ABSTRACT

JAHNKE, NATHAN JOSEPH. Preventing Postharvest Stress and Disease of Unrooted Cuttings and Liners (Under the direction of Dr. John M. Dole).

Vegetative cuttings are used for asexual propagation around the world to produce new plants in a cost and time effective manner, while preserving cultivar characteristics such as plant habit or flower color. However, control of the postharvest environments of cutting shipments remains a major challenge in floriculture production. This research determined how three postharvest factors: ethylene, *Botrytis cinerea*, and storage temperature affected plant health and propagation success of unrooted cuttings and liners.

Cultivars of *Pelargonium* × *hortorum* (zonal geraniums) and *P. peltatum* (ivy geraniums) exhibited varying levels of leaf yellowing in response to ethylene treatments of 0.1 and 1.0 $uL \cdot L^{-1}$. Cultivars also differed in susceptibility to *B. cinerea*. Ivy geranium cultivars 'Great Balls of Fire Light Lavender' and 'Great Balls of Fire Lavender' were more sensitive to ethylene treatments and susceptible to *Botrytis* than zonal cultivars. Zonal geraniums had measureable differences in leaf yellowing and susceptibility with "Patriot Bright Red' and 'Americana Coral' being less sensitive and less susceptible than 'Patriot Rose Pink', 'Tango Tango', Tango Dark Red, and 'Americana Red'. Pre-treating shoot tip cuttings of geranium with 1.0 $\mu L \cdot L^{-1}$ ET before inoculating with *Botrytis* increased the amount of damage *Botrytis* caused after 4 days of incubation, but did not increase leaf yellowing of any cultivar. A pre-treatment of 1-methylcyclopronene increased ethylene production by cuttings, but reduced leaf yellowing and kept *Botrytis* damage to levels similar to controls. Non-inoculated cuttings produced at NCSU as well as those produced

commercially developed symptoms of *Botrytis* infection after incubation, meaning latent spores or infections can cause damage to cuttings if subjected to conducive conditions and time.

Geranium rooted cuttings (liners) developed symptoms of *Botrytis* infection after 4 days of storage at 15°C, which was comparable to inoculated treatments of 10^4 and 10^6 spores/mL of *Botrytis* after 6 day of storage. Simulated shipping with variable temperatures ranging from 10 - 30°C did not affect *Botrytis* development, but caused significant leaf yellowing by day four when compared to liners stored at a stable temperature of 15°C. Cultivars did not differ in susceptibility to *Botrytis*, but 'Patriot Rose Pink' was more prone to leaf yellowing throughout the shipping duration.

Storing cuttings of *Euphorbia pulcherrima* cultivars 'Prestige Red' and 'White Star' for 8 days at 10°C significantly reduced fresh weight, rooting, and carbohydrate content. After 4 days of storage cuttings had reduced weight and carbohydrate content, but root ratings were comparable to the unstored control. While white poinsettias are often thought to be less durable as a potted plant, 'White Star' cuttings rooted better and had higher levels of fructose and glucose when stored for 0, 2, 4, 6, and 8 days at 10°C than 'Prestige Red'. Multivariate correlation showed glucose and reducible sugars (fructose + glucose) to be the best predictors of rooting.

Cultivars of *Pelargonium* and *Euphorbia* have differences in durability to shipping conditions. Inoculum *Botrytis* may be able to infect and cause damage during extended shipping and storage, while turgidity and carbohydrates losses reduce cutting quality and performance. Suppliers and growers need to continue to be conscientious of cutting quality

and cleanliness before shipping to ensure rooting success. Future postharvest research will need to address how containers or pretreatments of cuttings could better protect perishable vegetative cuttings from environmental effects and disease.

