# Evaluation of Calcium Sources for the Management of Botrytis Blight on Petunia Flowers

Katherine Bennett[1], Guido Schnabel[1], and James E. Faust[1]

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Abstract. **Previous studies have demonstrated the efficacy of calcium (Ca) spray applications derived from Ca chloride for reducing botrytis (*Botrytis cinerea*) infection severity on petunia (*Petunia ×hybrida*) flowers. This study examines the effects of six Ca sources for their efficacy in reducing Botrytis blight on petunia flowers and their potential to cause spray damage or phytotoxicity. In the first experiment, the six Ca sources evaluated were laboratory-grade and commercial-grade Ca chloride, Ca nitrate, Ca ethylenediaminetetraacetic acid chelate, Ca amino acid chelate, and Ca silicate. In the second experiment, petunia flowers that were 0, 1, 3, 5, or 7 days old at the time of the Ca spray applications were evaluated for spray damage severity. For both experiments, treatments were applied to flowering plants. For the evaluation of Botrytis blight efficacy, flowers were excised and inoculated with botrytis spores 24 hours after the Ca spray application, and were evaluated every 12 hours for 72 hours. Laboratory-grade and commercial-grade Ca chloride at 1250 mg·L$^{-1}$ Ca were the most effective Ca sources evaluated for decreasing Botrytis blight severity while not causing spray damage at any flower age. Spray damage to the flowers from the Ca chloride application increased when Ca concentrations increased to 2000 mg·L$^{-1}$, but no additional benefit was observed for reducing Botrytis blight severity compared with the 1250-mg·L$^{-1}$ Ca application. The results demonstrate that several Ca sources reduce Botrytis blight severity significantly; however, selection of the Ca source is important for minimizing the risk of spray damage.**

Botrytis (*Botrytis cinerea*), a necrotrophic plant pathogen, infects more than 200 hosts worldwide, causing significant losses within agricultural systems (Williamson et al. 2007). Bedding plant producers experience problems with Botrytis blight in all stages of production, from propagation to postproduction. When flowers are present on bedding plants, such as petunia (*Petunia ×hybrida*), botrytis infects the flowers rapidly under conducive environmental conditions, resulting in the disease referred to as Botrytis blight or gray mold. Botrytis blight is a significant issue during transportation of bedding plants to the retail environment, which may take up to 2 d. Plants are typically irrigated immediately before shipping, then are packed tightly onto shipping carts and loaded into trucks. This situation creates a high-relative humidity environment conducive to the development of Botrytis blight (Samarakoon et al. 2016). Latent infections may occur during shipping where botrytis spores have infected plant tissue in the greenhouse but remain quiescent until the environment is ideal for sporulation (Muñoz et al. 2019). Symptoms of Botrytis blight typically occur as tan necrotic spotting on the flower petals that coalesce as the disease progresses, resulting eventually in the complete collapse of the flower petal tissue (Bennett et al. 2020b).

Calcium (Ca) is a key component in cell wall formation for stabilization and strength, with a high portion of the total Ca located in the cell walls. Calcium is also involved in intracellular cation–anion balance and acts as a secondary messenger during stress events (Marschner 2012). Calcium uptake from the soil solution occurs from the mass flow of water. Transpiration drives water movement through the plant, supplying the plant tissues with Ca (White and Broadley 2003); however, transpiration rates of flowers and fruit are relatively low because of their large volume-to-surface area ratio and/or low stomatal density (Marschner 2012). Fruit and flowers are supplied mainly with nutrients through the phloem, where Ca transport is low. Increasing Ca in the nutrient solution does not provide adequate Ca distribution to low-transpiring plant tissues, such as petunia flowers, whereas spray applications increase the Ca concentration significantly and reduce the disease severity in flower petal tissues (Bennett et al. 2020b).

Álvarez (2012) evaluated three sources of Ca on flowers and stems of cut rose (*Rosa ×hybrida*): Ca chloride, Ca oxide, and Ca amino acid chelate at concentrations up to 1000 mg·L$^{-1}$ Ca. All Ca sources provided reductions in

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[1]Department of Plant and Environmental Sciences, Clemson University, E143 Poole Agriculture Center, Clemson, SC 29634, USA

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J.E.F. is the corresponding author. E-mail: jfaust@clemson.edu.



