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The Effect of Calcium on Botrytis Blight of Petunia Flowers

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THE EFFECT OF CALCIUM ON BOTRYTIS BLIGHT OF PETUNIA FLOWERS

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ABSTRACT

During greenhouse production, petunia plants are frequently exposed to conditions optimal for botrytis blight, caused by *Botrytis cinerea*. Once plants are flowering and ready to be shipped to retail, they are irrigated and tightly packed onto shipping carts, creating a humid environment conducive for *Botrytis* growth. At the retail environment, petunia flowers exhibit symptoms of botrytis blight, e.g., tan necrotic spots that coalesce making the plants unsellable. Fungicide applications are the primary strategy for *Botrytis* management; however, fungicide resistance is a common phenomenon that results in lack of fungicide efficacy. Alternative *Botrytis* management strategies are necessary. The goal of this thesis was to explore the potential of calcium (Ca) to reduce botrytis blight on petunia flowers. In the first part of this study, increasing Ca concentrations in a calcium chloride spray solution reduced botrytis blight severity up to 93%. In Chapter 1, three factors that may have been the source of the disease reduction were explored: Ca, the chloride ion (Cl), and/or the electrical conductivity (EC) of the solution. The results demonstrated that Ca was the sole active ingredient in calcium chloride causing the reduction in botrytis blight severity. In Chapter 2, two methods for delivering Ca to plant tissues were compared, namely fertigation and spray applications. Calcium fertigation applications were ineffective for increasing Ca concentration in flower petals and for reducing botrytis blight severity. In contrast, Ca spray applications increased Ca concentration in flower petal tissue and reduced botrytis blight severity in the flowers. In Chapter 3, six calcium sources were evaluated for their potential for reducing botrytis blight while not causing spray damage. Calcium chloride, either lab grade or commercial

grade, provided the highest disease reduction with the lowest spray damage potential at 1250 $mg \cdot L^{-1}$. Higher rates (2000 $mg \cdot L^{-1}$ Ca) did not improve the reduction in disease infection and did cause spray damage to flowers; however, flower buds were not damaged by the 2000 $mg \cdot L^{-1}$ Ca treatments. We conclude from this study that spray applications of 1250 $mg \cdot L^{-1}$ Ca from $CaCl_2$ are safe to spray on both flower buds and open flowers without risk of spray damage for maximum botrytis blight efficacy. The results from this research project demonstrate the effectiveness of Ca spray applications as an additional management strategy for decreasing flower susceptibility to botrytis blight.

DEDICATION

To God, with you, all things are possible.

To my parents, for all the unconditional love and support in everything that I do. I wouldn’t be who I am today, without you.

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CHAPTER ONE
LITERATURE REVIEW

Introduction

Floriculture is an important sector of the ornamental horticulture industry that includes crops such as bedding plants, herbaceous perennials, cut flowers, cut foliage, potted flowering plants, and indoor foliage plants (Jerardo, 2007). Amongst the world's most popular bedding plants is the petunia with its diversity of color and morphology (Griesbach, 2007).

Botanical history

Solanaceae is an important family that includes several important ornamentals, medicinal and nutrition species. *Petunia*, a solanaceous genus, is native to South America. The garden petunia, *Petunia* ×*hybrida* was obtained by the hybridization of two unidentified species in 1834 in Britain. The spread of the cultivated petunia began in Europe and today it is grown around the world (Stehmann et al., 2009).

Economic importance

The floriculture industry was valued at $4.37 billion dollars in 2015 (USDA, 2016). In the U.S. market, petunia wholesale value annually exceeds $130 million dollars. Seeds are typically sown in greenhouses during the winter months and flower during spring (Stehmann et al., 2009). In recent years, petunia flower meltdown, caused by *Botrytis cinerea*, has become a significant issue for commercial producers. The symptoms occur during shipping where necrotic spots appear on flower petals that

rapidly coalesce resulting in collapsed flowers as the plants arrive for display in retail garden centers.

Diseases of Petunia

There are several plant fungi, oomycetes, bacteria and viruses that infect petunia. The following are the principle diseases: botrytis blight of petunia flowers is caused by the organism *Botrytis cinerea*. This pathogen causes spotting on the flower petals that can develop to complete petal necrosis. The organism *Phytophthora nicotianae* causes damping off as well as crown and stem rot on petunia. *Pythium spp.* cause damping off during propagation, crown and/or stem rot. Powdery mildew can be caused by several genera. Viruses are primarily a problem with vegetatively propagated petunias (Daughtrey et al., 1995).

Calcium uptake

Calcium (Ca), a divalent cation essential for plant growth, is necessary for structural roles in the cell wall and membranes. Calcium also acts as a counter-cation for inorganic and organic anions in the vacuole and as an intracellular messenger responding to several developmental and environmental signals (White and Broadley, 2003). Calcium is taken up by the roots from the soil solution and delivered to the shoot through the xylem via mass flow (Bangerth, 1979). Movement of Ca from the roots to the xylem is restricted to the root tip and to regions where lateral roots are initiated. In these regions of the root, the casparian band is non-continuous which allows for the movement of solutes into the stele and into the xylem. The casparian band restricts symplastic

movement of solutes into endodermal cells which suggests that Ca uptake occurs via the apoplast to the xylem (White, 2001).

Calcium deficiencies rarely occur in nature; however, they frequently occur in several horticulture crops that include fruits, vegetables, roots, and young leafy shoots (Shear, 1975). These deficiencies occur due to the imbalanced distribution of Ca to these tissues. Reproductive tissues, storage organs, and young leaves contain significantly less Ca than mature leaves from the same plant. Movement of Ca into tissue is largely dependent on transpiration as Ca moves through the xylem (White and Broadley, 2003). Due to the lack of transport proteins involved in Ca uptake, Ca primarily moves passively with the mass flow of water through the plant vascular system (White, 2001).

Calcium distribution within cells and intercellular spaces

A high proportion of the total Ca is found in the cell wall (Marschner, 2012). High Ca concentrations can be found in the middle lamella of the cell wall, the exterior surface of the plasma membrane, the endoplasmic reticulum, and the vacuole. Calcium levels in the cytosol are usually low with concentrations in the range of 0.1- 0.2 µM of free Ca (Evans et al., 1991). The low cytosolic concentration may be essential for different reasons, such as preventing the precipitation of inorganic phosphate, competition with other cations such as magnesium, and as a prerequisite for Ca to function as a secondary messenger (Marschner, 2012).

Most of the functions of Ca as a structural component are related to its ability to form stable, but reversible, linkages predominately in the cell wall and plasma membrane. Calcium occurs in cell walls and membranes in the form of Ca-pectate which

binds the cell walls through Ca-pectate bonds in the middle lamella (Tuteja and Mahajan, 2007). These Ca-pectic bonds are broken by the enzyme polygalacturonase. In Ca-deficient tissue, polygalacturonase activity is increased (Konno et al., 1984). Under low Ca concentrations, membranes become leaky, resulting in the loss of cellular compartmentation, and the cross linkage of Ca with pectin in the middle lamella is weakened (Marinos, 1962).

Cells walls provide a physical barrier against plant pathogens from the internal contents of plant cells. Polysaccharides, such as pectic material, in the cell wall are crosslinked by both ionic and covalent bonds into a network that provides strengthening to resist physical penetration by the pathogen. Many pathogens release enzymes like polygalacturonases and pectate lyases which degrade the polysaccharides. Calcium interaction with polysaccharides, such as polygalacturonic acid, allows for the cementing of the middle lamella thus facilitating increased plant resistance to pathogen invasion (Vorwerk et al., 2004).

Calcium distribution in plant tissues

Plants grown with sufficient Ca in natural habitats have shoot Ca concentrations from 0.1% to 5.0% Ca dry weight. (Marschner, 2012). Plants showing symptoms of Ca deficiency may be due to uneven or localized Ca deficiencies (Anghileri and Tuffet-Anghileri, 1982). Increases in concentration of Ca in the nutrient solution leads to an increase in the Ca content of the leaves whereas in low-transpiring organs, such as in fleshy fruits or flowers, this is not necessarily the case as they are supplied predominately by the phloem (Marschner, 2012). The restriction of Ca in the phloem supplied to fruit

allows for rapid cell expansion and membrane permeability to occur, allowing the fruit to ripen more quickly. High growth and rapid cell expansion of reproductive tissue causes the tissue to fall below the critical value necessary for cell wall stabilization and membrane integrity (Marschner, 2012).

Calcium uptake is primarily driven by transpiration. Altering the relative humidity of the environment is one management strategy employed to promote increased transpiration. However, as flowers and fruits typically have reduced transpiration rates, Ca distribution to these tissues is low. Tipburn of lettuce, a Ca related disorder, was induced when inner leaves were artificially enclosed allowing for decreased transpiration and vapor pressure deficit, which caused Ca in the inner leaves to decrease from 1.43 to 0.63 $mg \cdot g^{-1}$ dry weight (Barta and Tibbits, 1986). This indicated that Ca movement to leaves is driven by the transpiration. In poinsettia, decreased relative humidity from 90% to 60% did not affect the incidence of bract edge burn (Strømme et al., 1994) which may be attributed to the low stomatal density of bracts (Nell and Barrett, 1986). In cut roses, Ca concentration in the petals, stems or leaves was not or slightly increased Ca, resulting in a low possibility of increasing Ca concentrations in tissue alone by altering the relative humidity (Baas et al., 2003).

Two primary methods are used for increasing Ca concentrations in plant tissue, through the nutrient solution or through spray applications. A typical petunia fertilization program provides 100 $mg \cdot L^{-1}$ N and between 17 to 33 $mg \cdot L^{-1}$ Ca at every irrigation event, depending on the formulation of pre-blended fertilizer used. The standard range for Ca concentrations in petunia leaf tissues ranges from 1.2% to 2.8% Ca, while Ca levels in

flowers are not typically measured or reported (Jones Jr. and Mills, 1996). Studies have demonstrated the effects of increasing Ca in the nutrient solution for increasing Ca in plant tissues. A study on cut lily demonstrated that increasing Ca from 40 to 240 $mg \cdot L^{-1}$ in the nutrient solution increased Ca concentration in the leaf tissue from 0.20% to 1.83% (Salazar-Orozco et al., 2010). In potted rose, as Ca increased in the nutrient solution from 44 to 176 $mg \cdot L^{-1}$, Ca concentrations of flowers and flower buds increased from 0.4% to 0.6% (Starkey and Pedersen, 1997). In poinsettia, Ca concentrations in leaves increased from 0.35% to 0.75% as the Ca supplied in the nutrient solution increased from 19 to 300 $mg \cdot L^{-1}$ (Jacques et al., 1990).

Calcium spray applications provide targeted distribution of Ca to tissue, such as flowers and fruits, that would otherwise have difficulty assimilating Ca from the nutrient solution. Álvarez (2012) evaluated spray applications from three different sources of Ca, including calcium chloride, calcium oxide and calcium + amino acid chelate at 250, 500 and 1000 $mg \cdot L^{-1}$ Ca and observed that Ca concentrations in leaves increased while flower petal tissue did not. Harbaugh and Woltz (1989), demonstrated that bract edge burn of poinsettia was related to Ca deficiency and could be reduced with foliar spray applications of 400 $mg \cdot L^{-1}$ Ca whereas increasing Ca levels in the growing medium did not reduce bract edge burn. Strømme et al. (1994), determined that bract edge burn was a localized Ca deficiency by sectioning and separating the outer margins of poinsettia bracts from the inner portion and performing nutrient analysis on these divided bracts. This study also showed an inverse linear relationship between Ca concentration in the tissue increased and bract edge burn decreased. Nell and Barrett (1986) identified that

low stomatal density of poinsettia bracts that causes decreased transpiration and the movement of Ca to these tissues. Weekly pre-harvest Ca spray applications of Ca-chelate at 200 $mg \cdot L^{-1}$ Ca on strawberries decreased botrytis blight during postharvest storage (Naphun et al., 1997). Kiwifruit sprayed up to three times during fruit development with 0, 1350, 2700, 4050, or 5400 $mg \cdot L^{-1}$ Ca from calcium chloride ($CaCl_2$) increased Ca concentration in the pericarp, core and skin by up to 200% (Gerasopoulos et al., 1996). These studies demonstrate the potential value of Ca spray applications for increase flower petal tissue that may be susceptible to *Botrytis* infection.

