

Calcium Application Method Impacts Botrytis Blight Severity on Petunia Flowers

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Abstract. Two application methods of calcium (Ca), fertigation and spray, were investigated regarding their effects on Botrytis blight on petunia (*Petunia ×hybrida*) flowers. Plants were grown for 6 weeks with three nutrient solutions consisting of 0, 100, or 200 mg·L⁻¹ Ca and weekly calcium chloride (CaCl₂) sprays of 0, 750, or 1500 mg·L⁻¹ Ca for a total of nine treatment combinations. Flowers were harvested, inoculated with *Botrytis* spores, placed in humidity chambers, and evaluated for Botrytis blight severity. Disease severity decreased by 57% and 70% when flowers were treated with Ca spray applications of 750 and 1500 mg·L⁻¹ Ca, respectively; however, no change in disease severity occurred across the Ca fertigation applications. Ca concentration in the flower petal tissue increased with the Ca spray applications: the flower petal Ca concentration increased from 0.26% to 0.65% of tissue dry mass (DM) as the Ca spray application rate increased from 0 to 1500 mg·L⁻¹. However, no change was observed across the Ca fertigation treatments. Leaf tissue Ca concentration increased from 2.1% to 3.2% DM as the fertigation solution increased from 0 to 200 mg·L⁻¹ Ca, whereas spray application had no significant effects of leaf tissue Ca concentration. The results demonstrate that spray application is a more effective technique than fertigation application to provide higher Ca tissue concentrations in flowers, and that the Ca concentration in flower petal tissue is an important consideration when evaluating tissue susceptibility to Botrytis blight. Because of the high rate of fungicide resistance to *Botrytis cinerea* found in commercial greenhouses, spray applications of CaCl₂ are an important disease management tool for commercial growers.

Botrytis cinerea is a ubiquitous plant pathogen that infects bedding plants during greenhouse production, resulting in latent infections that appear in the postharvest shipping environment. On arrival at the retail location, petunias frequently exhibit symptoms of Botrytis blight on flowers consisting of tan, necrotic spots that may coalesce and lead to tissue collapse. This phenomenon is termed petunia flower meltdown, and preventive fungicide applications are the primary management technique implemented

by commercial growers. However, fungicide resistance is becoming more problematic, as demonstrated by *Botrytis* isolates recently collected from commercial greenhouses that were found to be resistant to six chemical classes of fungicides (Samarakoon et al., 2017a). Consequently, alternative management strategies to preventive fungicide applications are necessary.

Recent research has demonstrated the potential value of Ca nutrition to improve host tissue resistance to Botrytis blight (Samarakoon et al., 2017b). Calcium is an essential plant nutrient that has several functions in plant growth such as being a key structural component within the cell wall, a counter cation to anions in the vacuole, and a secondary messenger to biotic and abiotic stresses. Calcium uptake into plant tissue occurs passively via mass flow that is dependent on the water potential gradient from the soil solution through the plant to the surrounding atmosphere; therefore, Ca movement through the xylem into specific plant organs is dependent on the transpiration rates of those organs. Flower petals have low stomatal density or are lacking stomata; therefore, transpiration rates are relatively low and transport of Ca through the xylem to the flower petal is subsequently low (Roddy et al., 2016). Furthermore, Ca movement through the phloem is relatively low

because of Ca binding with phosphate, although adequate Ca levels for growth do exist in the phloem (Clarkson, 1984).