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**Units**

| To convert U.S. to SI, multiply by | U.S. unit | SI unit | To convert SI to U.S., multiply by |
|---|---|---|---|
| 29.5735 | fl oz | mL | 0.0338 |
| 0.3048 | ft | m | 3.2808 |
| 3.7854 | gal | L | 0.2642 |
| 0.4075 | gal/100 ft$^2$ | L·m$^{-2}$ | 2.4542 |
| 2.54 | inch(es) | cm | 0.3937 |
| 1 | mmho/cm | dS·m$^{-1}$ | 1 |
| 28.3495 | oz | g | 0.0353 |
| 1 | ppm | mg·L$^{-1}$ | 1 |
| 10.7639 | W/ft$^2$ | W·m$^{-2}$ | 0.0929 |
| (°F − 32) ÷ 1.8 | °F | °C | (°C × 1.8) + 32 |

Botrytis blight severity at 500 and 1000 mg·L$^{-1}$ Ca; however, Ca chloride was the only source effective at 250 mg·L$^{-1}$ Ca. De Capdeville et al. (2005) evaluated Ca sulfate spray applications and reported that disease severity decreased as Ca concentration of the spray solution increased from 0 to 800 mg·L$^{-1}$ Ca. Bract-edge burn of poinsettia (*Euphorbia pulcherrima*) is caused by a localized Ca deficiency along the margins of poinsettia bracts. Spray applications of Ca chloride at 360 mg·L$^{-1}$ Ca (Strømme et al. 1994) and Ca nitrate at 432 mg·L$^{-1}$ Ca (Harbaugh and Woltz 1989) were effective at reducing bract-edge burn and the subsequent botrytis infection of poinsettia, and no spray damage was reported.

Based on previous work, several sources of Ca have been evaluated for improving plant quality or reducing disease severity on vegetable, fruit, and ornamental crops; however, studies evaluating multiple sources of Ca at rates greater than 1000 mg·L$^{-1}$ Ca have not been done for flowering crops. Exploring greater rates of Ca is important to determine whether additional benefits could be obtained in terms of heightened disease management. Greater Ca concentrations may have negative consequences in regard to spray damage, so it was also necessary to address this issue. Because bedding plants have flowers of varying ages on individual plants, we hypothesized that spray damage may be related to flower age, so this factor was included in our study. Therefore, the objectives of our study were to compare Ca sources for their effect on Botrytis blight of petunia flowers 1) to determine whether there was any benefit from applying greater Ca concentrations than those reported previously, 2) to identify the risk of spray damage that may result from Ca application, and 3) to evaluate the potential for spray damage as flowers age.

## Materials and methods

Two experiments were conducted to quantify the effect of different Ca sources for their potential spray damage effects and their efficacy for reducing Botrytis blight severity on petunia flowers of different ages. The first experiment examined the potential spray damage to petunia flowers after spray treatments of six Ca sources applied at each of three rates. The second experiment examined the same Ca products and rates for their reduction of Botrytis blight severity after inoculation of petunia flowers with botrytis spores.

**General procedures.** Petunia 'Dreams Red' plugs were received from a commercial grower and transplanted into 6-inch-diameter (1.4-L) round containers containing a peat-based growing medium (Fafard 3B; Sun Gro Horticulture, Agawam, MA, USA) with an average starting concentration of 2.7 g Ca per container. A constant liquid fertigation program was used with 15N–2.2P–12.5K water-soluble fertilizer (Peter's Excel Cal-Mag Special 15-5-15; Scotts-Sierra, Marysville, OH, USA) providing 150 mg·L$^{-1}$ nitrogen and 50 mg·L$^{-1}$ Ca at each irrigation event by hand watering as needed. Plants were grown in a glass greenhouse at Clemson University, Clemson, SC, USA (lat. 34.7°N, long. 82.8°W), with the environment controlled by a climate-control computer (Argus Control Environmental Systems, White Rock, BC, Canada). Heating and cooling set points for Expt. 1 were 22 and 19 °C, and for Expt. 2 were 22 and 16 °C, respectively. Retractable curtains providing 55% shade were engaged when solar radiation measured outside the greenhouse exceeded 800 W·m$^{-2}$. For Expt. 1, conducted in January and March, plants were grown under a long-day photoperiod provided with daylength extension lighting from metal halide lamps from 0900 to 2400 HR, when solar radiation measured outdoors was more than 200 W·m$^{-2}$, to promote flowering of this facultative long-day species. No daylength extension lighting was provided for Expt. 2 because the experiment was conducted in September.