Botrytis cinerea

Botrytis blight of petunia is caused by the pathogen *Botrytis cinerea* which is a ubiquitous airborne, necrotrophic pathogen that causes infection by killing host cells to obtain nutrients. This pathogen attacks over 200 crop hosts and causes serious losses worldwide. *Botrytis cinerea* mainly hosts dicotyledonous plant species in both temperate and subtropical regions. The pathogen can cause soft rotting of all aerial plant parts in the greenhouse or field as well as in the post-harvest environment. *B. cinerea* produces enzymes targeting the cell wall. Evidence suggests that the pathogen also triggers the host to induce programmed cell death as a remediation for attack (Williamson et al., 2007).

Disease cycle. *Botrytis cinerea* can persist in the greenhouse year-round on living or dead tissue as conidia, mycelium, or sclerotia. The ellipsoid conidia, born on conidiophores, move through the air and germinate at optimal temperatures of 22 to 28 °C, however some growth does occur from 0 to 35 °C, and relative humidity above 93% or free water is necessary for germination. Spores land on tissue where infection may

occur directly, through natural wounds and openings by conidial germ tubes or hyphal growth from previously colonized dead plant tissue or debris. Free water can facilitate germination at 22 °C in as little as 4 h. Once infection occurs, the tissue becomes necrotic and under optimal conditions sporulation will occur producing the characteristic gray fuzzy appearance (Daughtrey et al., 1995). Sclerotia, a melanized structure important for survival of this pathogen, develop in dying host tissue (Daughtrey et al., 1995). The sclerotia protect the internal mycelium from desiccation, ultraviolet radiation and microbial attack over time (Backhouse and Willetts, 1984). The cycle continues as newly infected tissue is colonized and sporulation occurs (Williamson et al., 2007).

Disease management. *Botrytis cinerea* is considered a "high-risk" pathogen due to its propensity for developing resistance to new chemistries used for disease management. In greenhouses, *Botrytis* isolates with multiple chemical class resistance have been discovered, decreasing the efficacy of fungicides (Konstantinou et al., 2015; Samarakoon et al., 2017). The current strategies for *Botrytis* management include chemical, cultural and biological approaches. Fungicide applications are the current standard for *Botrytis* management. However, as awareness to fungicide resistance increases, alternative strategies become necessary. Cultural practices can also be used to manage levels of inoculum present in greenhouses (Elad and Shtienberg, 1995). Preventative measures are most commonly taken to alter the microclimate in the canopy. Dense plant canopies, that cause higher humidity with decreased air flow, create a more conducive environment for *Botrytis*. Spacing of plants can be utilized to decrease canopy density, increase airflow, and reduce humidity throughout the crop. Timing of irrigation, sufficient ventilation,

heating, and increased air circulation are management strategies that can be implemented to shorten the duration of leaf wetness as this is a strict requirement for *B. cinerea* spore germination (Daughtrey et al., 1995). Flower size may also contribute to increased susceptibility to *Botrytis* infection. As flowers are bred to be larger, the Ca concentration in the petals in rapidly expanding flower tissue decreases, decreasing the integrity of the flower petals. A preliminary experiment was performed screening thirty-seven different cultivars of varying flower size (2.5 to 7.0 cm in diameter) and color. After evaluation of *Botrytis* infection following inoculation, results indicate that larger petunia flowers exhibited increased susceptibility to *Botrytis* infection.

Effects of calcium on botrytis blight

Several studies have been conducted evaluating the effect of Ca on *Botrytis*. A study on potted roses demonstrated that a continuous supply of Ca from 44 to 176 $mg \cdot L^{-1}$ in the nutrient solution decreased botrytis blight incidence as Ca concentration in the flowers increased (Starkey and Pedersen, 1997). Volpin and Elad (1991) examined two cut rose cultivars dipped in calcium sulfate or $CaCl_2$ solutions containing 120 $mg \cdot L^{-1}$ Ca and found that both Ca sources reduced botrytis blight severity in both cultivars. Two studies have been conducted on cut roses examining the effect of Ca spray on botrytis blight. Álvarez (2012) evaluated three different sources, $CaCl_2$, calcium oxide, and calcium + amino acid chelate. All three sources reduced botrytis blight severity at 500 and 1000 $mg \cdot L^{-1}$ Ca; however, $CaCl_2$ was the only Ca source to reduce disease severity at 250 $mg \cdot L^{-1}$ Ca, indicating differences between Ca sources. The second study performed by De Capdeville et al. (2005) observed decreased botrytis blight severity as Ca spray

applications increased from 100 to 800 $mg \cdot L^{-1}$ Ca. A study performed on poinsettia cuttings demonstrated that Ca spray applications of 400 and 800 $mg \cdot L^{-1}$ Ca decreased *Botrytis* severity (Samarakoon et al., 2017). Studies have also been performed on fruit crops evaluating the effect of Ca on *Botrytis* infection. In strawberries, García et al. (1996), demonstrated that postharvest dips in $CaCl_2$ significantly reduced gray mold decay of fruit. Weekly pre-harvest Ca spray applications of Ca-chelate on strawberries at 200 $mg \cdot L^{-1}$ Ca decreased *Botrytis* infection during postharvest storage (Naphun et al., 1997). A study on table grapes demonstrated the efficacy of spray applications of 3600 $mg \cdot L^{-1}$ Ca from calcium chloride applied before harvest in reducing the incidence of botrytis blight after 30 d storage (Nigro et al., 2006). Chardonnet et al. (2000) evaluated the effects of pressure infiltration of 0 and 21,600 $mg \cdot L^{-1}$ Ca from $CaCl_2$ on 'Red Delicious' apples on the pathogenicity of different isolates of *B. cinerea*. Fruit were evaluated 5, 6 and 7 days after inoculation and at each evaluation the Ca treatment was significantly lower than the control for each isolate tested. These studies demonstrate that several sources of Ca can influence *Botrytis* infection, demonstrating possible benefit for the management of botrytis blight on petunia.

Purpose of this study

Greenhouse producers have expressed difficulties managing *Botrytis* infection on petunia during production and in shipping. The goal of this study was to determine the effect of Ca on the severity of botrytis blight on petunia. The first part of the study evaluates the effect of increasing rates of Ca as well as the mechanism by which $CaCl_2$ spray applications reduce botrytis blight. This was evaluated by determining the effect of

Ca concentration, the chlorine concentration, and the electrical conductivity of the solutions on botrytis blight severity. The second study examines the most effective method for delivering Ca to flower petal tissue by comparing fertigation and spray applications of Ca at a range of different rates. The third study evaluates different sources of Ca for their potential spray damage to flower petals of petunia and the efficacy of these products for reducing botrytis blight severity.

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CHAPTER TWO

THE EFFECT OF CALCIUM SPRAY APPLICATIONS ON *BOTRYTIS* INFECTION OF PETUNIA FLOWERS

Abstract

Previous studies have demonstrated the effect of Calcium (Ca) on *Botrytis* infection of horticultural crops. Botrytis blight on petunia flowers causes significant losses in the postharvest environment. Infection occurs during greenhouse production and symptoms are expressed during transport. This phenomenon is termed petunia flower meltdown due to the rapid collapse of flower petal tissue. The objective of this study was to determine the effect of Ca spray applications on botrytis blight severity in petunia flowers. For the first experiment, petunia 'Pretty Grand Red' plants were sprayed twice per week for two weeks with calcium chloride ($CaCl_2$) at rates of 0, 400, 800 and 1200 $mg \cdot L^{-1}$ Ca. A fungicide (cyprodinil 37.5% and fludioxonil 25%) was used as an additional control treatment. Twenty-four hours after the last treatment, freshly opened flowers were harvested, placed into a humidity chamber with 99% relative humidity, and inoculated with a *Botrytis cinerea* spore suspension ($1x\ 10^4$ $conidia \cdot mL^{-1}$). Disease progression was recorded every 12 h for 72 h. Botrytis blight severity decreased as Ca concentration increased from 0 to 1200 $mg \cdot L^{-1}$ Ca. The calcium treatments provided better disease control than the fungicide treatment due to the fungicide resistance of the isolate used in the study. A second experiment was performed to determine if the beneficial response to $CaCl_2$ application was influenced by chlorine (Cl) or the electrical

conductivity (EC) of the spray solutions. This experiment proved that Ca is the sole source of the reduction in botrytis blight severity following treatment with $CaCl_2$. These results demonstrate the benefit of $CaCl_2$ spray applications for management of botrytis blight of petunia flowers.

Additional index words:

calcium chloride, disease management, electrical conductivity, petunia flower meltdown

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Introduction

Botrytis cinerea, the causal agent of botrytis blight, is a ubiquitous plant pathogen that infects over 200 crop species worldwide. Although there are fungicides available for *Botrytis* management, many chemical classes have low efficacy due to high levels of fungicide resistance in commercial greenhouses (Williamson et al., 2007). *Botrytis* becomes a significant threat in greenhouses during periods with relative humidity above 93% (Williamson et al., 2007) and temperatures between 10-20 °C; however, infection can also occur at 2 °C and above 25 °C (Elad and Shtienberg, 1995). Moderate temperatures and/or rainy weather during the spring bedding plant season negatively affects retail sales which causes growers to hold flowering plants in the greenhouses.

During this time plants grow closer together forming a denser canopy, and the oldest flowers begin to senesce. These conditions tend to increase the amount of inoculum present when the plants are moved into the postharvest environment resulting in the proliferation of infection on petunia flowers, causing significant losses in the retail environment (Kessler, 2004).

Botrytis cinerea produces cell wall degrading enzymes that play an important role in tissue maceration. Polygalacturonases are the first cell-wall degrading enzymes produced by *Botrytis* that bind with polygalacturonic acid (pectin material) in the middle lamella (Cabanne and Donéche, 2002). Calcium may act as a competitive inhibitor for the binding site of pectin material, causing gel formation in the middle lamella due to Ca cross-linking in the pectin chains (Conway and Sams, 1984) resulting in polygalacturonic acid becoming less accessible to polygalacturonase enzymes (Cabanne and Donéche, 2002).

Calcium is required for structural roles in cell walls and membrane development within plants (White and Broadley, 2003). Calcium deficiencies are common in horticultural crops (Shear, 1975; White and Broadley, 2003) and usually result from inefficient uptake and distribution of Ca throughout different tissues (Poovaiah, 1988). Passive uptake of Ca via mass flow of water through the plant is dependent of the transpiration. Flower buds and fruits are low transpiring organs due to their low stomatal density and/or large volume to surface area ratio. Similarly, young leaves that are enclosed in heads, such as in head lettuce, do not have high transpiration rates, making them particularly susceptible to Ca deficiencies. Reproductive tissues, e.g., fruit and

flowers, are mainly supplied with nutrients through the phloem where transport of Ca is low (Marschner, 2012). Localized Ca deficiencies have been described in 1) leafy greens, such as tipburn of lettuce (Thibodeau and Minoti, 1969), 2) fruits, such as blossom end rot of watermelon (Waters and Nettles, 1961) and bitter pit of apple (DeLong, 1936; Drake et al., 1966), 3) vegetables, such as blossom end rot of tomato (Evans and Troxler, 1953; Millikan et al., 1971) and pepper (Hamilton and Ogle, 1962; Miller, 1961) and 4) ornamentals, such as bract edge burn of poinsettia (Strømme et al., 1994). Increasing the Ca concentration in the nutrient solution can provide sufficient Ca in leaves that transpire throughout their life cycle but not necessarily in low transpiring and rapidly expanding organs and reproductive tissues (Marschner, 2012).