Preventing Postharvest Stress and Disease on Unrooted Cuttings and Liners

by

Nathan Joseph Jahnke

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APPROVED BY:

_____

Dr. John M. Dole
Committee Chair

_____ _____

Dr. H. David Shew Dr. Hamid Ashrafi

DEDICATION

This thesis is dedicated to the Triune God the creator of the world and everything in it, the author and perfecter of my faith. He gave me strength when I was weak, courage when I was meek, and made me fruitful when I remained in Him. John 15:5

Soli Deo Gloria

BIOGRAPHY

Nathan Joseph Jahnke was born on October 9, 1992 to Clark and Susan Jahnke in West Fargo, North Dakota. He owes his love of plants to his father who helped him build his own greenhouse and start his own business selling vegetables and annuals. This experience inspired him to study horticulture after high school at North Dakota State University. There he completed his Bachelor's degree in December of 2013 in Horticulture. As a student he worked under his adviser Dr. Harlene Hatterman-Valenti in high value crops for three years and completed a published tissue culture research project on Wisteria under Dr. Todd West. Nathan started his Master's degree at North Carolina State University in August 2015 and will be continuing under the direction of Dr. John Dole to pursue a PhD. starting August 2017.

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Introduction

Vegetative propagation of plant material is a fast and cost effective propagation method used by the floriculture industry. An estimated 1.5 billion unrooted cuttings travel into the United States and Canada at an approximate market value of $250 million (M. Miller, unpublished). Vegetative-propagation allows growers to reproduce plants quickly and in a manner that preserves selected characteristics. Shoot-tip cutting is the most popular method of propagation in the floriculture industry, and is used to produce over 50% of the varieties commercially available (Gibson and Dole, 2006). Many floriculture crop species are propagated by vegetative cuttings, including carnations (*Dianthus caryophyllus*), chrysanthemum (*Chrysanthemum* × *grandiflora*), New Guinea impatiens (*Impatiens hawkeri*), poinsettia (*Euphorbia pulcherrima*), petunia (*Petunia* × *hybrida*), and geranium species (*Pelargonium*). Of these, geraniums and poinsettias are two of the most important cutting-propagated crops and comprise a large portion of the floriculture market (USDA, 2016).

Cuttings and plugs are shipped around the globe from countries in Central America and Africa because of favorable growing conditions and the reduced cost of labor in those countries (Gibson and Dole, 2006). Changes in temperature, presence of free water and pathogens and levels of ethylene (ET) are some important factors to consider when shipping top quality cuttings (Gibson and Dole, 2006). Unrooted cuttings typically endure a two to four-day shipment in cardboard containers, during which time cuttings can face extreme temperatures and humidity. Stressed cuttings can be more susceptible to disease and can have reduced rooting. Carbohydrates play a role in the overall quality and adventitious root formation during propagation of geranium and poinsettia (Druege et al., 2004; Einfield, 2011). In enclosed areas,

natural plant production of ET causes the phytohormone to accumulate, resulting in various levels of damage to plant material (Faust et al., 2011). Water condensation on tissue allows for germination of *B. cinerea* spores (Gibson and Dole, 2006). These potential problems, coupled with the unreliability of shipping durations and care while in transit increases the chance that cuttings will reach a grower in poor condition. Testing the effects of *B. cinerea* and ET on disease development of cuttings and liners within the box atmosphere will provide suppliers and growers with a better understanding of how *Botrytis* develops during storage and shipping. The length of time unrooted cuttings spend in shipping or storage is also a variable that can adversely affect plant quality, as carbohydrates and turgidity are important for rooting and sticking into propagation media.

The causal pathogen – *Botrytis cinerea*

The necrotroph and saprophyte, *Botrytis cinerea*, causes millions of dollars of plant damage pre and postharvest each year (Vrind, 2005; Elad, 2004). It is a major concern for many crops because *B. cinerea* can attack hundreds of plant species (Elad, 2004). Diseased unrooted cuttings cause profit losses for both suppliers and growers. Growers also have to file a claim which sets back schedules while waiting for new plant material to be shipped. The life cycle of *B. cinerea* is almost always asexual with the production of conidia that infect host material (Williamson et al., 2007). There are a variety of techniques used by *B. cinerea* to infect and spread throughout host tissue including, but not limited to: the host's hypersensitive response (HR) and hormone signals (Elad et al., 2004; Williamson et al., 2007). Fungicides are an important method of control for this organism. However, frequent use of fungicides, to manage disease caused by *Botrytis* has caused an increase of resistant strains to develop in plant production systems (Rosslenbroich and Stuebler, 2000; Williamson et al., 2007). During

postharvest shipping, cuttings and their environment are uncontrollable without climate controlled transportation. Knowledge of when and how this fungus infects and spreads during shipping and storage is crucial to developing methods of control during the vegetative propagation stages of production in the floriculture industry.