Several studies have demonstrated the effect of increasing Ca in nutrient solutions on Ca concentration in leaf and flower tissue of floriculture crops. For Oriental hybrid lilies (*Lilium ×hybrida*), increasing Ca concentration provided in the nutrient solution from 0 to 140 mg·L⁻¹ Ca increased Ca concentration in leaves from 0.20% to 1.83% dry mass (DM); however, flower Ca concentrations were not measured (Salazar-Orozco et al., 2011). Calcium concentration in poinsettia (*Euphorbia pulcherrima*) leaves increased from 0.35% to 0.75% as Ca concentration delivered in the nutrient solution increased from 18 to 300 mg·L⁻¹ Ca (Jacques et al., 1990). However, poinsettia bracts have low stomatal density and exhibit differences in Ca concentration along the margins of bracts compared with near the mid vein (Nell and Barrett, 1986), and increasing Ca in the nutrient solution was not effective for increasing Ca concentration in bract margin (Strømme et al., 1994). Roses (*Rosa ×hybrida*) supplied with nutrient solutions containing 160 to 280 mg·L⁻¹ Ca did not affect the Ca concentration of the flower petals (Baas et al., 2000). Volpin and Elad (1991) demonstrated in roses that variations can occur in Ca accumulation in different tissues over time; for example, increasing Ca in the nutrient solution from 100 to 200 mg·L⁻¹ increased the Ca concentration in leaf tissue, but not in flowers, after 4 weeks. However, after 6 weeks, increasing the Ca concentration from 100 to 200 mg·L⁻¹ increased Ca concentration in flowers but not in leaves. Starkey and Pedersen (1997) demonstrated that increasing the Ca supplied in nutrient solutions by 44 to 176 mg·L⁻¹ increased Ca concentration of the flowers and buds of potted roses from 0.4% to 0.6% Ca and increased Ca concentration in leaves from 0.7% to 1.6%. Although increasing Ca concentration in flower tissue is possible through the nutrient solution, previous research demonstrated that this method does not yield consistently positive results.

Calcium spray applications provide an additional strategy for increasing Ca concentration in low transpiring tissues such as flowers or fruits. Calcium spray applications of 432 mg·L⁻¹ Ca alleviated bract-edge burn in poinsettia, which is caused by a localized Ca deficiency occurring on the margins of the bracts (Harbaugh and Woltz, 1989). As the Ca concentration of the bract margin increased from 0.12% to 0.17% DM, bract-edge burn decreased. Tomato plants supplied with a low rate of Ca (11.6 mg·L⁻¹) in the nutrient solution and a high rate in the spray solution (1200 mg·L⁻¹ Ca) had increased Ca concentrations in the fruit and decreased incidence of blossom end rot (Schmitz-Eiberger et al., 2002).

Calcium can be delivered to plants by the fertigation solution delivered to the roots or by spray application to the aboveground tissues. The objective of this study was to

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Table 1. Calculated values of nutrients for the three calcium (Ca) treatments supplied in the fertigation solutions and the measured values of pH and electrical conductivity (EC). Sodium chloride was added to the 0 and 100 mg L⁻¹ Ca solutions to provide the same EC for each solution.

Ca	N	P	K	Mg	S	Fe	Mn	B	Cu	Zn	Cl	Na	EC (dS·m ⁻¹)	pH
0	100	20	150	50	40	1.05	0.53	0.26	0.53	0.53	420	271	2.5	6.0
100	100	20	150	50	40	1.05	0.53	0.26	0.53	0.53	385	133	2.5	6.0
200	100	20	150	50	40	1.05	0.53	0.26	0.53	0.53	355	0	2.5	6.0