Numerous studies have examined the effect of Ca on *Botrytis* on horticultural crops with varying results. In cut roses, increasing Ca concentration in the nutrient solutions decreased botrytis blight severity and increased Ca content of the petals (Baas and Marissen, 2000; Bar-Tal et al., 2001; Volpin and Elad, 1991). Similarly, in potted roses, botrytis blight incidence decreased as Ca increased in the nutrient solution and the flower petal tissue (Starkey and Pedersen; 1997). Two similar studies evaluated pre-harvest spray applications of Ca in cut roses, from calcium sulfate, which caused decreased botrytis blight severity in flower petals (De Capdeville et al., 2005; Nabigol, 2012). Bract edge burn disorder of poinsettia begins as necrotic spots along the margin of the bracts, which is caused by a localized Ca deficiency (Strømme et al., 1994). The necrotic tissue can then be infected with *Botrytis* resulting in coalesced lesions that are termed bract edge burn (Barrett et al., 1995). This phenomenon can be corrected with

weekly Ca spray applications during bract development (Woltz and Harbaugh, 1985). Calcium spray applications have also increased the strength of the leaves of unrooted cuttings of poinsettia and geranium, and a subsequent reduction in *Botrytis* infection severity was also observed in poinsettia leaves (Samarakoon et al., 2017). Calcium spray applications, dips and vacuum infiltration of fruits have also been evaluated for reducing *Botrytis* severity. In grapes, pre-harvest Ca spray applications significantly reduced storage rot from *B. cinerea* (Nigro et al., 2006). Postharvest dips or vacuum infiltration of Ca on apples increased Ca content of apples and reduced decay caused by *B. cinerea* (Conway et al., 1993).

Calcium chloride spray solutions provide a means of increasing Ca content in plant tissues; however, chlorine, an essential plant micronutrient, is also supplied as chloride (Cl^-) during these applications. In plants, Cl^- is involved in maintaining turgor pressure and regulation of solutes in the vacuole. Chloride has functions in the stabilization of membrane potential, regulation of pH gradients, and electron excitation during photosynthesis. Chlorine deficiency is rarely observed in nature or in agricultural systems; however, toxicity can occur at high concentrations. The minimum chlorine concentration required in plant tissue has been suggested to be 1 $g \cdot kg^{-1}$ dry weight (Marschner, 2012). Chlorine, a common component of disinfectant products, has been tested for its effectiveness against reducing *Botrytis* inoculum; however, few studies have been conducted on the response of *B. cinerea* to Cl^- nutrition. Studies on *B. cinerea*, as well as other fungi, have been performed evaluating the pathogens response to Cl^- foliar sprays with varying results. A study on grapes showed that spray applications of

potassium chloride (KCl) and sodium chloride (NaCl) did not provide any reduction in the development of botrytis blight, while the $CaCl_2$ treatment provided significant reduction in botrytis blight development (Nigro et al. 2006). Kettlewell et al. (2000) investigated the use of KCl sprays for powdery mildew disease (*Erysiphe gramminis*) of wheat and suggested an osmotic effect on the causal agent for disease reduction.

The EC of $CaCl_2$ solutions applied to plants can be relatively high and have the potential to affect plant pathogens due to the osmotic effects from the high EC of the solution. For example, 300 $mg \cdot L^{-1}$ $CaCl_2$ and NaCl solutions reduced the germination of *B. cinerea* spores; however, the EC of the solutions were not measured (Boumaaza et al., 2015). inorganic salts have been shown to suppress various fungal pathogens (Deliopoulos et al., 2010).

Based on the literature demonstrating the effect of Ca spray applications on leaf tissue strength and botrytis blight we hypothesized that Ca may be beneficial for the management of petunia flower meltdown. The objectives of this study were to determine the effect of $CaCl_2$ spray applications on botrytis blight severity on petunia flowers and to determine if any beneficial response was due to Ca, Cl and/or the EC of the spray solution.

Materials and Methods

Two experiments were conducted to quantify the effect of Ca spray applications on the resistance of petunia flowers to *Botrytis*. The first experiment examined the response of harvested petunia flowers following spray applications of $CaCl_2$. The second

experiment was conducted to determine if the responses to $CaCl_2$ treatments observed in Expt. 1 were due to Ca, Cl, and/or to the EC of the $CaCl_2$ solutions.

Botrytis *isolation, culture maintenance, and preparation of conidial suspension.* Petunia plants were received from a commercial grower and plant tissue symptomatic of a *Botrytis* infection was removed and placed in a plastic bag containing a moist paper towel and incubated on a laboratory bench at 22 °C until sporulation was observed in 1 to 5 days. A pure culture was obtained by isolating spores from the incubated plant tissue and plating them on a petri dish (100 x 15 mm) with potato dextrose agar (PDA) medium (Difco laboratories, Sparks, MD) under sterile conditions. Mycelia from the leading edge of the colony was transferred to new PDA plates. This process was repeated until a pure mycelium culture of a single *Botrytis* isolate was obtained. The isolate was incubated until sporulation occurred and matured (7-10 d). For long-term storage, conidia were harvested by pipetting 3 mL of sterile aqueous solution of 0.01% Tween 80 (Sigma-Aldrich Corporation, St. Louis, MO) and 15% glycerol onto the petri dish. Then the spore solution was transferred to 2 mL cryogenic vials (Nalgene Corporation, Rochester, NY) and stored in an ultra-low temperature freezer at -80 °C. To obtain fresh spores for inoculation, the stored spores in cryogenic vials were retrieved from the -80 °C freezer and the solution was pipetted onto PDA and allowed to grow for 7 to 10 d. From each incubated PDA plate, mature spores were placed into solution by pipetting 5 mL of sterile de-ionized water onto PDA plates and using a sterile stir rod to lightly press on spores to get them to release from the conidiophores and go into solution. The spore solution was then pipetted from the PDA plate into sterile de-ionized water to prepare a suspension

measured to $1x10^4$ conidia/mL using a hemocytometer (Bright-line 3110, Hausser Scientific, Horsham, PA) by placing 25 µL on each side of the hemocytometer.

General procedures. Petunia plugs (Expt. 1: *Petunia ×hybrida* 'Pretty Grand Red', Expt. 2: *Petunia ×hybrida* 'Dreams Red') were transplanted in 1.4 L round containers containing a peat-based growing medium (Fafard 3B, Conrad Fafard, Inc., Agawam, MA) to provide a supply of flowers for the experiments. The plants were grown in a glass greenhouse at Clemson University, SC, USA (lat. 35°N) with the environment controlled by a climate-control computer (Argus Control Environmental Systems, White Rock, BC, Canada). Heating and cooling set points were 22 and 27 °C, respectively. Plants were shaded with retractable curtains providing 55% shade when solar radiation measured outside of the greenhouse exceeded 800 $W \cdot m^{-2}$. For Expt. 1, plants were grown under long days provided with daylength extension lighting with metal halide lamps when solar radiation measured outdoors was <200 $W \cdot m^{-2} \cdot s^{-1}$ from 900 to 2400 HR to promote flowering of this facultative long day plant during October and November. Expt. 2 was conducted during June and July under the ambient photoperiod and no supplemental lighting. A constant liquid fertigation program was used with Peter's Excel Cal-Mag Special (15% N, 5% P_2O_5, 15% K_2O, 5% Ca, 2% Mg; Scotts-Sierra, Marysville, OH) providing 150 $mg \cdot L^{-1}$ N and 50 $mg \cdot L^{-1}$ Ca at each irrigation event. All open petunia flowers were removed the day before the final spray application to allow for harvesting of freshly open flowers for the experiment.

Effect of calcium chloride sprays (Expt. 1). Calcium chloride (anhydrous 96% purity; Thermo Fisher Scientific, Waltham, MA) was dissolved in deionized water to provide 0, 400, 800 or 1200 $mg \cdot L^{-1}$ Ca treatments. Spray applications were made between 1600 and 1700 HR and applied at a rate of 204 $mL \cdot m^{-2}$ using hand sprayers. The $CaCl_2$ applications were made twice per week for two weeks. A fungicide control contained two active ingredients, cyprodinil 37.5% and fludioxonil 25% (Switch®, Syngenta, Greensboro, NC), applied at the recommended rate (449 $mg \cdot L^{-1}$) for ornamentals. The fungicide spray applications were made twice a week for two weeks from 1600 to 1700 HR. Five plants per treatment were treated with each of the Ca rates and the fungicide treatment while ten plants were treated with deionized water and later were divided into two control groups: non-inoculated and inoculated with a conidial suspension. The experiment was conducted twice.

Separating the effect of calcium, chloride and electrical conductivity (Expt. 2). Calcium chloride and KCl (99% purity, Thermo Fisher Scientific, Waltham, MA) solutions were mixed in deionized water to provide EC treatments of 3.0 and 6.0 $mS \cdot cm^{-1}$ for each salt. The 3.0 $mS \cdot cm^{-1}$ $CaCl_2$ solution provided 800 $mg \cdot L^{-1}$ Ca and 1420 $mg \cdot L^{-1}$ Cl, while the 6.0 $mS \cdot cm^{-1}$ solution provided 1600 $mg \cdot L^{-1}$ Ca and 2840 $mg \cdot L^{-1}$ Cl (Table 4.1). The 3.0 $mS \cdot cm^{-1}$ KCl solution provided 960 $mg \cdot L^{-1}$ K and 871 $mg \cdot L^{-1}$ Cl, while the 6.0 $mS \cdot cm^{-1}$ solution provided 1920 $mg \cdot L^{-1}$ K and 1743 $mg \cdot L^{-1}$ Cl. Two additional KCl solutions were mixed in deionized water to provide the same Cl concentrations as delivered in the $CaCl_2$ solutions, i.e., 1420 and 2840 $mg \cdot L^{-1}$ Cl. The EC of these two KCl solutions were measured at 4.2 and 8.2 mS/cm. Five plants were treated with each of the $CaCl_2$ and KCl

applications while ten plants were treated with deionized water and later were divided into two control groups: non-inoculated and inoculated with a conidial suspension. The experiment was conducted twice

Botrytis *inoculation and evaluation*. One day following the last treatment spray application, 4 to 5 newly open flowers per plant with 3 cm of pedicel were harvested between 1900 to 2000 HR. Flowers were immediately placed in 9 mL vials filled with 9 mL of deionized water. These flowers were placed into 32.5×15.0×17.5 cm humidity chambers (BioTransport Carrier, Nalgene Corp., Rochester, NY). Three humidity chambers were used per treatment with six flowers per chamber. Each chamber contained a piece of polystyrene foam with holes in which the vials were held upright. Water (500 mL) was placed in the bottom of each chamber to provide a high relative humidity (99.9%) as measured with a psychrometer (RH300, Extech Instruments, Nashua, NH). Before the chamber lids were sealed, the flowers were inoculated with hand sprayers providing 1 mL of $1x10^4$ inoculum solution per flower and incubated for 72 h at 22 °C. Disease progression data were collected every 12 h by taking pictures of the flowers and blindly rating the individual flowers in the images at the end of each experiment. Infection severity was rated on a 1-9 scale based on the area of infected corolla (1 = no infection; 9 = complete necrosis).