**Evaluation of Ca sources for spray damage severity (Expt. 1).** Six Ca sources mixed at three rates each plus an additional control consisting of deionized water were evaluated for the potential spray damage caused on petunia flowers. Six weeks after liners were transplanted, the effect of flower age was evaluated by marking individual flowers with a tag on the day they opened. At the time of the spray application, 0-, 1-, 3-, 5-, and 7-d-old flowers were sprayed once to determine the effect of flower age on the susceptibility to spray damage. Day 0 refers to unopened flower buds that open the day after the spray application. Flowers were evaluated 1, 3, 5, and 7 d after spray application. Spray treatments consisted of laboratory-grade Ca chloride (anhydrous 96% purity; Thermo Fisher Scientific, Waltham, MA, USA), commercial-grade Ca chloride (80% purity; TETRA Chemicals, The Woodlands, TX, USA), Ca nitrate (19% Ca; Hydro Agri North, Tampa, FL, USA), Ca amino acid chelate (6% Ca, Metalosate® Calcium; Albion Laboratories, Layton, UT, USA), and Ca salt of ethylene-diamine-tetraacetic acid chelate [Ca-EDTA (9% Ca; Brandt Sequestar, Springfield, IL, USA)] at 500, 1250, or 2000 mg·L$^{-1}$ Ca. Calcium silicate (99% purity; Alfa Aesar Chemicals, Ward Hill, MA, USA) was applied at rates of 143, 285, or 427 mg·L$^{-1}$ Ca because of its low solubility in water. A spray application containing deionized water only was used as a control. The spray rate for each application was 0.5 gal/100 ft$^2$, and applications were done between 0800 and 1100 HR using hand sprayers. Electrical conductivity (EC) and pH of the solutions were measured (Table 1). Five plants were used per treatment and two flowers per flower age per plant for a total of 10 flowers per plant and 10 flowers per treatment. Petunia plants were arranged on the greenhouse bench in a completely randomized design. Individual flowers were evaluated visually 1, 3, 5, and 7 d after the spray application using a 1- to 5-point scale (1 = 0%, 2 = 1% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = 76% to 100% of the petal area was damaged) for spray damage. The experiment was conducted twice.

**Evaluation of Ca sources for Botrytis blight efficacy (Expt. 2).** The same Ca sources, rates, and control group as described in Expt. 1 were evaluated for their efficacy for reducing Botrytis blight severity on petunia flowers. There were 20 treatments in this experiment; two additional controls were included: an non-inoculated and an inoculated control. These treatments received a deionized water spray and did not receive any additional Ca from spray applications. Six plants were used per treatment and were arranged on the greenhouse bench in a completely randomized design. A single spray application was done 24 h before inoculation with botrytis spores. The Ca sprays were applied between 1600 and 1830 HR at a rate of 0.5 gal/100 ft$^2$ using hand sprayers.

**Table 1. Measured pH and electrical conductivity (EC) values of six calcium spray solutions applied at three rates for the purpose of evaluating their effect on botrytis severity and potential spray damage on petunia (*Petunia ×hybrida*) flowers.**

