2.1	3.5	4.1	3.8
K^+	2.5	2.5	4.6	4.9	5.1	4.0	6.0
$N\text{-}NO_3^-$	4.6	7.8	7.6	9.4	10.0	35.0	57.0
$N\text{-}NH_4^+$	3.2	0.7	4.2	1.8	0.9	0.7	1.1
P_2O_6	1.0	1.0	...	...	...	...	...
$H_2PO_4^-$	...	...	1.5	1.2	1.4	0.2	0.3
Na^+	ND[y]	ND	2.1	0.9	4.3	3.2	5.2
Mg^{++}	ND	ND	1.6	2.8	1.6	0.8	1.2
Cl^-	ND	ND	6.3	7.1	6.4	4.6	6.5
SO_4^{--}	ND	ND	0.6	0.5	0.6	...	...
Coratin[z]	4.5	4.5	0.09	0.09	0.09	0.09	0.09

[x] Treatment in each experiment varied in calcium concentration. Consequently, concentrations of K^+, $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ varied.
[y] ND = Not determined.
[z] Coratin: Mo^{2+}, 0.02%; Fe^{3+}, 0.28%; Cu^{2+}, 0.03%; Bo^{2+}, 0.20%; Mn^{2+}, 0.42%; Zn^{2+}, 0.12%. Specific weight, 1.0 kg/L.

To examine the occurrence and development of natural, latent infestation, rose flowers (experiment 2, Table 1) of export quality were picked from Mercedes and either inoculated with a conidial suspension of *B. cinerea* as described, or not inoculated. Experiments were arranged in randomized complete block designs, with four replicates. Flowers of export quality were picked randomly twice (Golden Times) or three times (Mercedes). Results presented are from a representative experiment.

Calcium content. Five symptomless flowers of Golden Times were washed with double-distilled water and dried for 48 h at 65 C. Plant material was weighed, ground in a coffee mill, and digested in hot concentrated HNO_3 + $HClO_4$ (1:1, v/v) for 1 h. The final solution of the plant material was diluted $1{:}10^4$ (v/v) in a 0.0005% $LaCl_3$ solution. Calcium concentration was measured with an atomic absorption spectrophotometer (460 Perkin Elmer, Norwalk, CT), and Ca^{++} concentration was expressed as percentage of dry weight.

Production of ethylene. Measurements were carried out according to Elad (4). Five symptomless flowers of Mercedes were placed in 100-ml Erlenmeyer flasks, and the flasks were sealed with rubber stoppers. Flasks were incubated for 2 h at 20 ± 2 C, and ethylene was measured with a gas chromatograph (Series 750, Gow-Mac Instrument Co., Logan, UT) fitted with a flame ionization detector. The rate of ethylene production was expressed as nanoliters per gram fresh weight per hour; purified ethylene was used as a standard.

Polygalacturonase activity of *B. cinerea*. A 3-mm-diameter mycelial disk from the growing margin of a PDA culture of the pathogen was transferred to 50 ml of synthetic liquid growth medium (19) in 250-ml Erlenmeyer flasks on a shaker (100 rpm, 20 ± 2 C); the medium contained Na-pectate as the only carbon source and $CaCl_2$ concentrations of 0–3.0 mM. Cultures were incubated for 2 days, when growth was measured as mycelial dry weight (dried for 48 h at 65 C). Fungal-free samples of 1 ml were dialyzed against 0.1 M acetate buffer (pH 4.2) for 24 h at 4 C. Activity of polygalacturonic enzymes was measured with Na-pectate as substrate and dinitrosalicylic acid (DNS) as reagent (10). Color change was read on a spectrophotometer (Spectrum 21, Bausch & Lomb GMBH, Unterfohning, Germany) at 575 nm. Polygalacturonic acid was used as a standard. Activity was expressed as enzyme units (EU) per milliliter solution. One EU was defined as the amount of enzyme that released 1 μmol of reducing groups per minute at 30 C. Specific enzyme activity was expressed as EU ml^{-1} mg^{-1} dry weight of mycelium. The experiment was arranged in a completely randomized design with four replicates, and was repeated once. Results are from a representative test.