Data analysis. Data analysis was performed using JMP Pro version 13.2.0 (SAS Institute Inc., Cary, NC). ANOVA was used to determine treatment effects and Fisher's LSD student's T test was used to compare means between treatments at $p<0.05$. Treatments

were analyzed using area under disease progression curve (AUDPC) and the trapezoidal rule to evaluate total reduction of *Botrytis* severity for each treatment compared to the control. To calculate the AUDPC, the following equation was used:

$$AUDPC = \sum^{n} \; [t(y - y_{LAG})/2] + [t(y_{LAG} - 1)]$$

where *n* equals the number of assessments, *t* is the time interval between each evaluation (12 h), *y* is the severity rating, and y_{lag} is the *y* value at the previous rating time. The six time intervals are summed to calculate the total area under the curve.

For Expt. 1, the data set consisted of a 2x7 factorial model evaluating the two replications and 7 spray treatments. Fisher's LSD student's T test was used to compare means for the factor levels at $p<0.05$. For Expt. 2, the data set consisted of the single factor of spray treatment to evaluate as replications were combined. A one-way ANOVA was performed to analyze the effect of spray treatment on reduction of *Botrytis* blight severity. Fisher's LSD student's T test was used to compare means for the factor level at $p<0.05$.

Results and Discussion

Botrytis blight severity decreased as the Ca concentration supplied in the $CaCl_2$ solution increased from 0 to 1200 mg·L^{-1} Ca (Fig. 2.1). The 800 mg·L^{-1} Ca treatment was statistically the same as the non-inoculated control while the 1200 mg·L^{-1} Ca treatment had a lower Botrytis blight severity than the non-inoculated control. All three $CaCl_2$ treatments provided better *Botrytis* control than the fungicide treatment which was not different from the inoculated control, i.e., the fungicide had no effect on *Botrytis*

infection. The AUDPC decreased as the Ca application rate increased (Table 2.1). The KCl treatments that provided an equivalent solution EC or an equivalent concentration of Cl compared to the $CaCl_2$ treatments showed no significant differences when compared to the inoculated control. Therefore, Ca is solely responsible for the reduction in botrytis blight severity.

Two mechanisms are suggested to explain the effectiveness of Ca to reduce *Botrytis* infection in horticultural crops. First, Ca binds with pectic material in the middle lamella to stabilize the cell wall that makes it more difficult for fungal penetration into the tissue (Gislerød, 1997). Tomato fruits sprayed with Ca resulted in significantly increased levels of membrane and cell wall-bound Ca as well as an increase in free Ca within the fruit, which also demonstrated a reduction of blossom end rot. (Schmitz-Eiberger et al., 2002). Second, the binding of Ca to pectin in the middle lamella renders *B. cinerea* incapable of utilizing pectin as a source of carbon, causing a reduction in polygalacturonase activity (Volpin and Elad, 1991). Volpin and Elad (1991) observed complete inhibition of polygalacturonase activity in liquid culture containing 120 $mg \cdot L^{-1}$ Ca. Nigro et al. (2006) showed that multiple salts reduced polygalacturonase activity, but $CaCl_2$ demonstrated the highest efficacy.

Spray applications are a method employed for applying Ca directly to susceptible tissues instead of relying on Ca transport from the roots. Two studies have shown the benefits of Ca sprays on reducing botrytis blight severity on cut flower roses. Álvarez (2012) tested three sources of Ca: $CaCl_2$, calcium oxide and calcium chelate + amino acid at a rate of 0, 250, 500 and 1000 $mg \cdot L^{-1}$ Ca. The Ca source did not affect the Ca

concentration of the tissue. The 1000 $mg \cdot L^{-1}$ Ca treatment significantly increased the Ca concentrations of the leaves while no changes in Ca content of the petals were observed. Despite Ca concentrations in rose flower petal tissue not increasing from Ca spray applications, a significant reduction in botrytis blight severity was observed. Differences were observed in the efficacy of the Ca sources for reducing disease severity. The calcium chelate + amino acid and calcium oxide sources provided significant reductions in infection area from the control at both 500 and 1000 $mg \cdot L^{-1}$ Ca. Compared to the other Ca sources, $CaCl_2$ was effective at reducing botrytis blight at all rates tested including the 250 $mg \cdot L^{-1}$ Ca. De Capedeville (2005) demonstrated that pre-harvest Ca sulfate sprays at five different rates of 100, 200, 400 and 800 $mg \cdot L^{-1}$ Ca provided significant reductions compared to the 0 $mg \cdot L^{-1}$ Ca in botrytis blight severity in cut roses. Foliar Ca spray applications are useful for management of bract edge burn of poinsettia. Bract edge burn was significantly reduced in susceptible cultivars when 360 $mg \cdot L^{-1}$ Ca sprays from $CaCl_2$ were provided. Leaf margins were analyzed for Ca following spray applications, and it was determined that Ca content above 0.16% prevented bract edge burn (Strømme et al., 1994). This indicates that Ca sprays may be necessary for overcoming localized Ca deficiencies in susceptible cultivars that do not distribute Ca at sufficient levels. Pre-harvest sprays of Ca are also beneficial in grapes for managing storage rots (Nigro et al., 2006). In tomatoes, blossom end rot was reduced with Ca sprays that increased bound and free Ca within the fruit (Schmitz-Eiberger et al., 2002).

Botrytis cinerea exhibits incredible genetic plasticity that allows for fungicide resistance to occur rapidly when under selection pressure from fungicide applications

(Oliveira et al. 2017; Williamson et al, 2007). The results presented in this manuscript showed inadequate control of *Botrytis* from the fungicide treatment. Further testing of this isolate resulted in the discovery of the first isolate from greenhouse-grown ornamentals displaying resistance to six chemical classes of fungicides commonly used for *Botrytis* management (Samarakoon et al., 2017). The isolate was recovered from a shipment of petunia plants received from a commercial grower. Similarly, nurseries producing strawberry transplants have been identified as a primary source of inoculum, and the isolates were resistant to as many as six chemical classes (Oliveira et al. 2017). Calcium sprays provide a useful strategy to employ for management of botrytis blight, especially when dealing with fungicide resistant isolates.

In conclusion, the results from this study clearly demonstrate that Ca is the factor influencing the reduction in botrytis blight severity. Increasing the Ca concentration in the spray solution provides decreased botrytis blight severity up to 1200 $mg \cdot L^{-1}$, especially when dealing with fungicide resistance. This study demonstrates the potential usefulness of implementing Ca sprays during the production phase to help reduce botrytis blight during shipping. Petunias are grown during spring months when conditions favorable for *Botrytis* development may occur for days at a time, Ca spray applications provide another management strategy for growers to use in addition to conventional fungicides.

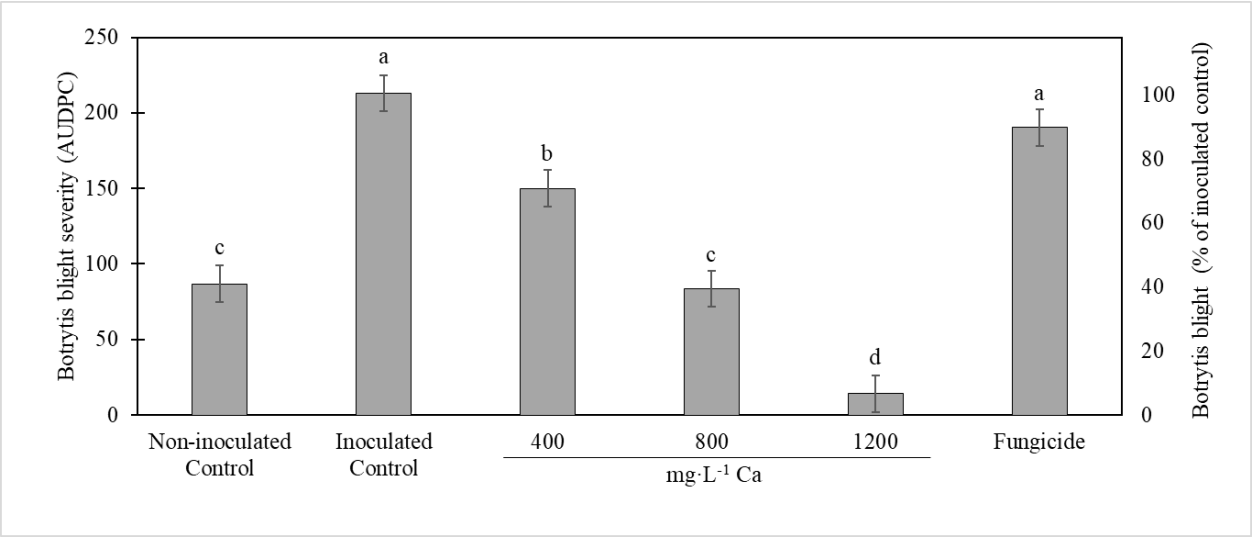

Figure 2.1 Evaluation of botrytis blight severity on petunia flowers treated with calcium chloride spray applications or a fungicide (active ingredients: cyprodinil 37.5% and fludioxonil 25%) and then inoculated with a conidial suspension. Botrytis blight severity is expressed as a sum of the area under the disease progress curve (AUDPC). Letters indicate significantly different responses between treatments using an LSD test ($\alpha = 0.05$). Error bars represent ± 1 SE.

Table 2.1 Evaluation of botrytis blight severity on petunia flowers treated with calcium chloride ($CaCl_2$) or potassium chloride (KCl) spray applications and then inoculated with a conidial suspension of *Botrytis*. The KCl treatments provided equivalent concentrations of chloride (Cl) or electrical conductivity (EC) compared to the $CaCl_2$ treatments. Botrytis blight severity is expressed as a sum of the area under the disease progress curve (AUDPC).

Treatment	EC	Ca	K	Cl	Botrytis blight severity
	($dS \cdot m^{-1}$)		($mg \cdot L^{-1}$)		(AUDPC)
Control	0	0	0	0	305 a[z]
$CaCl_2$	3.0	800	0	1420	167 b
	6.0	1600	0	2840	73 c
KCl	3.0	0	960	871	320 a
	6.0	0	1920	1743	304 a
	4.2	0	1565	1420	301 a
	8.2	0	3129	2840	313 a

[z] Letters indicate significantly different responses using an LSD test ($\alpha = 0.05$).

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CHAPTER THREE

THE EFFECT OF CALCIUM APPLICATION METHODS ON BOTRYTIS BLIGHT OF PETUNIA FLOWERS

Abstract

Two application methods of calcium (Ca), fertigation and spray, were investigated in regard to their effect on botrytis blight in petunia (*Petunia ×hybrida*) flowers. Plants were grown for 6 weeks with three nutrient solutions consisting of 0, 100 or

200 $mg \cdot L^{-1}$ Ca and weekly calcium chloride ($CaCl_2$) sprays of 0, 750 or 1500 $mg \cdot L^{-1}$ 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 57.3% and 70.3% when flowers were treated with Ca spray applications of 750 and 1500 $mg \cdot L^{-1}$ Ca, respectively; however, no change in disease severity occurred across the Ca fertigation applications. Similarly, Ca concentration in the flower petal tissue increased with the Ca spray applications but no change was observed with the Ca fertigation treatments, e.g., 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 $mg \cdot L^{-1}$. The results demonstrate the importance of Ca concentration in flower petal tissue for reducing botrytis blight and that Ca spray applications provide higher Ca tissue concentrations in flowers than Ca fertigation applications. Calcium spray applications are an effective tool for managing botrytis blight of petunia flowers.