| Calcium source | Molecular formula | Calcium ($mg·L^{-1}$)[i] | pH | EC ($dS·m^{-1}$)[i] |
|---|---|---|---|---|
| Calcium chloride (laboratory grade) | $CaCl_2$ | 500 | 6.7 | 2.3 |
| — | — | 1,250 | 7.1 | 5.0 |
| — | — | 2,000 | 8.2 | 8.5 |
| Calcium chloride (commercial grade) | $CaCl_2$ | 500 | 7.5 | 2.8 |
| — | — | 1,250 | 8.8 | 5.8 |
| — | — | 2,000 | 9.4 | 8.9 |
| Calcium nitrate | $Ca(NO_3)_2$ | 500 | 6.9 | 3.2 |
| — | — | 1,250 | 6.7 | 7.8 |
| — | — | 2,000 | 6.5 | 11.5 |
| Calcium amino acid chelate | — | 500 | 6.5 | 2.5 |
| — | — | 1,250 | 6.5 | 5.6 |
| — | — | 2,000 | 6.4 | 10.1 |
| Calcium ethylenediaminetetraacetic acid chelate | $C_{10}H_{14}CaN_2Na_2O_8$ | 500 | 6.6 | 1.6 |
| — | — | 1,250 | 6.8 | 3.1 |
| — | — | 2,000 | 6.9 | 6.7 |
| Calcium silicate | $Ca_2SiO_4$ | 143 | 8.9 | 0.2 |
| — | — | 285 | 9.5 | 0.4 |
| — | — | 427 | 9.6 | 0.6 |

[i] 1 $mg·L^{-1}$ = 1 ppm, 1 $dS·m^{-1}$ = 1 mmho/cm.

Twenty-four hours after the Ca spray application, three freshly opened flowers per plant were harvested with 3 cm of pedicel, for a total of 18 flowers per treatment. Flowers were placed immediately into vials filled with 9 mL deionized water to maintain turgor and hold flowers upright. Flowers were then placed immediately into a 2.8 × 0.8 × 0.6-m (length × width × height) humidity chamber with high relative humidity (97.8%) measured using a psychrometer (RH300; Extech Instruments, Nashua, NH, USA) after the chamber was sealed. Trays (53 × 26 cm) were placed in the bottom of the chamber and filled with water to maintain the humidity in the chamber. Flowers were then inoculated individually with a $1 \times 10^4$ conidia/mL botrytis spore suspension using hand sprayers providing ~1 mL of inoculum suspension per flower. The non-inoculated flowers were sprayed with deionized water and then placed in the chamber after the inoculated treatments to avoid inoculate solution drift onto the negative control. The chamber was then sealed and data were collected every 12 h for 72 h by taking pictures and performing a blind rating of flowers for Botrytis blight severity from the pictures after each experiment replication ended. Infection severity was rated on a scale of 1 to 9 points (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 flower petal area infected). Flowers were arranged in the chamber with six flowers per treatment grouped together with three locations per treatment randomized within the humidity chamber. The same isolate was used for this study with the same preparation, storage, and maintenance as described by Bennett et al. (2020b). The experiment was conducted twice.

**Data analysis.** Data analysis was performed using statistical software (JMP Pro version 13.2.0; SAS Institute Inc., Cary, NC, USA). For each experiment, analysis of variance (ANOVA) was used to determine treatment effects, and Tukey's honestly significance difference test was used to compare means among treatments at $P < 0.05$. For Expt. 1, a two-factor ANOVA was performed to evaluate the effect of spray treatment and flower age and their interaction. For Expt. 2, a one-factor ANOVA was performed to analyze the effect of spray treatment on Botrytis blight severity as quantified by calculating the area under the disease progression as described by Bennett et al. (2020b). The area under the disease progress curve is a cumulative measure from which a single value is calculated for each flower, and these values were used in the ANOVA.

## Results and discussion

Spray damage severity differed among the Ca sources (Fig. 1). The laboratory-grade and commercial-grade Ca chloride products at 500 and 1250 $mg·L^{-1}$ Ca and Ca nitrate applied at 500 $mg·L^{-1}$ Ca demonstrated the lowest spray damage severity with no significant damage to flowers. All Ca sources sprayed at the rate of 2000 $mg·L^{-1}$ Ca exhibited spray damage. Calcium-EDTA exhibited the greatest amount of spray damage. Van Engelen et al. (2011) reported that the EDTA component of Ca-EDTA causes spray damage at concentrations greater than 200 $mg·L^{-1}$. When applying 500 $mg·L^{-1}$ Ca from Ca-EDTA, the concentration of the EDTA component is 4500 $mg·L^{-1}$. In poinsettia cuttings, phytotoxic responses were observed from Ca-EDTA spray applications at 160 $mg·L^{-1}$ Ca (Samarakoon and Faust 2019). Calcium silicate exhibited high spray damage severity at all three concentrations applied. The Ca concentrations were considerably less in the Ca silicate treatments than the other Ca sources, so the spray damage was likely caused by the silicon. The Ca silicate solutions had a high pH (Table 1) but were not unique compared with the high rates of Ca chloride, so pH is not a likely cause of the spray damage.