Life cycle

Botrytis cinerea is in the Ascomycota and is rarely seen in its sexual stage (Williamson et al., 2007). Winter survival structures include sclerotia and mycelium on plant debris. From these two sources, asexual spores are released to infect new hosts. Conidia, the asexual spore, is the main form of dispersal forming on the ends of conidiophores produced by mycelium and sclerotia. Like most Ascomycota, dispersal of spores is regulated by temperature and humidity. As humidity declines and temperature rises conidiophores release conidia into the air (Elad et al., 2004; Williamson et al., 2007). Colonization often occurs on dead or weakened plant material giving the pathogen easy access to nutrients.

To gain access to plant cells, enzymes along with infection cushions are used to penetrate host tissue (Choquer et al., 2007; Elad et al., 2004). The main source of infiltration occurs through wounds or natural openings (Hausbeck et al., 1991). After infection, one method of killing cells is through the secretion of phytotoxic compounds (Choquer et al., 2007; Williamson et al., 2007). *B. cinerea* also uses the host's protective hypersensitive response (HR) to its benefit. Plants protect themselves by producing harmful oxidative species within cells, causing cell death to prevent invading pathogens access to healthy, living cells. This HR benefits *Botrytis* development due to its ability to colonize dead tissue (Choquer et al., 2007; Williamson et al., 2007).

Infection factors – water, temperature, plant age, inoculum concentration

A variety of factors play a role in the interaction between *B. cinerea* and the host's susceptibility. Free water on plant tissue, temperature, plant age, and plant organs as well as the previously described HR are important in the disease development process. Free water is what many plant pathogens need to infect and spread. Many storage and postharvest conditions provide high humidity and water condensation. As periods of wetness increase in duration, disease incidence and severity increases (Sirjusingh and Sutton, 1996). Onion leaves inoculated with *Botrytis squamosa* increased lesion numbers the longer dew remain on leaves (Alderman and Lacy, 1983). Temperatures between 20 – 30°C and a leaf wetness of over 4 hours creates an environment for grey mold to thrive (Elad et al., 2004). If leaf wetness on young tissue (2 week) and old tissue (10 week) are kept under 4 hours or under 8 hours for 4-week old tissue then sporulation incidence can be significantly reduced (Sirjusingh and Sutton, 1996). Sirjusingh and Sutton (1996) also showed that overall, sporulation of *B. cinerea* on geranium leaves was slowest and the least when temperatures were below 10°C.

As plants grow, young tissue starts out soft and over time builds up a cuticle. Followed up by another test of inoculum concentration and leaf age, Sirjusingh et al. (1996), reported similar results showing sporulation was achieved more quickly on 1 week old leaves than on older tissue. Host leaves, at an age of 4 weeks old, required the longest wetness durations, and highest temperatures for sporulation to occur (Sirjusingh and Sutton, 1996; Sirjusingh et al., 1996). Older plant tissue is more susceptible to infection by conidia than recently matured tissue (Elad et al., 2004). Plant parts that are sensitive to wounding such as young or old tissue show symptoms in four days (Hausbeck and Pennypacker, 1991). Conidia easily infect and sporulate on petals of geranium at concentrations as low as 10 conidia per ml, while healthy leaves require 10^3 to 10^6 conidia per ml (Sirjusingh et al., 1996).