Statistical analysis. Disease indices (DI) were transformed according to $x = \arcsin(\sqrt{DI}/10)$. A Student's t test was used for separation of disease index and calcium-content means in the greenhouse experiment conducted on Golden Times. Standard deviations are presented for ethylene production. Analysis of variance was performed on data from other experiments, and means were with Duncan's multiple range test ($P = 0.05$).

RESULTS

Postharvest treatment of cut rose flowers with calcium. After 3 days in humid conditions, rose flowers from Mercedes treated with $CaSO_4$ or $CaCl_2$ had 45% less disease than untreated flowers. A similar effect was obtained with $CaSO_4$ on Jaguar, whereas only 20% less disease resulted from treatment with $CaCl_2$ (Fig. 1).

Treatment with 1 mM $CaCl_2$ resulted in 60% less disease on Golden Times 3 and 5 days after inoculation (DI of the control was 1.3 and 2.5 at 3 and 5 days, respectively) (data not shown). Disease severity of flowers treated with 10–50 mM $CaCl_2$ was up to 35% less than that of untreated flowers, but differences were not significant (data not shown). $CaCl_2$ concentrations of 10–20 mM caused up to 40% less disease on flowers of Mercedes 7 days after inoculation, whereas 1 and 50 mM had no influence as compared with the control (data not shown).

Rose plants fertilized with calcium. DIs of flowers of Golden Times 3 and 5 days after inoculation were significantly lower on inoculated, nonpredisposed flowers treated with 5 mM calcium than on those treated with 2.5 mM calcium (Fig. 2). Exposure of flowers from plants fertilized with 2.5 mM calcium to storage at 4 and 10 C did not significantly influence the severity of Botrytis blight as compared with flowers exposed to 20 C (Fig. 3). Flowers from plants fertilized with 5 mM calcium and predisposed to 10 C had significantly ($P = 0.03$) lower DIs at 3 and 5 days post-inoculation than flowers fertilized with 2.5 mM calcium and predisposed at 10 C.

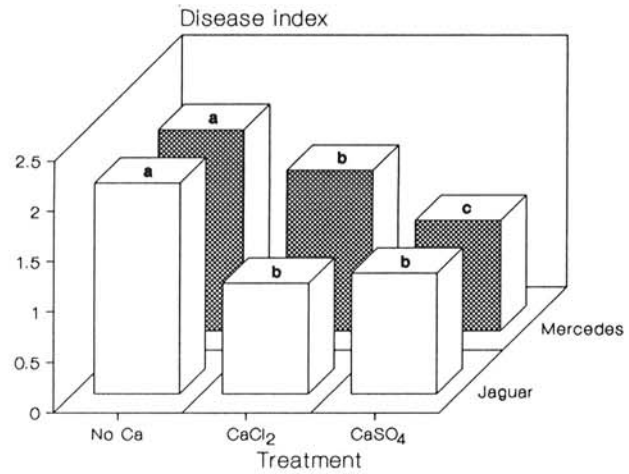

Fig. 1. The influence of 3 mM $CaCl_2$ or $CaSO_4$ in distilled water, on Botrytis blight severity (index from 0 = healthy to 5 = flowers decayed and covered with fungus) on cut rose flowers of cultivars Mercedes and Jaguar 3 days after inoculation. Flower stems were immersed for 48 h in the solutions, then transferred to distilled water and high relative humidity. Within treatments, bars with the same letter do not differ significantly at $P \leq 0.05$ according to Duncan's multiple range test.