Spray damage severity varied with flower age (Fig. 1). Flowers that were 0 d old (i.e., unopened flower buds)

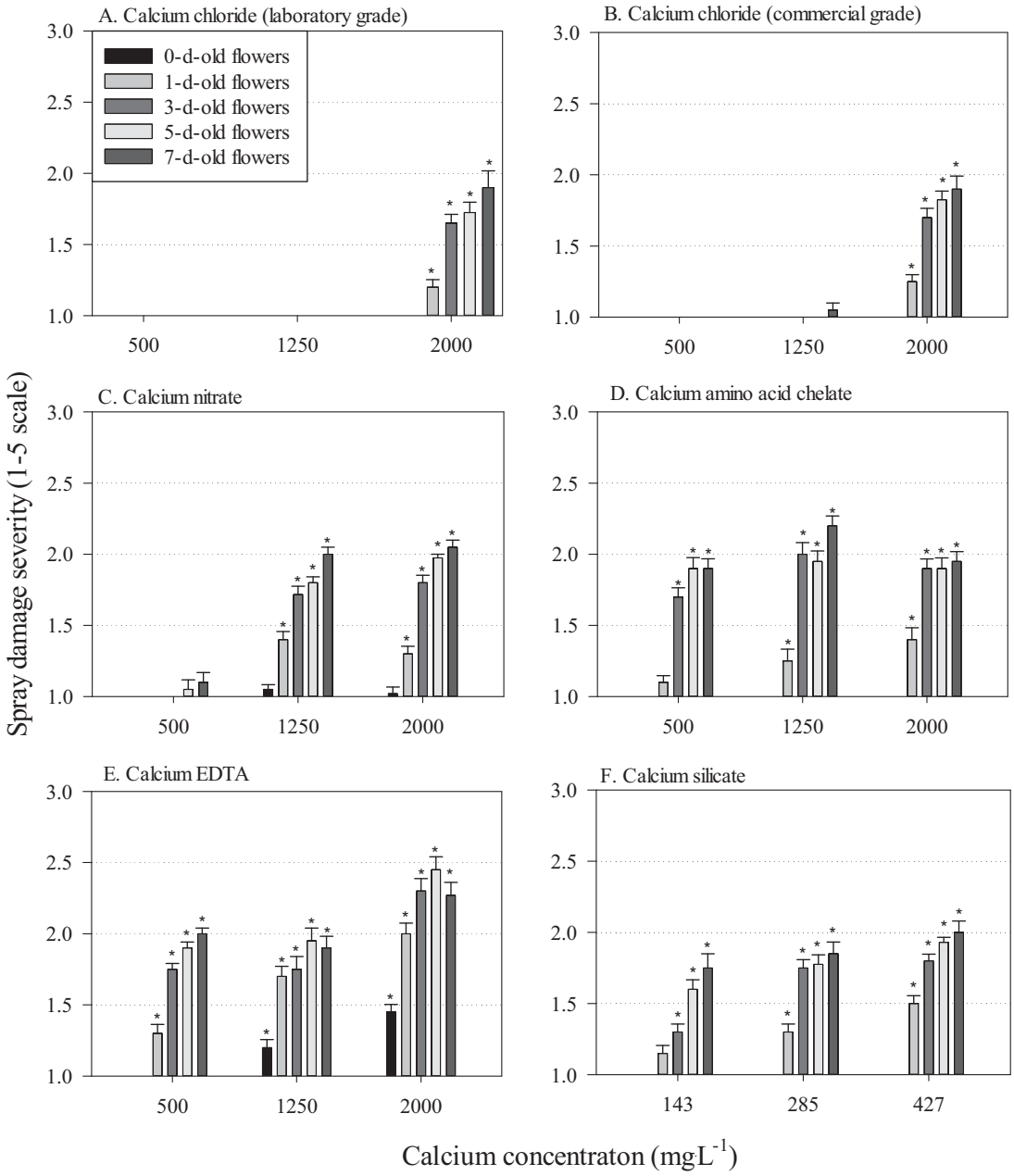


**Fig. 1. Evaluation of six calcium sources at three application rates for their resulting spray damage on different-age petunia (*Petunia ×hybrida*) flowers ($n$ = 20). Spray damage severity was rated 24 h after spray application using a 1 to 5 scale (1 = 0%, 2 = 1% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, 5 = 76% to 100% petal tissue damage area). Least squared means were calculated based on Tukey's honestly significance difference test (α = 0.05), and asterisks identify treatments significantly different from the control, which had no spray damage severity. EDTA = ethylenediaminetetraacetic acid. Error bars represent +1 standard error; 1 mg·L$^{-1}$ = 1 ppm.**

were the least susceptible to spray damage from any Ca source. The only Ca source to cause significant damage on flower buds was Ca-EDTA, and the damage occurred at 1250 and 2000 mg·L$^{-1}$ Ca. All sources, except for Ca-EDTA, can be sprayed on flower buds without any damage at the highest rate tested in this study. Therefore, spray applications can have greater Ca concentrations before open flowers are present; however, after flowers are open, Ca concentrations in the spray solution should be decreased because the flower petal