Additional index words

Botrytis cinerea, fertigation, disease management, petunia flower meltdown

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Introduction

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. Upon 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 preventative fungicide applications are the primary management technique implemented by commercial growers. However, fungicide resistance is becoming more problematic in the commercial industry, and *Botrytis* isolates resistant to six chemical classes of fungicides have recently been identified in commercial greenhouses (Samarakoon et al. 2017a). Consequently, alternative management strategies to preventative 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 (White and Broadley, 2003). 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, thus transpiration rates are relatively low and transport of Ca through the xylem to the flower petal is subsequently low (Roddy et al., 2016). Also, Ca movement through the phloem is relatively low because of Ca binding with phosphate, although adequate Ca levels for growth in the phloem do exist (Clarkson, 1984).

Several studies have demonstrated the effect of increasing Ca in nutrient solutions on Ca concentrations in leaf and flower tissue of floriculture crops. Increasing Ca concentrations provided in the nutrient solutions from 0 to 140 $mg \cdot L^{-1}$ Ca to lilies increased Ca concentration of leaves from 0.20% to 1.83% DM, however flower Ca concentrations were not measured (Salazar-Orozco et al., 2011). Calcium concentration in poinsettia leaves increased from 0.35% to 0.75% as Ca concentrations delivered in the nutrient solution increased from 18 to 300 $mg \cdot L^{-1}$ Ca (Jacques et al., 1990). However, poinsettia bracts have low stomatal density and exhibit differences in Ca concentration along the margins of bracts compared to near the mid vein (Nell and Barrett, 1986) and

increasing Ca in the nutrient solution was not effective in increasing Ca concentrations in bract margins (Strømme et al., 1994). Roses supplied with nutrient solutions containing 160 or 280 $mg \cdot L^{-1}$ Ca did not increase Ca concentration of the flower petals (Baas et al., 2000). Volpin and Elad (1991) demonstrated in roses that variation can occur in Ca accumulation in different tissues over time. Increasing Ca in the nutrient solution from 100 to 200 $mg \cdot L^{-1}$ increased Ca concentrations in leaf tissue, but not in flowers after 4 weeks. However, after six weeks the opposite occurred, increasing the Ca concentration from 100 to 200 $mg \cdot L^{-1}$ increased Ca concentrations in flowers, but not in leaves. Starkey and Pedersen (1997) demonstrated that increasing the Ca supplied in nutrient solutions in a range from 44 to 176 $mg \cdot L^{-1}$ increased Ca concentration of the flowers and buds of potted roses from 0.4% to 0.6% Ca, and increased Ca concentrations in leaves from 0.7% to 1.6%. Although increasing Ca concentrations in flower tissue is possible through the nutrient solution, previous research demonstrates that this method does not yield consistent results.

Calcium spray applications provide an additional strategy for increasing Ca concentrations in low transpiring tissues such as flowers or fruits. Calcium spray applications of 432 $mg \cdot L^{-1}$ 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%, bract edge burn decreased. Schmitz-Eiberger et al. (2002) demonstrated that tomato plants supplied with a very low rate of Ca (11.6 $mg \cdot L^{-1}$) in the nutrient solution and spray applications of 1200 $mg \cdot L^{-1}$ Ca, significantly increased Ca concentrations of fruit and

decreased blossom end rot. This study also reported significant increases in membrane-bound Ca and free Ca from the Ca spray application. These studies demonstrate the potential benefits of Ca sprays for increasing Ca in flower tissue.

Calcium can be delivered to plants by the fertigation solution delivered to the roots or by spray application to the above ground tissues. The objective of this study was to determine the effect of Ca delivery method and Ca concentration in the application solutions on Ca concentration in petunia leaf and flower petals and on the susceptibility of the flowers to botrytis blight.

Materials and Methods

A 3x3 factorial treatment design was utilized to compare two Ca application methods by providing three concentrations of Ca via each of the two application methods: fertigation solutions (0, 100 and 200 $mg \cdot L^{-1}$ Ca) and spray applications (0, 750 and 1500 $mg \cdot L^{-1}$ Ca) for a total of nine treatment combinations. Calcium concentrations were measured in flower and leaf tissue, and flowers were inoculated with *Botrytis* spores to evaluate the effect of the Ca treatments on botrytis blight severity.

Botrytis *isolation, culture maintenance, and preparation of conidial suspension.* The same isolate of *Botrytis* and preparation of the conidial suspension was prepared according to methods described in Chapter 1.

General procedures. Petunia plugs (*Petunia* ×*hybrida* 'Dreams Red'*)* were received from a commercial grower and transplanted in 1.4 L containers filled with a peat-based growing medium (Fafard 3B, Conrad Fafard, Inc., Agawam, MA) with an average starting Ca concentration of 2.7 g of Ca per container. Plants were grown in a

glass greenhouse at Clemson University, SC, USA (lat. 35°N). Heating and cooling setpoints were 21 and 24 °C, respectively. Long days were provided with daylength extension lighting with metal halide lamps when solar radiation was <200 $W \cdot m^{-2}$ from 900 to 2400 HR to promote flowering of this facultative long day species from February to May. During the six weeks that Ca treatments were provided, the relative humidity averaged 54.9% $\pm$ 13.5. The plants were fertilized at each irrigation event for 3 weeks after transplant with Jack's Professional LX Ca-Mg (15% N, 5% P_2O_5, 15% K_2O, 4% Ca, 2% Mg; JR Peters Inc. Allentown, PA) providing 100 $mg \cdot L^{-1}$ N and 26 $mg \cdot L^{-1}$ Ca.

Three weeks after transplant, the fertigation and spray application treatments were initiated. There were 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. In order to avoid confounding the Ca fertigation treatments and 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 $dS \cdot m^{-1}$, which was the initial EC of the 200 $mg \cdot L^{-1}$ Ca solution. The calculated nutrient concentrations of the fertigation solutions and pH and EC measurements are shown in Table 3.1. Calcium spray applications occurred weekly during the same 6 weeks as when fertigation treatments were applied. For the spray application treatments, $CaCl_2$ (anhydrous 96% purity; Thermo Fisher Scientific, Waltham, MA) was dissolved in deionized water to provide 0, 750 or 1500 $mg \cdot L^{-1}$ Ca. The spray application rate was 204 $mL \cdot m^{-2}$, and the applications were made 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 fully expanded green leaves and open flowers. All flowers were harvested without their peduncle and sepals. The petal tissue was then dried in an oven at 60 °C, ground to a fine powder, and sent for nutritional analysis (ICP-OES; Thermo scientific) performed at USDA-ARS, Toledo, Ohio.

For the last 3 weeks of the experiment, 3-4 freshly-opened flowers per plant were harvested 24 h after the weekly spray application and inoculated with *Botrytis* spores (10^4 spores·mL^{-1}). All open flowers were removed the day before each spray application 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=0-2%, 3=2-5%, 4=5-10%, 5=10-25%, 6=25-50%, 7=50-75%, 8=75-100%, 9=100% of flower petal infected). The experiment was conducted three times.

Botrytis *inoculation and evaluation*. Twenty-four hours after each weekly Ca spray application, 3-4 freshly opened flowers per plant were harvested, inoculated, and evaluated according to the methods described in Chapter 1.

Data analysis. Data analysis was performed using JMP Pro version 13.2.0 (SAS Institute Inc., Cary, NC). ANOVA was used to determine treatment effects for the nutritional analysis of flowers and leaves. The data set consisted of two separate ANOVA tables for leaves and flowers, both consisting of a 3x3 factorial model for the three spray treatments and three fertigation treatments. Fisher's LSD student's T test was used to compare means between treatments at $p<0.05$.

For botrytis blight severity data, analysis of variance (ANOVA) was used to determine treatment effects and Fisher's LSD student's T test was used to compare means between treatments at $p<0.05$. Individual treatments were analyzed using area under disease progression curve (AUDPC) as previously described in chapter 1 for evaluation of botrytis blight severity. Regression analysis was also performed using JMP's Quadratic Fit for botrytis blight severity data.

Results and Discussion

The main effect of fertigation application was the only significant factor to affect Ca concentration within petunia leaf tissue (Table 3.2). Leaf tissue Ca concentration increased from 2.1% to 3.2% DM as the fertigation solution increased from 0 to 200 $mg \cdot L^{-1}$ Ca (Fig. 3.1A). For petunia flowers, the main effect of spray application was the only significant factor affecting the Ca concentration in the tissue. The Ca concentration in flower petal tissue increased from 0.26% to 0.65% DM as the spray solution increased from 0 to 1500 $mg \cdot L^{-1}$ Ca (Fig. 3.1B). No interaction between the fertigation and spray application treatments was observed.

Botrytis blight severity decreased by 70.3% as the Ca concentration in the spray application treatment increased from 0 to 1500 $mg \cdot L^{-1}$ Ca (Fig. 3.2). The Ca concentrations delivered in the fertigation solution did not affect botrytis blight severity (Table 3.2). No significant interaction between the fertigation and spray application treatments occurred.

During commercial production of petunias, delivery of Ca to plants primarily occurs through the nutrient solution. A typical nutrition program for petunia production is

delivered in a constant liquid fertilization approach providing a nutrient solution containing 100 $mg \cdot L^{-1}$ N. The Ca concentrations in these solutions typically range from 17 to 33 $mg \cdot L^{-1}$ depending on the formulation of pre-blended fertilizer used and the amount of Ca in the irrigation water. The standard range for Ca concentrations in leaf tissue of petunia ranges from 1.2% to 2.8% Ca DM, (Jones Jr. and Mills, 1996), while in our study petunia leaf tissue concentrations ranged from 2.2% to 3.3% Ca DM from the 0 to 200 $mg \cdot L^{-1}$ Ca fertigation application treatments, respectively. Calcium concentrations in petunia leaves increased 19.9% when the Ca provided in the nutrient solution increased from 0 to 100 $mg \cdot L^{-1}$, and when Ca concentrations in the nutrient solution increased from 100 to 200 $mg \cdot L^{-1}$ Ca, the Ca concentrations in the leaves increased by an additional 16.8%. Petunia plants only supplied with Ca in the nutrient solution, regardless of Ca concentration, had flower petal tissue of 0.26% Ca, which was twelve times lower than the Ca concentration of leaves from plants that were provided with 200 $mg \cdot L^{-1}$ Ca in the nutrient solution. These results demonstrate that constant liquid fertilization is an ineffective method of increasing Ca concentrations in petunia flower petals.

Ca spray applications effectively increased petal Ca concentrations and demonstrate the capability for reducing botrytis blight severity in petunia flowers. This is attributed to direct application of Ca from the spray application on flowers that allows flower petal tissue to acquire Ca whereas uptake and distribution from the roots is inadequate. The results demonstrate that supplying Ca in the fertigation solution is not sufficient enough for increasing Ca in flower tissue or decreasing botrytis blight severity.

As a result, Ca spray applications become necessary to make petunia flowers less susceptible to botrytis blight.