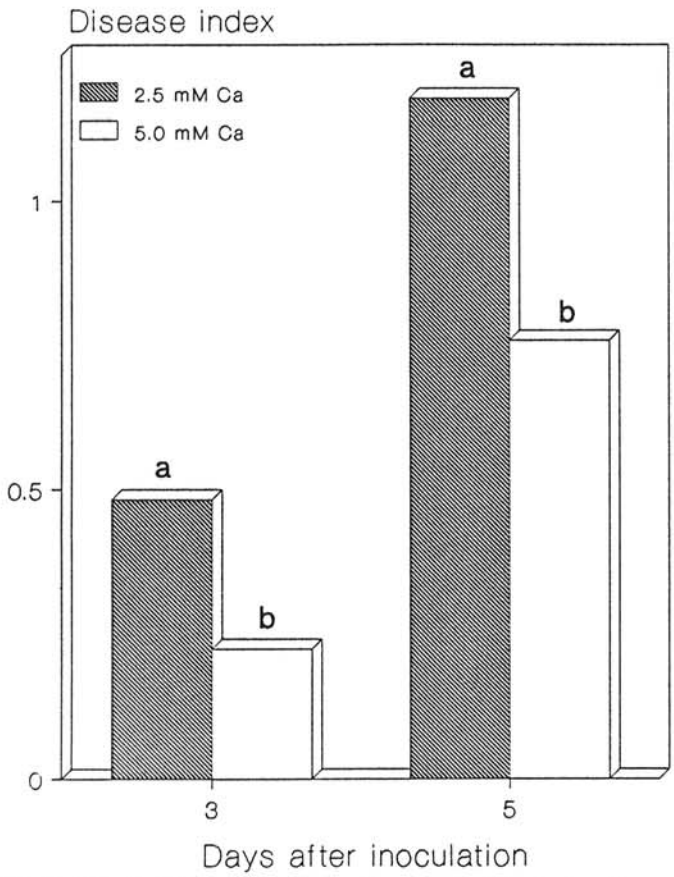

Fig. 2. Botrytis blight severity (index from 0 = healthy to 5 = flowers decayed and covered with fungus) of cut rose flowers (cultivar Golden Times, experiment 1, Table 1) picked in a commercial greenhouse from plants fertilized with 2.5 and 5.0 mM calcium. Flowers were inoculated with *Botrytis cinerea* and placed in a chamber at high relative humidity (>97%). Within each day, bars with the same letter do not differ significantly at $P < 0.05$ according to the Student's t test.

DIs of flowers of Mercedes tended to be greater on inoculated flowers than on naturally infested flowers after 3 days of incubation under humid conditions (Fig. 4). In both groups, disease was suppressed significantly ($P = 0.02$) by fertilization with 3.5 mM as compared with 1.2 mM calcium. In the inoculated group, disease severity on flowers from plants fertilized with 2.1 mM calcium did not differ from those fertilized with 3.5 mM calcium. In the naturally infected group, disease severity on flowers that received 2.1 mM calcium did not differ significantly from the 1.2 or the 3.5 mM treatment. Suppression of disease severity 3 days after inoculation by fertilizing with 3.5 mM as compared with 1.2 mM calcium was relatively higher in naturally infected flowers (55% reduction) than in inoculated flowers (30% reduction).

DIs of cut rose flowers from Mercedes (experiment 3, Table 1) from plants fertilized with 3.8 mM calcium and 6.0 mM potassium were 0.6 at 5 days after inoculation and 0.8 at 6 days after inoculation. DIs of flowers from plants fertilized with 4.1 mM calcium and 4.0 mM potassium were 0.4 and 0.5 at 5 and 6 days after inoculation, respectively.

Calcium content. Calcium content of leaves, stems, and flowers from rose plants of Golden Times (experiment 1, Table 1) after 4 and 6 wk of treatment in the greenhouse is given in Table 2. On both sampling dates, the calcium content of the flowers from the plants fertilized with 5 mM calcium tended to be higher than that achieved with 2.5 mM, but most differences were not significant.

Ethylene production. Production of ethylene 1–7 days after harvest by rose flowers of Mercedes (experiment 2, Table 1) from the plants fertilized with 1.2 mM calcium was higher than from those treated with 2.1 or 3.5 mM calcium (Fig. 5). Differences between the 2.1 or 3.5 mM calcium treatments were not significant at any harvest day.