tissue becomes more susceptible to spray damage. Flowers exhibited the greatest amount of spray damage on 3-, 5-, and 7-d-old flowers. Because individual petunia flowers survive for ~9 d, if spray damage were to occur, these flowers would senesce and the plants would be completely undamaged ~1 week after spraying. Thus, the persistence of the spray damage is relatively limited.

Calcium chloride, Ca nitrate, and Ca amino acid chelate reduced Botrytis blight severity significantly by an average of 85%, 79%, and 76%, respectively, as the Ca concentration increased from 0 to 1250 mg·L$^{-1}$ Ca (Fig. 2). No significant benefit was

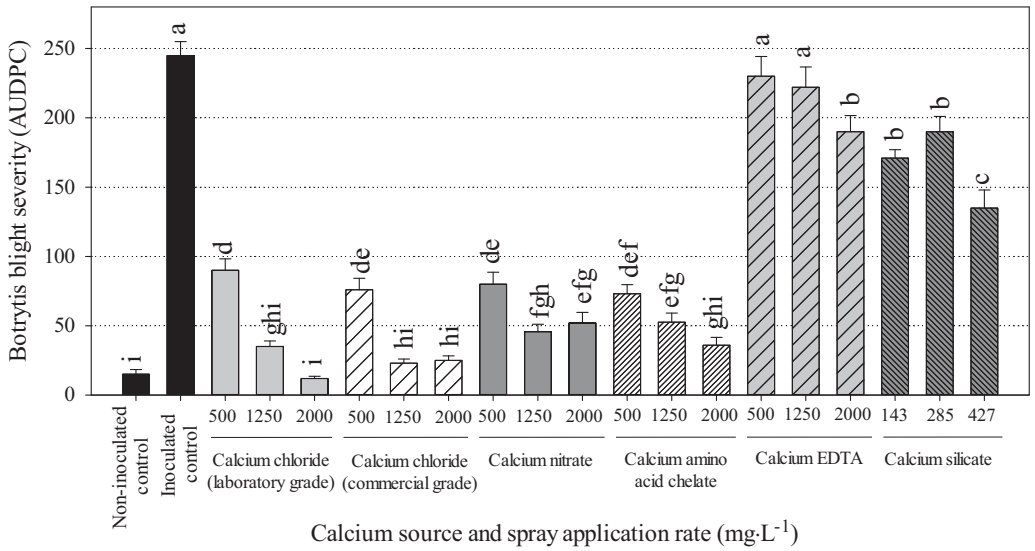


**Fig. 2. Evaluation of six calcium sources at three application rates for their effect on Botrytis blight severity of petunia (*Petunia ×hybrida*) flowers after inoculation with botrytis spores ($n$ = 36). Inoculated and non-inoculated control groups were included. Botrytis blight severity is expressed as the sum of the area under the disease progression curve (AUDPC). Least squared means were calculated based on Tukey's honestly significance difference test ($\alpha$ = 0.05). Lowercase letters indicate significantly different treatments across calcium sources and application rates. EDTA = ethylenediaminetetraacetic acid. Error bars represent +1 standard error; 1 mg·L$^{-1}$ = 1 ppm.**