Infection factors – virulence

Botrytis cinerea uses enzymes and phytotoxic compounds to increase pathogenicity. To get through the waxy cuticle it produces lipases and cutinases (Williamson et al., 2007). After breaching the cuticle, endopolygalacteruronases, a type of pectinase, degrades the pectin layer around epidermal cells (Choquer et al., 2007; Williamson et al., 2007). Super dismutase is an important enzyme which produces H_2O_2, which accumulates in the cell causing cell death (Choquer et al, 2007; Williamson et al., 2007). H_2O_2 is a reactive oxygenating species (ROS), which is usually produced by plants in an HR. The HR is supposed to inhibit pathogens and disease development by killing the surrounding living cells. Due to the saprophytic nature of *B. cinerea,* it takes advantage of the HR by producing ROS and colonizing the resulting dead cells (Choquer et al. 2007, Elad et al., 2004; Williamson et al., 2007). Phytotoxic compounds and proteins are also secreted to kill cells and advance fungal colonization of host tissue (Williamson et al., 2007).

Infection factors – host resistance and defenses

Phytohormones as well as secondary metabolites are commonly used in plant defense systems to combat pathogens. Ethylene as well as methyl jasmonate and salicylic acid have been known to work in signaling pathways to induce defense (Padney et al., 2016). Genes coding for defenses have been upregulated by applications of jasmonates (JA), ET and salicylic acid (SA) (AbuQamar et al., 2006). AbuQamar et al. (2006), Hoffman et al. (1999), Thomma et al. (1999), and Zhao et al. (2012) researched genes upregulated by hormones that were able to increase resistance to pathogens. It is important to determine if plants and have the genes and pathways that can be upregulated through applications of these various compounds.

Ferrari et al. (2007) and El Oirdi et al. (2011) reported that the named elicitors did not provide complete host resistance to *B. cinerea* in *Arabidopsis* and tomato. *Arabidopsis* sprayed with oligogalacturonides (OG), endogenous elicitors, had a slower progression of infection due to the upregulation of the PHYTOALEXIN DEFICIENT3 gene (Ferrari et al., 2007). *Arabidopsis* mutants "impaired in signaling mediated by SA, ET or JA" still showed resistance when sprayed with OG. In tomato, the signaling pathway for JA can be suppressed by an exopolysacchride (EPS) produced by *B. cinerea* (El Oirdi et al., 2011). EPS causes the accumulation of SA, which competes with JA. This antagonistic interaction caused tomatoes to become less resistant. Plants with signaling pathways of JA, SA and ET may still be more resistance than plants without them, because wild types of *Arabidopsis* proved to have significantly better resistance than mutants deficient in those pathways (Ferrari et al., 2007). These studies all point out that there are a variety of pathways, interconnecting factors, and differences in species when testing disease resistance.

Ethylene

Ethylene is an odorless, clear gas that is produced naturally by plants. It acts as a phytohormone regulating many different processes such as senescence and defense signaling (Xu and Zhang, 2015). ET can cause epinasty, yellowing of green tissue, leaf, petal and flower drop, and stunting (Gibson and Dole, 2006). It has a wide variety of uses in for plant management such as floral bud removal or ripening fruit and it can be effective at low concentrations (Gibson et al., 2000). There are a variety of anti-ethylene or ethylene inhibitors commercially available to help control ET and its effects on plants (Gibson and Dole, 2006). These tools usually work by blocking ET receptors in plant cells making plants unable to sense ET in the atmosphere.

Production and sensitivity in plants

Plants produce small amounts of ET during normal growth and development (Gibson et al., 2000). Ethylene is produced for maturation and development, but also in response to different stresses such as drought or pest damage (Abeles et al., 2012). Plants are known to produce more ET than normal when ET receptors are blocked using antiethylene agents or inhibitors such as 1-methylcyclopropene (1-MCP) (Kadner and Druege, 2004; Blankenship and Dole, 2003). 1-MCP along with other compounds such as silver thiosulfate and aminoethoxyvinylglycine are used during shipping and handling of fruits, cut flowers and other horticulture products to protect plant tissue from ethylene effects such as premature ripening or petal shattering (Blankenship and Dole, 2003). Floriculture species have a wide range of ET sensitivity (Gibson et al., 2000). Important species produced as vegetative cuttings such as *Pelargonium* species and New Guinea Impatiens (*Impatiens hawkerii*) are both highly susceptible to ET while poinsettia (*Euphorbia pulcherrima*) is moderately affected (Gibson et al. 2000).