Polygalacturonase activity of *B. cinerea*. Growth of *B. cinerea* in liquid medium containing Na-pectate as the sole carbon source, and 3.0 mM calcium was significantly inhibited ($P = 0.01$) after

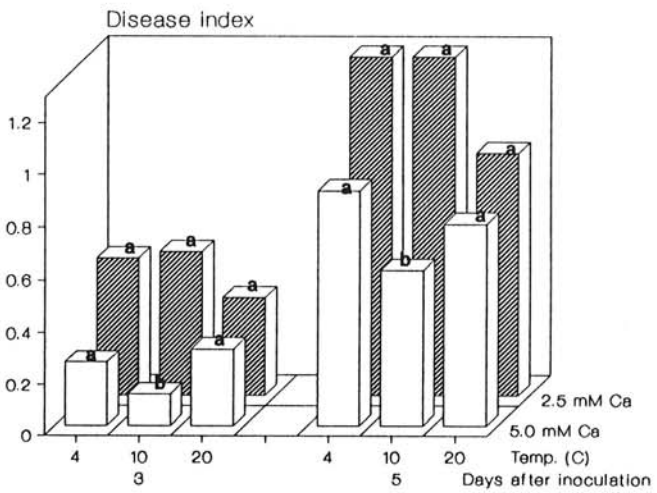

Fig. 3. Botrytis blight severity (index from 0 = healthy to 5 = flowers decayed and covered with fungus) of cut rose flowers (cultivar Golden Times, experiment 1, Table 1) picked in a commercial greenhouse from plants fertilized with 2.5 and 5.0 mM calcium and stored at 4, 10, or 20 C for 2 days, then inoculated with conidia of *Botrytis cinerea* and placed in a chamber at high relatve humidity (>97%). Within calcium treatments, bars with the same letter do not differ significantly at $P < 0.05$ at each day and temperature, according to Duncan's multiple range test. There were no significant differences between storage temperatures at each day.

2 days of growth as compared with growth of the pathogen in a medium containing Na-pectate and 0 or 0.5 mM calcium (Table 3). In medium containing 3.0 mM calcium, no polygalacturonase activity could be detected. Addition of 0.5 mM calcium to the medium did not inhibit growth but caused a 56% reduction in specific enzyme activity of the pathogen as compared with the control.

DISCUSSION

Development of Botrytis blight on rose petals occurs in two stages. Initially, infection is followed by a very limited colonization and then cessation of development, which is not visible macroscopically. In the second stage, small to spreading necrotic lesions develop when environmental and host conditions are suitable (5). The transition of host tissue from resistant to susceptible is not yet understood. Senescence of rose petal tissue probably plays a role in this change. The role of ethylene in the development of Botrytis blight already has been demonstrated (4). Moreover, inhibitors of ethylene production or activity can decrease disease severity (4,6). Calcium recently was shown to inhibit development of Botrytis blight in cut rose flowers, which was associated with the ability of calcium to inhibit ethylene production by the flowers (6). In the present study, cut roses fertilized with nutrient solutions containing higher concentrations of calcium ions produced less ethylene.

Physiological stresses that increase membrane permeability of plant tissue enhance disease development, with exudate leakage serving as a nutrient source for the pathogen (23,24). Addition of calcium ions to plant tissue defers plant senescence (8,20) and limits membrane permeability (22). Additionally, a number of plant diseases are inhibited by the addition of calcium ions, inhibition being associated with the ability of calcium to strengthen the cell wall and inhibit degradation by pectinolytic enzymes (3,15).