Two modes of action are suggested for the effect of Ca on *Botrytis.* The first mode of action 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 Donéche, 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 concentrations in vitro from 0 to 120 $mg \cdot L^{-1}$ Ca. Similar results have been found in *Botrytis* isolates from grapes when grown in vitro with Ca ranging from 145 to 582 $mg \cdot L^{-1}$ (Nigro et al., 2006). Cabanne and Donéche (2002) reported 90% inhibition of polygalacturonase activity in an in vitro solution containing 40 $mg \cdot L^{-1}$ Ca, which is a concentration similar to naturally occurring Ca concentrations in grapes, and 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 effect 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 that causes the necrotic spots to coalesce. Harbaugh and Woltz (1989) demonstrated that weekly Ca spray applications of 432 $mg \cdot L^{-1}$ 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 from increasing Ca concentration in the nutrient solution. De Capdeville et al. (2005) also demonstrated that Ca spray applications of 400 and 800 $mg \cdot L^{-1}$ Ca were effective in reducing botrytis blight severity by 68% and 76%, respectively. Álvarez et al. (2012) demonstrated a significant reduction in botrytis blight severity from naturally occurring Botrytis populations following $CaCl_2$ spray applications of 1000 $mg \cdot L^{-1}$ Ca.

The results from Chpt. 1 demonstrate that neither the chloride anion nor the EC of the spray solution contribute to the reduction in botrytis blight following spray applications of $CaCl_2$. The reduction of botrytis blight severity on petunia flowers following $CaCl_2$ applications is solely due to the Ca in the solution.

In conclusion, the results from this study demonstrate the effectiveness of Ca spray applications for increasing Ca content of petunia flowers and the subsequent effect on botrytis blight severity. In contrast, increasing Ca in the fertigation solution was effective for increasing Ca concentration in the leaves whereas spray applications were not. Therefore, Ca spray applications are not necessary for increasing Ca concentrations of leaves which indicates that fertigation applications with increased Ca concentrations could be utilized for increasing Ca in leaves susceptible to *Botrytis*, although leaves were not evaluated in this study. Tissue analysis results suggest that Ca concentrations of petunia flower petals should not be less than 0.4% Ca DM for decreasing petal susceptibility to *Botrytis* infection. Petunias are grown during the spring months in greenhouses that may be exposed to conditions favorable for *Botrytis* growth for extended periods of time. This study demonstrates the potential usefulness of Ca spray

applications during the production phase as an option to *Botrytis* management to prevent petunia flower meltdown.

Table 3.1 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	pH
					($mg \cdot L^{-1}$)								($dS \cdot m^{-1}$)	
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

Table 3.2 ANOVA table comparison of calcium (Ca) application methods, fertigation application or spray application, on botrytis blight severity on flowers and Ca concentration in flower and leaf tissue.

	Significance		
Source	Botrytis blight severity	Ca in flower tissue	Ca in leaf tissue
Fertigation application	NS	NS	***
Spray application	***	***	NS
Fertigation × spray application	NS	NS	NS

NS, *** Nonsignificant or significant at the $P < 0.001$, respectively.

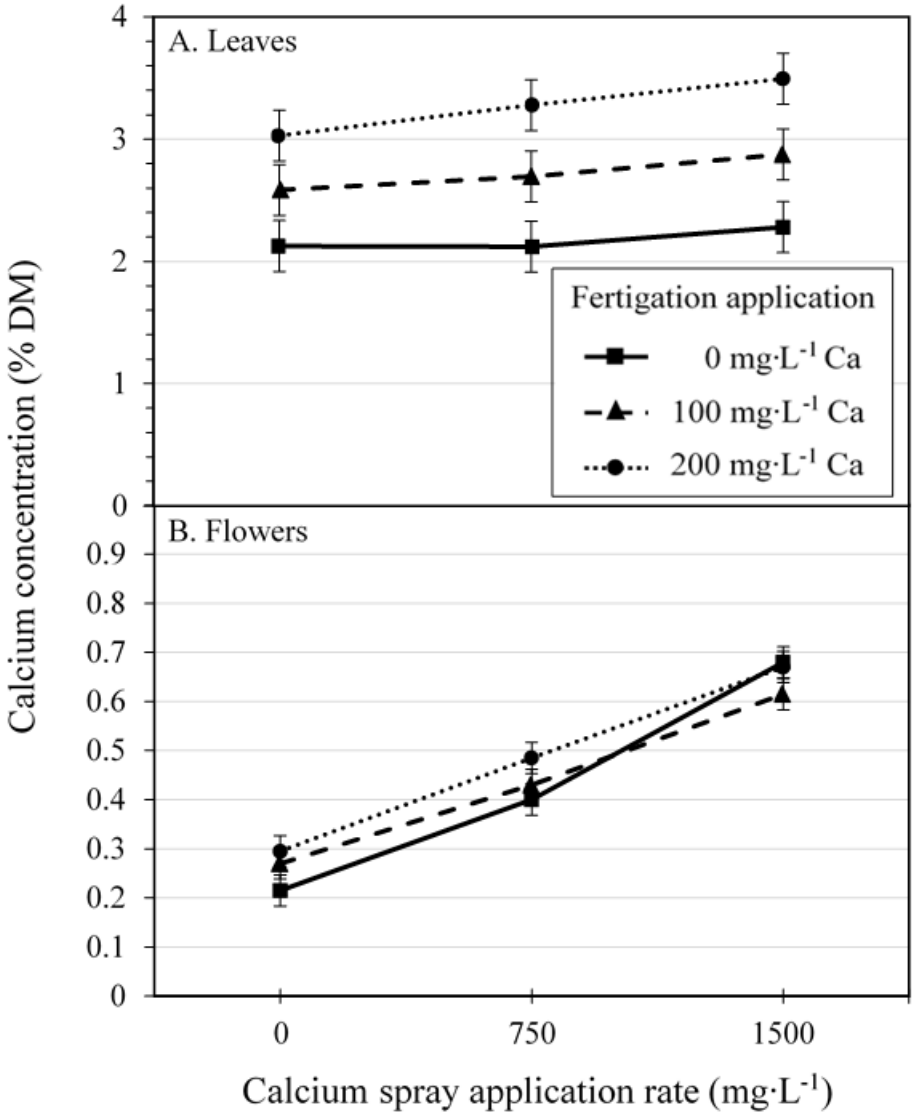

Figure 3.1 Measure of calcium (Ca) concentration in petunia leaves (A) and flowers (B) on plants grown with three different Ca concentrations provided during fertigation applications and spray applications for 3 weeks. Errors bars represent ± 1 SE.

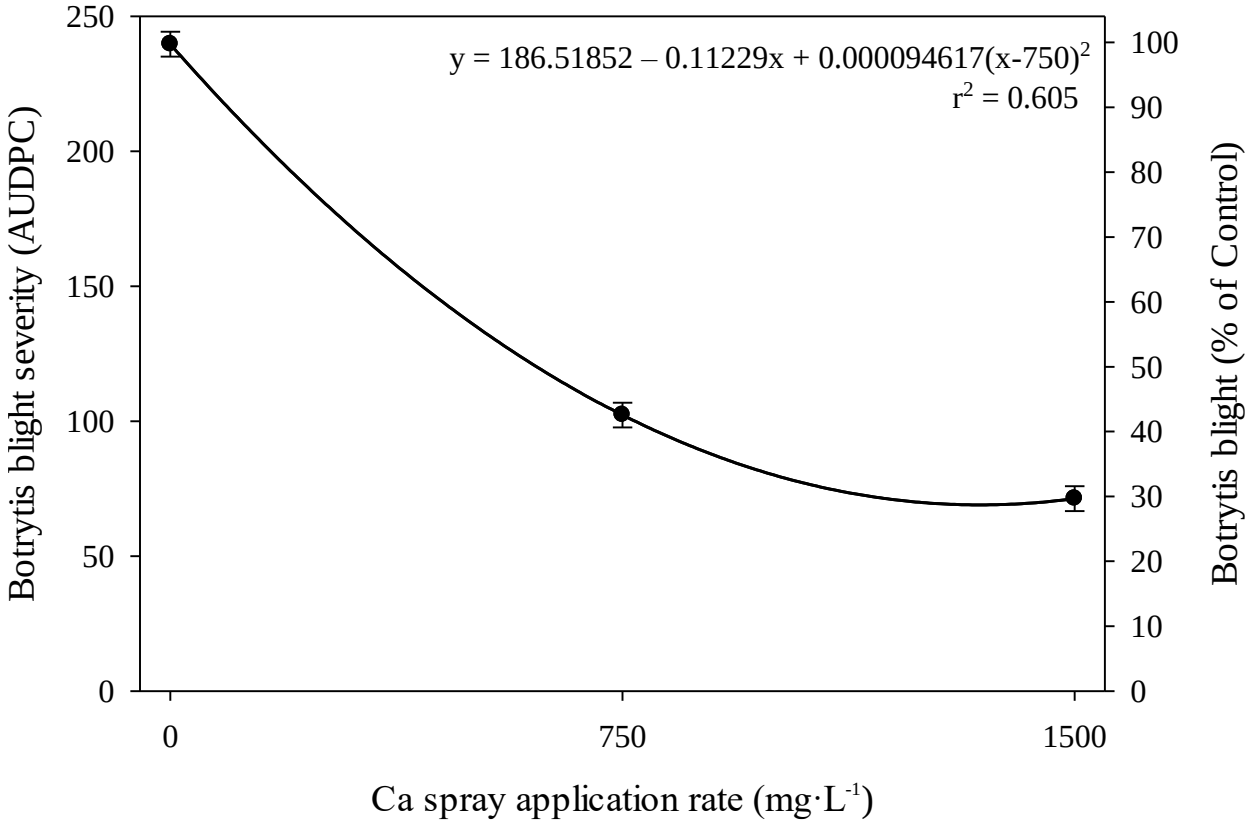

Figure 3.2 Evaluation of botrytis blight severity on petunia flowers following weekly calcium (Ca) spray applications using calcium chloride. Botrytis blight severity is expressed as the sum of the area under the disease progression curve (AUDPC) (left) and as a % of control treatment (right). Error bars represent +1 SE.

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CHAPTER FOUR

EVALUATION OF CALCIUM SOURCES FOR SPRAY DAMAGE AND BOTRYTIS BLIGHT EFFICACY ON PETUNIA FLOWERS

Abstract

Previous studies have demonstrated the efficacy of calcium (Ca) spray applications derived from calcium chloride ($CaCl_2$) for reducing *Botrytis* infection severity on petunia (*Petunia ×hybrida*) flowers. This study examined the effect of six Ca sources at three concentrations each for their efficacy in reducing botrytis blight on petunia flowers and for their potential to cause spray damage. The six calcium sources evaluated were lab grade and commercial grade $CaCl_2$, calcium nitrate ($Ca(NO_3)_2$), calcium chelate (Ca-EDTA), Ca-amino acid chelate, and calcium silicate (Ca_2SiO_4). Petunia flowers ages of 0, 1, 3, 5, or 7 d at time of the spray application were evaluated for spray damage severity 1, 3, 5, and 7 days after the spray application. For the evaluation of botrytis blight efficacy, flowers were harvested and inoculated 24 h after the spray application and evaluated every 12 h for 72 h. The lab grade and the commercial grade $CaCl_2$ at 1250 $mg \cdot L^{-1}$ Ca was the most effective Ca source evaluated for decreasing botrytis blight severity and without causing spray damage on any flower age. Spray damage increased when Ca concentrations were increased to 2000 $mg \cdot L^{-1}$ derived from $CaCl_2$, but no additional benefit was observed for reducing botrytis blight severity. The results demonstrate that several Ca sources reduce botrytis blight severity; however, selection of Ca source is important for minimizing the risk of spray damage.