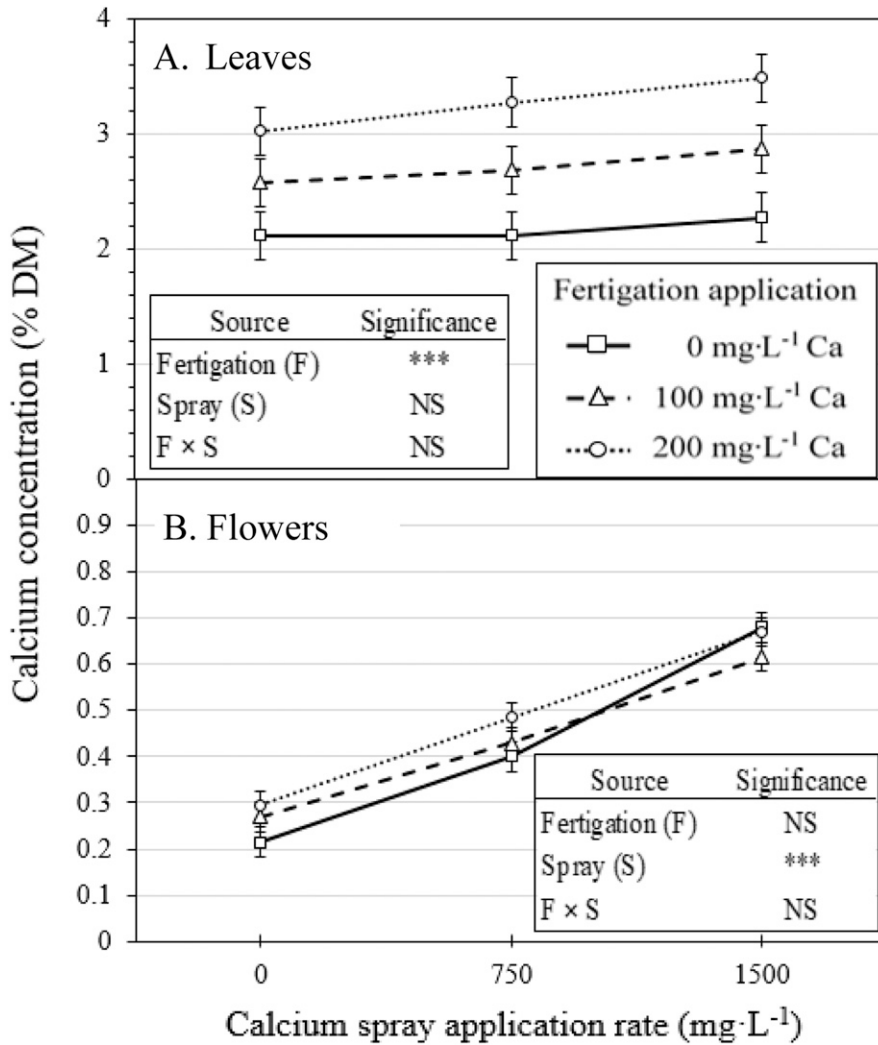


Fig. 1. Calcium (Ca) concentration (% dry mass, DM) in petunia leaves (A) and flowers (B) on plants grown with three Ca concentrations provided during daily fertigation applications and three Ca concentrations provided during weekly spray applications for 3 weeks. Analysis of variance results of the main effects are presented in each figure. Interactions were nonsignificant. Errors bars represent ± 1 SE.

determine the effects of Ca application methods and Ca concentrations in the application solutions on the Ca concentration in petunia leaf and flower petals and on the susceptibility of the flowers to Botrytis blight.

Materials and Methods

Two methods (fertigation and spray) of Ca application were compared; three concentrations of Ca were used for each method. More specifically, three fertigation solutions (0, 100, and 200 mg·L⁻¹ Ca) and three spray solutions (0, 750, and 1500 mg·L⁻¹ Ca) were applied, for a total of nine treatment combinations, using a

3 × 3 factorial arrangement. Calcium concentrations were measured in flowers and leaf tissue. Flowers were inoculated with *Botrytis* spores to evaluate the effects of Ca treatments on Botrytis blight severity.

Botrytis isolation, culture maintenance, and preparation of conidial suspension. The same isolate of *Botrytis* was used and the conidial suspension was prepared according to methods described by Bennett (2019).

General procedures. Petunia plugs (*Petunia ×hybrida* 'Dreams Red') were received from a commercial grower and transplanted in 1.4-L containers (one plant per container) filled with a peat-based growing medium

(Fafard 3B; Conrad Fafard, Inc., Agawam, MA) containing an initial Ca concentration of 1.5% Ca. Plants were grown in a glass greenhouse (lat. 35°N) with heating and cooling setpoints of 21 and 24 °C, respectively. Long days were provided with daylength extension lighting with metal halide lamps when solar radiation was <200 W·m⁻² from 0900 to 2400 HR to promote flowering of this facultative long day species from February to May. During the 6 weeks when Ca treatments were provided, the vapor pressure deficit averaged 1.24 ± 0.07 kPa. The plants were fertilized during each irrigation event for 3 weeks after transplantation with Jack's Professional 15-5-15 Calcium + Magnesium LX (15 N, 2.2 P, 12.5 K, 4 Ca, 2 Mg; JR Peters Inc., Allentown, PA), providing 100 mg·L⁻¹ N and 26 mg·L⁻¹ Ca.