Ethylene screening

A triple response assay is an effective method for screening plants for ethylene sensitivity. It has been used in soybeans and *Arabidopsis,* and has been adopted and proven to be effective for *Pelargonium* seedlings (Hoffman et al., 1999). By growing seeds of *Pelargonium* × *hortorum* on medium containing 1-aminocyclopropane-1-carboxylic acid (ACC), a biosynthetic precursor to ethylene, in the dark, hypocotyl length was reduced at 10 mM compared to higher concentrations (Clark et al., 2001). At 40 mM of ACC receptors were thought to be saturated, because responses were similar when using 80 mM. Of the cultivars tested, ‘Ringo 2000 Salmon’, ‘Multibloom Lavender’ and ‘Elite White’ were the least susceptible to ACC followed by ‘Pinto Pink’ and ‘Orbit Red’. The most susceptible cultivar was ‘Elite Scarlet’. These

sensitivities were confirmed when exogenous applications of ethylene to florets produced similar results (Clark et al., 2001).

Botrytis cinerea interaction

B. cinerea, along with multiple other plant pathogenic fungi, e.g. *Penicillium exicum* and *Rhizopus nigricans* are positively affected by ET in terms of germination, infection and disease development (Kepczynska, 1989). *B. cinerea* also produces ET using methionine, a precursor to ET (Chague' et al., 2002; Qadir et al., 1997). ET production is primarily reliant on methionine, and production rates are usually higher under light than darkness (Chague' et al., 2002). Rose petals inoculated with *B. cinerea* and 50 mM of methionine significantly increased in the number of necrotic areas (Elad, 1988). On bean, pepper and tomato leaves production of ET increased as necrotic areas increased in size (Elad, 1990).

Resistance

Evidence supporting interaction between ET and *Botrytis* species has led to the hypothesis that plants that are more sensitive to ET may be more prone to infection and have an increase in disease severity. The recently developed ability to sequence genomes and genetically modify organisms has allowed researchers to detect ET-sensitive genes, insert or delete them, and regulate them. ET-insensitive *Arabidopsis* plants deficient in the gene EIN2, which is required for the ethylene response pathway, showed significantly higher susceptibility to *B. cinerea* (Thomma et al., 1999). However, ET-insensitive *Arabidopsis* infected with *Phytophthora parasitica* were less susceptible than the normal, ET-sensitive plants (Thomma et al., 1999). ET-insensitive soybeans showed increased susceptibility to the fungi *Septoria glycines* and *Rhizoctonia solani*, but reduced susceptibility to *Pseudomonas syringae* and *Phytophthora sojae* (Hoffman, 1999). This means that the correlation between ET sensitivity and pathogen

susceptibility may not be the same for every plant species or even among varieties of the same species.

Hormone signaling is complex and may be related to expression of plant defenses. Plant defenses are often elicited by compounds such as chitosan, ET, JA and SA. Thomma et al. (1999) showed that *Arabidopsis* mutants, coi1-1, insensitive to methyl jasmonate have an even higher susceptibility to *B. cinerea* than just ET-insensitive plants. Thomma et al. (1999) deduced that *Arabidopsis* needs the "JA and ET-dependent pathways" to induce plant defense genes. Hoffman et al. (1999) eluded to "ET signaling being a factor for gene defense signaling pathways" in their study on ET-insensitive soybeans. Although elicitors have been linked to plant defense, Ferrari et al. (2007) reported that resistance to *B. cinerea* was independent of ET, JA and SA on tomato leaves. Studies on tomato report that together pretreatments of ET and SA reduced resistance, while tomatoes pretreated with just ET had increased resistance to *B. cinerea* (Diaz et al., 2002).