Additional index words:

Petunia flower meltdown, *Botrytis cinerea*, disease management

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Introduction

Botrytis cinerea, a necrotrophic plant pathogen, infects over 200 hosts worldwide causing significant losses within agricultural systems (Williamson et al., 2007). Bedding plant producers experience problems with *B. cinerea* in all stages of production, from propagation to shipping. When flowers are present on petunia, *B. cinerea* rapidly infects the flowers under conducive conditions, resulting in botrytis blight. Botrytis blight is a significant issue during transportation of plants to the retail environment which may take up to two days. Plants are irrigated prior to shipping, packed tightly onto shipping carts and loaded into trucks, creating a conducive environment for the development of botrytis blight. 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. Symptoms of botrytis blight typically occur as tan necrotic spotting on the flower petals that coalesce as the disease progresses eventually resulting in the complete collapse of the flower petal tissue.

Calcium is a key component in cell wall formation for stabilization and strength with a high part of total Ca in the plant located in the cell walls. Calcium is also involved in intracellular cation-anion balance and acts 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, 2001). However, transpiration rates of flowers and fruit are relatively low due their large volume to surface area ratio and/or low stomatal density (Marschner, 2012). Fruits and flowers are mainly supplied 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 shown above in chapter 2. However, Ca spray applications provide direct application of Ca to plant tissue that may otherwise have low Ca concentrations and have increased susceptibility to disease, such as flowers.

Previous studies have shown the benefit of spray applications of Ca using $CaCl_2$ for reducing *Botrytis* infection in petunia flower (Chapters 1 and 2). In cut roses, Álvarez (2012) evaluated three sources of Ca: $CaCl_2$, calcium oxide and calcium + amino acid chelate at concentrations up to 1000 $mg \cdot L^{-1}$ Ca. All sources provided reductions in botrytis blight severity at 500 and 1000 $mg \cdot L^{-1}$ Ca; however, $CaCl_2$ was the only source effective at 250 $mg \cdot L^{-1}$ Ca. De Capdeville (2005) evaluated calcium sulfate spray applications and reported that disease severity decreased as Ca concentration of the spray solution increased from 0 to 800 $mg \cdot L^{-1}$ Ca. Bract-edge burn of poinsettia is caused by a localized Ca deficiency along the margins of poinsettia bracts. Calcium spray

applications from $CaCl_2$ at 360 mg·L^{-1} Ca (Strømme et al., 1994) and $Ca(NO_3)_2$ at 432 mg·L^{-1} Ca (Harbaugh and Woltz, 1989) were effective at reducing bract-edge burn and the subsequent *Botrytis* infection of poinsettia. No spray damage was reported in either study.

Based on previous work, several sources of Ca have been evaluated for improving quality or reducing disease severity on several vegetable, fruit and ornamental crops; however, studies evaluating multiple sources of Ca at rates above 1000 mg·L^{-1} Ca, have not been done for flowering crops. The objectives of this study were to compare sources of Ca for their effect on botrytis blight of petunia flowers and to identify the risk of spray damage that may result from their application.

Material 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 response of potential spray damage to petunia flowers following spray treatments of six different Ca sources at each of three different rates. The second experiment examined the same products and rates for their reduction of botrytis blight severity following inoculation with *Botrytis* of petunia flowers.

General procedures. Petunia plugs (*Petunia ×hybrida* 'Dreams Red') were received from a commercial grower and transplanted into 1.4 L round containers containing a peat-based growing medium (Fafard 3B: Sun Gro Horticulture; Agawam, MA) with an

average starting Ca concentration of 2.7 g of Ca per container. A constant liquid feed fertigation program was used with Peter's Excel Cal-Mag Special (15% N, 5% P_2O_5, 15% K_2O, 5% Ca, 2% Mg; Scotts-Sierra, Marysville, OH) providing 150 mg·L^{-1} nitrogen and 50 mg·L^{-1} calcium at each irrigation event. Plants were grown in a glass greenhouse at Clemson University, SC, USA (lat. 35°N) with the environment controlled by a climate-control computer (Argus Control Environmental Systems, White Rock, BC, Canada). Heating and cooling set points or for Expt. 1 were 72 and 66 °C and for Expt. 2 were 72 and 62 °C, respectively. Retractable curtains providing 55% shade were engaged when solar radiation measured outside of the greenhouse exceeded 800 W·m^{-2}. For Expt. 1. conducted in January and March, plants were grown under long days provided with daylength extension lighting from metal halide lamps from 900 to 2400 HR when solar radiation measured outdoors was <200 W·m^{-2} to promote flowering of this facultative, long-day plant. No daylength extension was provided for Expt. 2 as the experiment was conducted under long days in September.

Evaluation of Ca sources for spray damage severity (Expt. 1.) Six Ca sources at three rates, with an additional control consisting of deionized water each were evaluated for their potential spray damage on petunia flowers. The effect of flower age was also evaluated by marking individual flowers with a tag on the day that they opened. At time of the single spray application, 0, 1, 3, 5, and 7 day-open flowers were sprayed to determine if there was an effect of flower age on the susceptibility to spray damage. Day 0 refers to unopened flower buds that will open the day after the spray application. Flowers were evaluated 1, 3, 5 and 7 days after the single spray application. Spray

treatments consisted of: lab grade $CaCl_2$ (anhydrous 96% purity; Thermo Fisher Scientific, Waltham, MA), commercial grade $CaCl_2$ (80% purity; TETRA Chemicals, The Woodlands, TX), $Ca(NO_3)_2$ (19% Ca; Hydro Agri North, Tampa, FL), Ca-amino acid chelate (6% Ca purity; Metalosate® Calcium: Albion Laboratories, Layton, UT), and calcium salt of ethylenediaminetetraacetic acid chelate (Ca-EDTA) (9% Ca purity; Brandt Sequestar, Springfield, IL) at 500, 1250 and 2000 $mg \cdot L^{-1}$ Ca. Calcium silicate (99% purity; Alfa Aesar Chemicals, Ward Hill, MA) was applied at a rate of 143, 285 and 427 $mg \cdot L^{-1}$ Ca, and a spray application containing only deionized water was used as a control. Different Ca concentrations from calcium silicate were used as the solubility of the compound is low. The spray rate was 204 $mL \cdot m^2$ and applications were made between 0800 and 1100 HR using hand sprayers. Electrical conductivity (EC) and pH of the solutions were measured (Table 4.1.). There were five plants per treatment and two flowers per flower age per plant, for a total of 10 flowers per plant and 10 flowers per treatment. Flowers were visually evaluated 1, 3, 5 and 7 days after the spray application using a 1-5 scale (1=0%, 2=1-25%, 3=26-50%, 4=51-75%, 5=76-100% petal tissue damage) for spray damage. The experiment was conducted twice.

Evaluation of Ca sources for botrytis blight efficacy (Expt. 2.) The same six Ca sources and rates as described in Expt.1 were evaluated for their efficacy for reducing botrytis blight severity on petunia flowers. Two additional controls were included, a non-inoculated and inoculated control. There were six plants per treatment. One spray application was made 24 h prior to inoculation with *Botrytis* spores. The sprays were

made between 1600 and 1830 HR at a rate of 204 $mL \cdot m^2$ using hand sprayers. The experiment was conducted twice.

Twenty-four hours after the spray application, three freshly open flowers per plant were harvested with 3 cm of pedicel, for a total of 18 flowers per treatment. Flowers were immediately placed into vials filled with 9 mL of deionized water to maintain turgor and hold flowers upright. Flowers were then immediately placed into a 285x80x60 cm humidity chamber with high relative humidity of 97.8%, measured with a psychrometer (RH300, Extech Instruments, Nashua, NH), when the chamber was sealed. Trays (53x26 cm) were placed in the bottom of the chamber and filled with water to maintain the humidity in the chamber. One liter round empty containers were placed in the trays filled with water to hold the polystyrene board above the water filled trays. Flowers were then inoculated individually with 10^4 *Botrytis* spore suspension using hand sprayers providing approximately 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 blindly rating flowers for botrytis blight severity from the photographs after each experiment replication ended. Infection severity was rated on a scale of 1-9 (1=0%, 2=0-2%, 3=2-5%, 4=5-10%, 5=10-25%, 6=25-50%, 7=50-75%, 8=75-100%, 9=100% of flower petal infected). Flowers were arranged in the chamber with six flowers per treatment grouped together with three locations randomized across the humidity chamber. The same isolate

was used for this study with the same preparation, storage and maintenance as described in Chpt. 1.

Data analysis Data analysis was performed using JMP Pro version 13.2.0 (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) was used to determine treatment effects and Fisher's LSD student's T test was used to compare means between treatments at $p<0.05$. For analyzing botrytis blight severity in Expt. 2, area under the disease progression curve was used with the trapezoidal rule to evaluate total reduction by treatments compared to the control. The same formula was used as discussed in Chapter 1.

For Expt. 1, a two factor ANOVA was performed to evaluate the effect of spray treatment and flower age and their interaction. Fisher's LSD student's T test was used to compare means for the factors levels at $p<0.05$. For Expt. 2 a two factor ANOVA was performed to analyze the effect of spray treatment for reduction of botrytis blight severity. Area under the disease progress curve (AUDPC) was utilized as describe above in Chapter 1. Fisher's LSD student's T test was used to compare means for the factors levels at $p<0.05$.

Results and Discussion

The spray damage severity differed between the Ca sources (Fig. 4.1). The lab grade and commercial grade $CaCl_2$ products at 500 and 1250 $mg \cdot L^{-1}$ Ca demonstrated the lowest spray damage severity with no damage to flowers. All Ca sources sprayed at the rate of 2000 $mg \cdot L^{-1}$ Ca exhibited spray damage. Ca-EDTA exhibited the highest amount of spray damage. Van Engelen et al., (2011) reported that the EDTA component of Ca-

EDTA causes phytotoxicity at concentrations >200 $mg \cdot L^{-1}$. When applying 500 $mg \cdot L^{-1}$ Ca from the Ca-EDTA, the concentration of the EDTA component of the formulation is 4,500 $mg \cdot L^{-1}$. In poinsettia cuttings, phytotoxic responses were observed from Ca-EDTA spray applications at 160 $mg \cdot L^{-1}$ Ca (Samarakoon and Faust, 2019). Calcium silicate exhibited high spray damage severity at all three concentrations applied. The Ca concentrations were lower in the Ca_2SiO_4 treatments than the other Ca sources, so the spray damage was likely caused by the silicon. The Ca_2SiO_4 solutions had a high pH (Table 4.1) but were not very different from the high rates of $CaCl_2$, so pH is not a likely cause of the spray damage.

Spray damage severity varied with flower age (Fig. 4.1). Zero-day old flowers (flower buds) were the least susceptible flower age to spray damage from any source. The only source to cause significant damage on flower buds was Ca-EDTA at 1250 and 2000 $mg \cdot L^{-1}$ 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 higher Ca concentrations before open flowers are present; however, once flowers are open, Ca concentrations in the spray solution should be decreased as the flower petal tissue becomes more susceptible to spray damage. Flowers exhibited the highest amount of spray damage on 3, 5 and 7 day-old flowers. Since petunias survive for approximately 9 d, if spray damage were to occur, these flowers would senesce and the plants would be completely undamaged one week after the spray.

Calcium chloride, $Ca(NO_3)_2$, and Ca-amino acid chelate significantly reduced botrytis blight severity by an average of 67% and 85% as the Ca concentration increased

from 500 to 1250 mg·L^{-1} Ca. No additional benefit was observed as Ca concentrations were increased from 1250 to 2000 mg·L^{-1} Ca. The Ca-EDTA and Ca_2SiO_4 treatments had a relatively small effect on botrytis severity compared to the other Ca sources.