Three weeks after transplantation, the fertigation and spray application treatments were initiated. The experiment used 12 plants per treatment, for a total of 108 plants. The fertigation treatments consisted of a constant liquid fertilization program providing 200 mL of fertigation solution per container each day between 0800 and 0830 HR for 6 weeks. To avoid confounding the Ca fertigation treatments with the electrical conductivity (EC) of the fertigation solution, the fertigation solutions were adjusted with sodium chloride (NaCl) so that all solutions had an EC of 2.5 dS·m⁻¹, which was the initial EC of the 200 mg·L⁻¹ Ca solution (Table 1). Calcium spray applications occurred weekly during the same 6 weeks when fertigation treatments were applied. For the spray application treatments, CaCl₂ (anhydrous 96% purity; Thermo Fisher Scientific, Waltham, MA) was dissolved in deionized water to provide solutions containing 0, 750, or 1500 mg·L⁻¹ Ca. The spray application rate was 204 mg·L⁻¹, and the applications were performed between 1600 and 1700 HR using hand sprayers.

After providing the nine combinations of fertigation and spray treatments for 3 weeks, two plants per treatment were randomly selected and destructively harvested for nutrient analysis of the fully expanded green leaves and open flowers. All flowers were harvested without their peduncle and sepals. The collected tissues were dried in an oven at 60 °C, ground to a fine powder, and submitted for nutritional analysis (ICP-OES; Thermo Scientific) performed at the Agricultural Research Service of the United States Department of Agriculture (USDA-ARS, Toledo, OH).

During the final 3 weeks of the experiment, three to four freshly opened flowers per plant were harvested 24 h after the weekly spray application and inoculated with *Botrytis* spores (10⁴ spores/mL). All open flowers were removed the day before each spray application

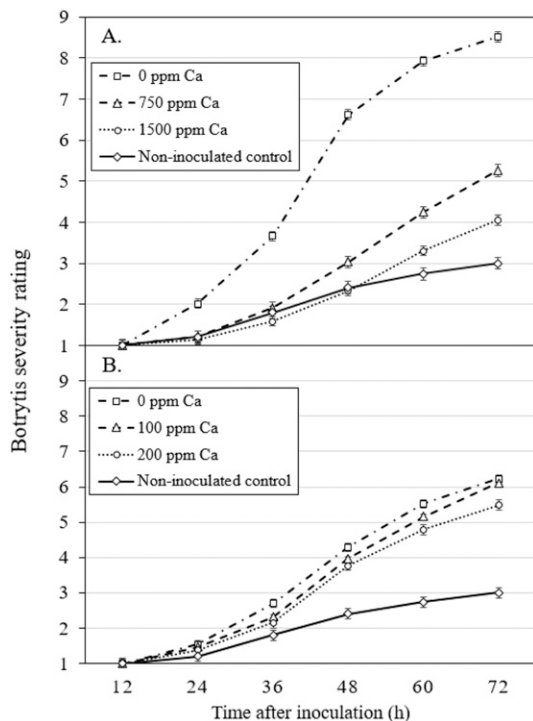


Fig. 2. The Botrytis severity rating (scale: 1 = 0%, 2 = 0% to 2%, 3 = 2% to 5%, 4 = 5% to 10%, 5 = 10% to 25%, 6 = 25% to 50%, 7 = 50% to 75%, 8 = 75% to 100%, 9 = 100% of the flower petal was infected) was recorded for individual flowers every 12 h for 72 h following inoculation with a conidial suspension. Main effect means are displayed for (A) the three spray applications (0, 750, or 1500 mg·L⁻¹ Ca) and (B) the three fertigation applications (0, 100, or 200 mg·L⁻¹ Ca). The noninoculated control is shown in both figures. Errors bars represent ±1 SE.

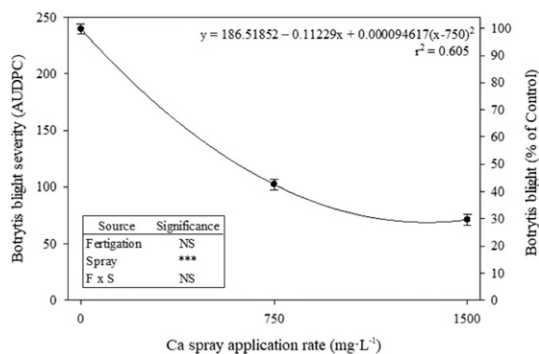


Fig. 3. Botrytis blight severity on petunia flowers following weekly calcium (Ca) spray applications using calcium chloride (CaCl₂). Botrytis blight severity is expressed as the sum of the area under the disease progression curve (AUDPC) (left) and as a percentage of the control treatment ±1 SE. Analysis of variance results for the main effects are presented. Error bars represent ±1 SE.