The RAP2.2 the gene in *Arabidopsis* that regulated and promoted resistance to *B. cinerea* (Zhao et al., 2012). This gene also needs the EIN2 gene to function, proven to promote resistance to *B. cinerea* due to its function in the ET response pathway (Thomma et al., 1999). When ET-insensitive mutants were treated with ET, the expression of RAP2.2 gene significantly increased while JA treatment did not increase expression (Zhao et al., 2012). El Oirdi et al. (2011) found contrasting evidence where tomato plants unable to produce JA showed increased susceptibility, but this may be due to the specific interaction JA has with systemin, a defense compound produced by the *Solanaceae* family, induced by JA. It was found that *B. cinerea* actually induced the SA pathway, which negatively affected the JA pathway (El Oirdi, 2011). These studies all point to the conclusion that different species and families of plants have various responses to *B.*

cinerea through different pathways and genetic defense systems. One solution, such as a broad application of ethylene or an ethylene inhibitor to all crops in hopes of controlling *Botrytis,* will not work.

Ethylene inhibitors

Aminoethoxyvinylglycine (AVG), 1-MCP, silver thiosulfate (STS) and 2,5-norbornadiene (NBD) are compounds used to inhibit responses to and the production of ET. AVG, an inhibitor of ACC synthase, blocks methionine's development into ET (Chague' et al. 2002; Wang. 1986). Chague' et al. (2002) found that *Botrytis* produces ET from methionine via α-keto γ-methylthiobutyric acid (KMBA) instead of the ACC pathway, because AVG had no effect on ET production by *B. cinerea*. When sprayed on petals of carnation and rose, AVG significantly "decreased disease incidence by 26 – 32% (Elad, 1988)." Elad (1990) also showed that a "concentration of 5×10^{-3} M AVG completely inhibited disease on tomato and bean leaves." This suggests that the production of ET by *Botrytis* may not be as much of a factor as much as the host's production of ethylene since plants use ACC pathway and *Botrytis* does not (Chague' et al., 2002).

1-MCP is one of the newest ET inhibitors. It binds to receptors and plants are only receptive to ET after producing new receptors (Sisler and Serek, 1997). Diaz et al. (2002) found that 1-MCP increased the number of expanding lesions on tomato plants. When applied before and during storage, 1-MCP caused significant increases in ethylene, especially as temperatures increased from 5°C to 20°C (Kadner and Dreuge, 2000). Ethylsorb (potassium permanganate) showed positive results when Elad (1993) found that removing ET from the atmosphere decreased disease severity on leaves of pepper and tomato. These results conflict with later experiments by Diaz et al. (2002), who found exogenous pretreatments of ET before inoculation

actually improved resistance in tomato. Diaz et al. (2002) points out that they used sealed containers while Elad (1993), used polyethylene bags, which allowed for the atmospheric exchange of ET.

STS is a liquid with silver ions that attach to and block ET receptors. Leaves of tomato, bean and pepper all showed a significant reduction in disease development when treated with 10^{-4} and 10^{-5} M STS (Elad, 1990). When rose cultivars were inoculated with 10^{3} ml^{-1} conidia, 5 mM of STS reduced disease severity (Elad, 1993). An STS application was found to significantly increase ethylene production during storage of cuttings when applied to stock plants, and this effect accumulated as temperatures rose from 5°C to 20°C (Kadner and Dreuge, 2000).