In our present study, $CaSO_4$ was more efficient than $CaCl_2$ in inhibiting disease on cut roses of Jaguar, whereas treatment differences were not significant on Mercedes. When different concentrations of $CaCl_2$ were applied to roses of Jaguar, 1 mM

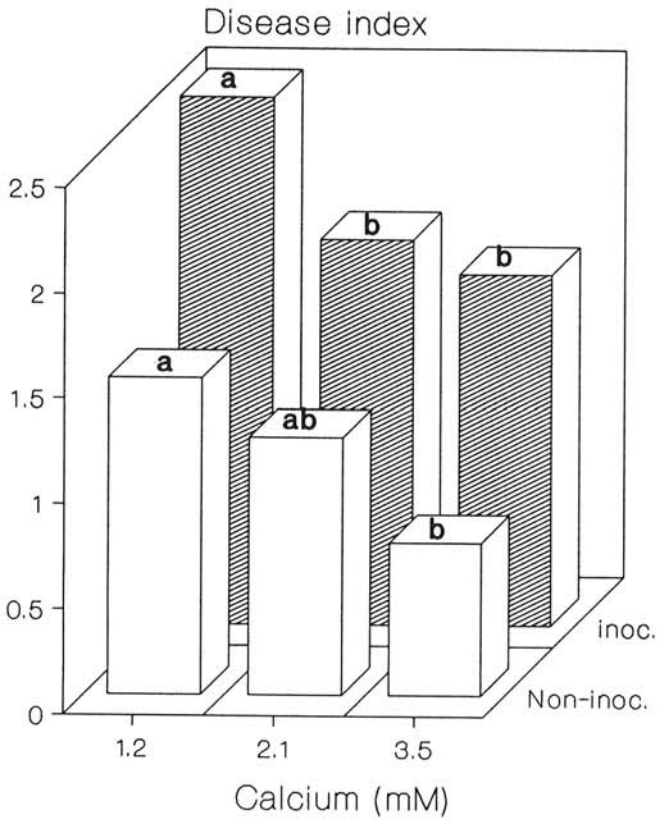

Fig. 4. Effect of calcium fertilization at concentrations of 1.2, 2.1, and 3.5 mM (experiment 2, Table 1) in a commercial greenhouse on postharvest Botrytis blight disease severity (index from 0 = healthy to 5 = flowers decayed and covered with fungus) of cut rose flowers (cultivar Mercedes). Flowers either were inoculated with *Botrytis cinerea* or not, and all were incubated in a chamber at high relative humidity (>97%). Within inoculation treatments, bars with the same letter do not differ significantly at $P < 0.05$ according to Duncan's multiple range test.

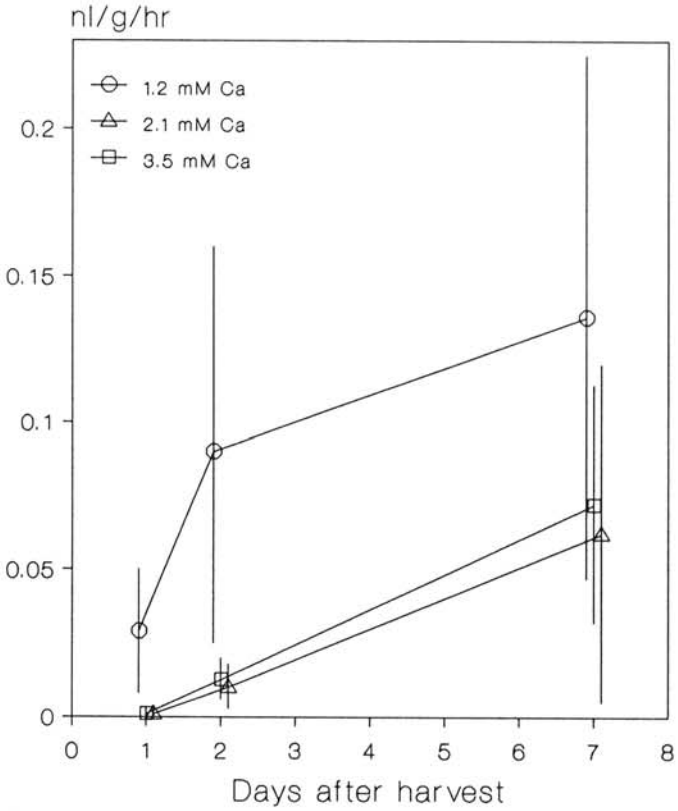

Fig. 5. Production of ethylene by flowers picked previously in a commercial greenhouse from rose plants (cultivar Mercedes; experiment 3, Table 1) fertilized with 1.2, 2.1, or 3.5 mM calcium. Ethylene production is expressed as nanoliter per gram fresh weight of flower per hour. Vertical bars = standard deviations.