so that only freshly opened flowers were used for Botrytis blight evaluation. Inoculated flowers were then evaluated every 12 h for 72 h using a rating scale (1 = 0%, 2 = 0% to 2%, 3 = 2% to 5%, 4 = 5% to 10%, 5 = 10% to 25%, 6 = 25% to 50%, 7 = 50% to 75%, 8 = 75% to 100%, 9 = 100% of the flower petal was infected). Botrytis inoculations were conducted during each week of the final 3 weeks of the experiment, for a total of three replications.

Data analysis. Data analysis was performed using JMP Pro version 13.2.0 (SAS Institute Inc., Cary, NC). An analysis of variance (ANOVA) was used to determine treatment effects for the calcium tissue analysis of flowers and leaves. The data set consisted of two separate ANOVA tables for leaves and

flowers, with both consisting of a 3 × 3 factorial model for the three fertigation treatments and three spray treatments.

For Botrytis blight severity data, an ANOVA was used to determine treatment effects. Treatments were analyzed using the area under the disease progression curve (AUDPC), as previously described by Bennett (2019). Regression analysis was also performed for Botrytis blight severity data using JMP Quadratic Fit.

Results and Discussion

The main effect of fertigation application was the only significant factor to affect Ca concentration within petunia leaf tissue

(Fig. 1A). Leaf tissue Ca concentration increased from 2.1% to 3.2% DM as the fertigation solution increased from 0 to 200 mg·L⁻¹ Ca. For petunia flowers, the main effect of the spray application was the only significant factor affecting the Ca concentration in the tissue (Fig. 1B). The Ca concentration in flower petal tissue increased from 0.26% to 0.65% DM as the spray solution increased from 0 to 1500 mg·L⁻¹ Ca. No interactions between the fertigation and spray application treatments on the leaf and flower petal were observed (Fig. 1).

The Botrytis severity rating for the inoculated control increased from 1 to 8.6 over the course of 72 h after inoculation, whereas the final ratings for 750 and 1500 mg·L⁻¹ Ca spray application treatments were 5.3 and 3.9, respectively (Fig. 2A). The noninoculated control was rated 3.0 after 72 h in the humid chambers. This value provided a baseline value for the naturally occurring disease pressure. The Botrytis severity rating for the three fertigation treatments ranged from 5.5 to 6.2 at the end of the evaluation period (Fig. 2B).

The AUDPC indicated that Botrytis blight severity decreased by 70.3% as the Ca concentration in the spray application treatment increased from 0 to 1500 mg·L⁻¹ Ca (Fig. 3). The Ca concentration delivered in the fertigation solution did not affect Botrytis blight severity. No significant interaction between the fertigation and spray application treatments on Botrytis blight severity occurred.

During commercial production of petunias, application of Ca to plants primarily occurs through the nutrient solution. A typical nutrition program for petunia production using constant liquid fertilization provides 100 mg·L⁻¹ N. The Ca concentration in these solutions typically ranges from 17 to 33 mg·L⁻¹, depending on the formulation of preblended fertilizer used. Additional Ca in the nutrient solution may result from naturally occurring Ca in the water source (e.g., Ca levels can vary from 0 to 100 mg·L⁻¹, depending on the water source) (Morr et al., 2006).

The standard range for Ca concentration in leaf tissue of petunia has been reported to be 1.2% to 2.8% DM (Jones and Mills, 1996); however, in our study, petunia leaves ranged from 2.2% to 3.3% DM with the 0 to 200 mg·L⁻¹ Ca fertigation application treatments. Calcium concentration in petunia leaves increased 19.9% when the Ca provided in the nutrient solution increased from 0 to 100 mg·L⁻¹; when Ca concentration in the nutrient solution increased from 100 to 200 mg·L⁻¹ Ca, the Ca concentration in the leaves increased by an additional 16.8%. Petunia plants supplied only with Ca in the nutrient solution, regardless of Ca concentration, had flower petal tissue of 0.26% Ca, which was 12-times lower than the Ca concentration of leaves from plants that were provided with 200 mg·L⁻¹ Ca in the nutrient solution. These results demonstrate that constant liquid fertilization is an ineffective method of increasing Ca concentration in petunia flower petals.