Two modes of action are suggested for the effect of Ca on *Botrytis*. First, within the middle lamella of the cell wall, Ca binds with polygalacturonic acid (pectin) creating Ca-pectate which causes a gelling affect 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 Donéche, 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 lab grade and commercial grade $CaCl_2$ products demonstrated the highest 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 observed at that rate; however, unlike $CaCl_2$, $Ca(NO_3)_2$ caused spray damage on flowers at 1250 mg·L^{-1} Ca. The Ca-amino acid chelate treatments at all three concentrations produced similar botrytis blight efficacy results compared to $CaCl_2$, but the potential spray damage increased on open flowers. Two Ca sources, Ca-EDTA and Ca_2SiO_4, provided lower efficacy and higher spray damage severity compared to the other Ca sources.

In conclusion, the results from this study suggest that Ca spray applications from $CaCl_2$ is the most effective source for reducing botrytis blight severity in addition to offering the lowest spray damage potential. Flower age should also be taken into consideration as the age of the flower increases, the potential for damage increases. Calcium sources containing EDTA should not be used while flowers are present on petunias, as damage is severe and persists as flowers continue to open, when immature buds have been sprayed. Several Ca sources provided similar reductions in botrytis blight; however, it is important to evaluate spray damage potential. Thus, we conclude that spray applications of $CaCl_2$ provide a safe and effective tool to reduce botrytis blight.

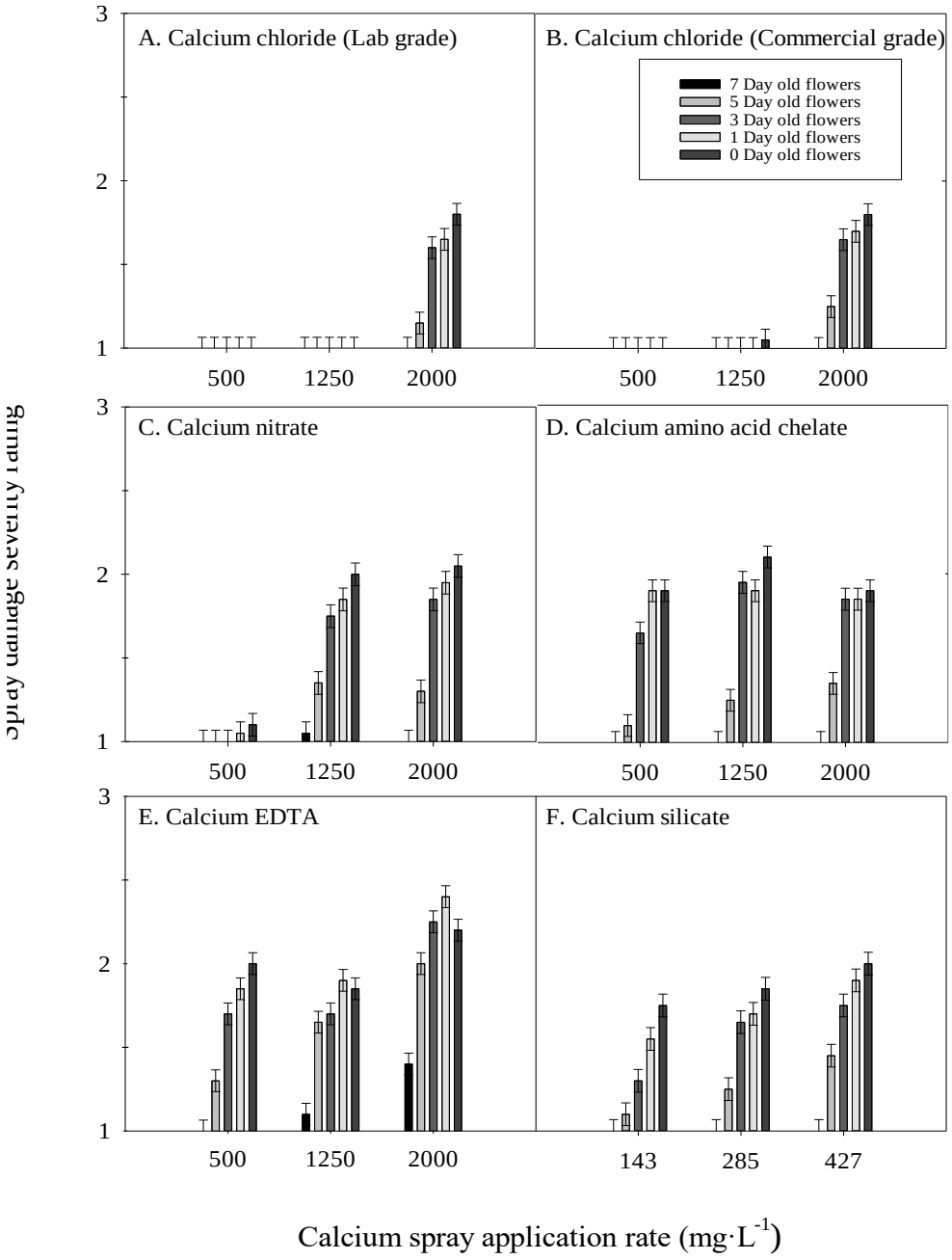
A. Calcium chloride (Lab grade)
B. Calcium chloride (Commercial grade)
7 Day old flowers
5 Day old flowers
3 Day old flowers
1 Day old flowers
0 Day old flowers
C. Calcium nitrate
D. Calcium amino acid chelate
E. Calcium EDTA
F. Calcium silicate
Spray damage severity rating
Calcium spray application rate (mg·L-1)
500
1250
2000
143
285
427
1
2
3

Figure 4.1 Evaluation of six calcium sources at three rates for spray damage on different aged petunia flowers (n=20). Spray damage severity was evaluated 24 h after spray application using a 1-5 scale (1=0%, 2=1-25%, 3=26-50%, 4=51-75%, 5=76-100% petal tissue damage). Least squared means were calculated based on LSD test (α= 0.05). Error bars represent $\pm$1 SE. Non-overlapping error bars indicate statistically significant treatment effects.

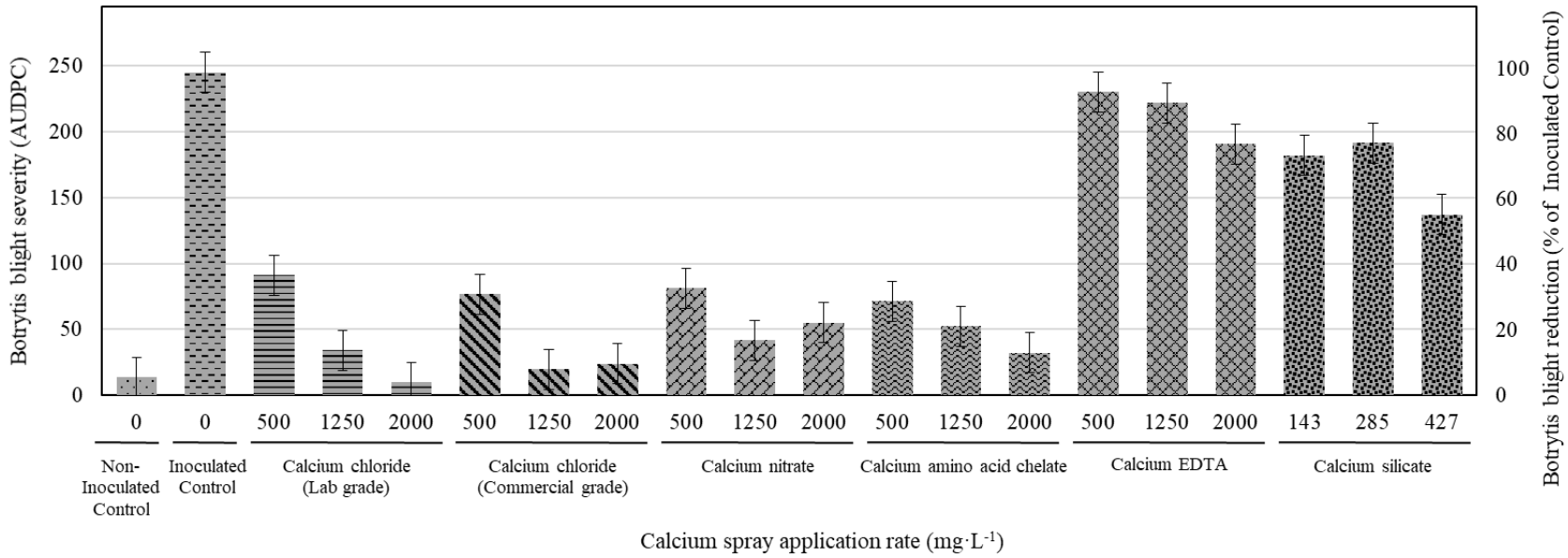

Figure 4.2 . Evaluation of calcium sources for their effect on botrytis blight severity of petunia flowers following inoculation with *Botrytis* spores. Six calcium sources were evaluated at three different rates and there were inoculated and non-inoculated control groups (n=36). Botrytis blight severity is expressed as the sum of the area under the disease progression curve (AUDPC) and as the percent botrytis blight reduction compared to the inoculated control. Least squared means were calculated based on an LSD test (α= 0.05). Error bars represent $\pm$1 SE. Non-overlapping error bars indicate statistically significant treatment effects.

Table 4.1 Measured pH and electrical conductivity (EC) values of calcium spray solutions.

Calcium source	Calcium ($mg \cdot L^{-1}$)	pH	EC ($dS \cdot m^{-1}$)
Calcium chloride (Lab grade)	500	6.7	2.3
	1250	7.1	5.0
	2000	8.2	8.5
Calcium chloride (Commercial grade)	500	7.5	2.8
	1250	8.8	5.8
	2000	9.4	8.9
Calcium nitrate	500	6.9	3.2
	1250	6.7	7.8
	2000	6.5	11.5
Calcium amino acid chelate	500	6.5	2.5
	1250	6.5	5.6
	2000	6.4	10.1
Calcium EDTA	500	6.6	1.6
	1250	6.8	3.1
	2000	6.9	6.7
Calcium silicate	143	8.9	0.2
	285	9.5	0.4
	427	9.6	0.6

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CONCLUSIONS

Calcium (Ca) was determined to be the sole source of the reduction in botrytis blight severity following spray applications of $CaCl_2$. Increased efficacy of the spray application for reducing botrytis blight can be achieved when Ca concentrations are increased to 800 and 1200 $mg \cdot L^{-1}$. However, increased efficacy was not achieved when Ca concentrations in the spray solution were increased from 1250 to 2000 $mg \cdot L^{-1}$ Ca.

Two main delivery methods for Ca delivery to plant tissue are through the irrigation solution with fertilizer, delivered to the roots and through foliar spray applications. When Ca spray applications were compared to fertigation applications, the Ca spray application was the only application method effective in increasing Ca concentrations in flower petal tissue, and for reducing botrytis blight severity. As the Ca concentration in the spray solution increased from 0 to 1500 $mg \cdot L^{-1}$ Ca the Ca concentration in the flower petal tissue increased linearly from 0.26% to 0.65% Ca DM. The results suggest that Ca concentrations in flower petal tissue should not be less than 0.4% Ca DM to increase flower resistance to *Botrytis* infection.

Six sources of Ca were evaluated for their potential efficacy for reducing botrytis blight severity, as well as for their potential spray damage. The lab grade and the commercial grade $CaCl_2$ products provided the highest reduction in botrytis blight, while exhibiting the least amount of spray damage up to 1250 $mg \cdot L^{-1}$ Ca. Spray damage was observed at 2000 $mg \cdot L^{-1}$, while no increased efficacy for botrytis blight was achieved. Based on these results, the recommended Ca source for spray applications is $CaCl_2$ at 1250 $mg \cdot L^{-1}$ Ca for reducing botrytis blight severity.