TABLE 2. Calcium content of different parts of rose plants (cultivar Golden Times) measured on two dates after the start of fertilization in a commercial greenhouse (experiment 2, Table 1)

Ca^{2+}	4 wk			6 wk		
(mM)	Stem	Flower	Leaf	Stem	Flower	Leaf
2.5	1.8 a[z]	1.8 a	1.6 b	3.0 a	1.9 b	3.3 a
5.0	3.3 a	2.2 a	3.6 a	3.0 a	3.3 a	4.4 a

[z] Within columns, figures followed by the same letter do not differ significantly at $P < 0.05$ by the Student's t test.

TABLE 3. Production of polygalacturonase and hyphae by *Botrytis cinerea* in liquid culture containing different calcium concentrations and Na-pectate as the sole carbon source, 2 days after transferring a mycelial disk to the medium

Ca^{2+}	Hyphal growth	Polygalacturonase activity	
(mM)	(mg dw[x]/100 ml)	EU[y] ml^{-1}	EU ml^{-1} mg^{-1} dw
0.0	3.4 a[z]	0.73 a	0.43 a
0.5	4.0 a	0.38 b	0.19 b
3.0	2.0 b	0.00 c	0.00 c

[x] Dry weight.
[y] EU = Enzyme units, defined as the amount of enzyme that releases 1 μM reducing groups per minute at 30 C.
[z] Within columns, figures followed by the same letter do not differ significantly at $P < 0.05$ according to Duncan's multiple range test.

caused the greatest suppression in disease severity, whereas 10–20 mM were needed for Mercedes. This difference between cultivars may be due to a difference in chloride tolerance. Both SO_4^{-2} and Cl^- ions can be toxic to plant tissue, but the degree of susceptibility to the anions varies with the plant and cultivar (11). Jaguar seems more susceptible to Cl^- ions than Mercedes.

Addition of calcium nitrate to the nutrient solutions used to fertilize rose plants of Golden Times and Mercedes reduced the severity of postharvest Botrytis blight. Susceptibility of cut flowers of Golden Times was increased by predisposition at 4 and 10 C for 2 days, but the effect was suppressed by calcium treatment. The effect of calcium was greater at 10 C. Cold storage of roses at 8 C shortens vase life, and storage at 3 C results in severe damage to the plasma membrane (7). The induced increase in flower susceptibility to Botrytis blight with cold storage could be due to increased leakage from plasma membranes. At 10 C, calcium may limit the damage caused to the membrane by the exposure to low temperature and thereby, inhibit leaching. At 4 C, calcium probably was not able to overcome the damage caused to the membrane.

Concomitant elevation of the K^+ content in the nutrient solution suppressed the effect of the calcium ions, probably by competition for cation uptake. Various cations are known to have an inhibitory effect on calcium uptake (14).

In medium containing Na-pectate as the carbon source and 3 mM calcium, growth of *Botrytis* and polygalacturonase activity were inhibited as compared with a Na-pectate medium without calcium. Pectate is an important compound in plant cell walls, and more than 60% of the calcium in the plant is found in cell walls (21). *Botrytis* produces at least four endopolygalacturonases (16). The inability of *Botrytis* to utilize pectate in the presense of calcium, therefore, is suggested as a mode of action of the Ca ions. In addition to strengthening membranes and inhibiting ethylene production, we conclude that calcium can influence flower susceptibility to *B. cinerea*.

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