Calcium spray applications effectively increased flower petal Ca concentration and reduced Botrytis blight severity in petunia

flowers. Direct applications of Ca from sprays allowed flower tissue to acquire Ca, whereas supplying Ca through fertigation did not increase Ca in flower tissue or decrease Botrytis blight severity. These results demonstrate that supplying Ca in the fertigation solution is not sufficient for increasing Ca in flower tissue or decreasing Botrytis blight severity of those flowers. As a result, Ca spray applications are necessary to reduce the susceptibility of petunia flowers to Botrytis blight.

Two modes of action are suggested for the effects of Ca on *Botrytis*. The first involves Ca binding with pectin (polygalacturonic acid chains) in the middle lamella forming Ca-pectate, which is important for strengthening cell walls. Crosslinks between Ca and pectin favor the formation of a gel that makes pectin spatially less accessible to polygalacturonases (Conway and Sams, 1984), which are enzymes produced by *Botrytis* to degrade host cell walls (Cabanne and Donèche, 2002). The second mode of action suggests a direct effect of Ca on polygalacturonase production and on *Botrytis* hyphal growth. Volpin and Elad (1991) showed that polygalacturonase activity and hyphal growth were inhibited by increasing Ca concentration in vitro from 0 to 120 mg·L⁻¹ Ca. Similar results have been found in *Botrytis* isolates from grapes when grown in vitro with Ca ranging from 145 to 582 mg·L⁻¹ (Nigro et al., 2006). Cabanne and Donèche (2002) reported 90% inhibition of polygalacturonase activity in an in vitro solution containing 40 mg·L⁻¹ Ca, which is a concentration similar to naturally occurring Ca concentration in grapes; they concluded that increasing Ca concentration in fruits may allow for increased resistance from the tissue by acting as an enzyme inhibitor.

Several studies have examined the effects of Ca on *Botrytis* infection. Bract-edge burn of poinsettia has the initial symptoms of necrotic spotting along bract margins due to a localized Ca deficiency that later succumbs to *Botrytis* infection and causes the necrotic spots to expand and coalesce. Harbaugh and Woltz (1989) demonstrated that weekly Ca spray applications of 432 mg·L⁻¹ Ca reduced the number of bract-edge burn lesions by 94%. Starkey and Pedersen (1997) reported a decrease in Botrytis blight of potted rose flowers and buds with increasing Ca concentrations in the nutrient solution. De Capdeville et al. (2005) also demonstrated that Ca spray applications of 400 and 800 mg·L⁻¹ Ca effectively reduced Botrytis blight severity of cut rose flowers by 68% and 76%, respectively. Álvarez

et al. (2012) demonstrated a significant reduction in Botrytis blight severity from naturally occurring *Botrytis* populations following CaCl₂ spray applications of 1000 mg·L⁻¹ Ca.

Results from a previous study determined that neither the chloride anion nor the EC of the spray solution contribute to the reduction in Botrytis blight following spray applications of CaCl₂ (Bennett, 2019). Therefore, the reduction of Botrytis blight severity on petunia flowers following CaCl₂ applications is solely due to Ca in the solution.

In conclusion, the results of this study demonstrate the effectiveness of Ca spray applications to increase Ca content of petunia flowers and decrease Botrytis blight severity. In contrast, increasing Ca in the fertigation solution effectively increased Ca concentration in the leaves, but increasing Ca in the spray applications did not. Therefore, Ca spray applications are not necessary to increase Ca concentration of leaves, and fertigation applications with increased Ca concentration could be used to potentially increase Ca in leaves of species that are susceptible to *Botrytis*. Tissue analysis results suggest that Ca concentration of petunia flower petals should not be less than 0.4% DM to decrease petal susceptibility to *Botrytis* infection. This study demonstrates the potential usefulness of Ca spray applications during greenhouse production as an alternative method of fungicide application for *Botrytis* management.

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