observed as the Ca concentration for any of these products increased from 1250 to 2000 mg·L$^{-1}$ Ca. The Ca-EDTA and Ca silicate treatments had a relatively small effect on botrytis severity compared with the other Ca sources.

Two modes of action are suggested for the effect of Ca on Botrytis blight. First, within the middle lamella of the cell wall, Ca binds with polygalacturonic acid (pectin) creating Ca pectate, which causes a gelling effect in the middle lamella that may decrease the accessibility of cell wall degrading enzymes (polygalacturonases) secreted by botrytis (Conway and Sams 1984). The second possible mode of action suggests a direct effect of Ca on the production of polygalacturonase by botrytis (Volpin and Elad 1991). Polygalacturonase activity in botrytis was inhibited by 90% when grown in an in vitro solution containing 40 mg·L$^{-1}$ Ca (Cabanne and Donèche 2002).

Four Ca products were similarly effective for reducing Botrytis blight; however, it is important to take into consideration the potential spray damage of each source. Both the laboratory-grade and commercial-grade Ca chloride demonstrated the greatest efficacy for reducing Botrytis blight from the 1250-mg·L$^{-1}$ Ca treatments without causing spray damage on buds or open flowers. Calcium nitrate was also effective for reducing Botrytis blight severity at 500 mg·L$^{-1}$ Ca, and spray damage was not significant at that rate; however, unlike Ca chloride, Ca nitrate caused spray damage on flowers at 1250 mg·L$^{-1}$ Ca. The Ca amino acid chelate treatments produced similar Botrytis blight efficacy results compared with Ca chloride, but the potential spray damage increased on open flowers. Two Ca sources, Ca-EDTA and Ca silicate, provided lower efficacy and greater spray damage severity compared with the other Ca sources.

The use of Ca to reduce Botrytis blight of floriculture crops began with the need to address bract-edge burn of poinsettia (Barrett et al. 1995). This phenomenon was determined to be a localized Ca deficiency along the outer margins of the bracts, which allowed for botrytis infection and further expansion of necrotic tissue. Weekly applications of Ca nitrate at a rate of 400 mg·L$^{-1}$ Ca throughout bract development became a standard practice through the 1990s and 2000s. In the past decade, these Ca applications have been mostly eliminated, as cultivars that are resistant to bract-edge burn have replaced older cultivars.

The use of Ca on other floriculture crops has rapidly become a standard practice among growers in the United States since our first report (Samarakoon et al. 2016); however, success with this approach appears to vary based on the plant species, tissues in question, and application methods. Flower petal tissue has a relatively low Ca concentration compared with leaf tissue (Muñoz 2022). So, foliar sprays effectively increase the Ca concentrations in flower petals whereas fertigation applications fail to deliver adequate Ca to these low-transpiring tissues (Bennett et al. 2020a, 2020b). Our observations suggest that the most benefit from Ca spray application occurs on species with large flower petals when there can be good contact with the application solution and the individual petals. Species with multiple layers of petals (e.g., double-flowering forms) can be difficult to provide adequate spray coverage to the inner petals before the flowers open completely. Thus, dip applications of cut flower rose into Ca solutions are more effective than spray applications (Muñoz 2022).

In conclusion, several Ca sources provided similar reductions in Botrytis blight; however, it is important to consider spray damage potential as well. The results from our study suggest that Ca chloride is the most effective source of those tested for reducing Botrytis blight severity and causing the least amount of spray damage. Flower age should also be taken into consideration because the potential for

damage increases as the age of the flower increases. Calcium sources containing EDTA should not be used while flowers are present on petunia plants because of the high risk of spray damage.

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