NBD, an early product used to block ET receptors, stopped spore germination of conidia of *B. cinerea*; the application of ethephon reversed the effects of NBD, inducing spore germination (Kepczynska, 1989). At 10 mM, NBD significantly reduced disease development on various rose cultivars, bean, pepper and eggplant, but not tomato (Elad, 1993). Diaz et al. (2002) and Elad (1993) and found that a NBD pretreatment actually increased the number of infected tomato plants. However, while an ET pretreatment made tomato less susceptible to *B. cinerea*, in Diaz et al. (2002) studies, Elad (1993) showed that ET significantly increased size of necrotic areas on tomato and pepper. The effects of anti-ethylene and ethylene inhibitory compounds on resistance are not consistent on all plant species. Generally, it seems the inhibition of ethylene signaling increases a plant's susceptibility to *Botrytis.*

Ethylene and ethylene inhibitor effect

STS caused phytotoxicity and significantly increased the decay of cuttings during the rooting process (Kadner and Dreuge, 2004). Ethylene showed a positive effect on rooting (+49% roots per cutting) when applied during storage at 20°C, compared to control cuttings (Kadner and

Dreuge, 2004). However, Leatherwood et al. (2016), saw a reduction in roots and root length on 'Rocky Mountain White' zonal geranium when treated with 1.0 $mL \cdot L^{-1}$ ET. Kadner and Dreuge (2004) report that at temperatures between 5°C and 20°C, treatment of *Pelargonium* cuttings with 1-MCP did not have a significant effect on leaf senescence or decay. 1-MCP treated cuttings had low levels of leaf abscission and similar chlorophyll content, as with un-stored and stored control cuttings (Rapaka et al., 2008). Rapaka et al. (2008) also showed that 1-MCP can significantly inhibit rooting of *Pelargonium* cuttings. Both the pre-storage treatments of 1-MCP and STS significantly decreased the root number on cuttings and increased number of dead cuttings when they were previously stored at 12°C (Kadner and Dreuge, 2004). In agreement with Kadner and Dreuge (2004), Serek et al. (1998) found 1-MCP significantly decreased rooting in *Pelargonium* cuttings. Although there was not a significant difference when pretreated cuttings that had been stored in dark conditions for 3 d, there was an increase in rooting compared to non-treated stored cuttings (Serek et al., 1998). Crawford (2013) also found that cuttings of 'Tango Dark Red Geranium' pretreated with 1-MCP then 1 $mg \cdot L^{-1}$ of ethylene and stored for 2 d had a higher root dry weight compared to the ethylene application. Rapaka et al (2008) pointed out that making cuttings insensitive to ET through 1-MCP decreased adventitious roots. They "speculate that postharvest ethylene sensitivity promotes carbohydrate transport toward the basal stem" to promote rooting.

Shipping Liners

After unrooted cuttings are received and stuck into plug trays in the United States, the resulting young plants (liners) can face additional adverse temperatures and humidity during shipping that result in reduced quality due to disease development, low carbohydrate status and water stress. Plugs are shipped in boxes that protect plants from compaction, but these boxes

allow ethylene to accumulate and humidity and temperature to be extremely variable. Kadner (unpublished data) found that shipments of *Pelargonium* had a mean temperature of 12°C during transportation. During storage "It is best to maintain nearly 100 percent humidity, and the temperature should be as low as the hardiness of the given species can tolerate" (Hartmann et al., 2002). Storage conditions could cause yellowing and stretching if plants are not received and planted immediately or placed in a cooler. Small or latent lesions of *B. cinerea* or spores on the plant surface could infect and cause large amounts of damage if shipments are delayed. If a reduction or application of ethylene can reduce fungal infection and disease incidence there may be potential to reduce economic losses when shipping plugs.

The resistance to pathogens and success of a plant after shipping depends on storage conditions, the state of the plant material entering storage and shipping, and the species/cultivar (Hartmann et al., 2002). During shipping and storage, darkness is an inevitable factor that has negative effect on plant health reducing photosynthetic rates, which coincide with reduced total protein, starch, sucrose, glucose and fructose concentrations in unrooted cuttings of geranium after only 1 day of simulated shipping in darkness at 25°C (Arteca et al., 1996). Arteca et al. (1996) also showed geranium cultivars vary in their responses to storage.

Pelargonium

As mentioned previously, *Pelargonium* is one of the most important cutting-propagated crops and comprises a large portion of the floriculture market. Currently, there is no research on the degree of susceptibility of *Pelargonium* spp. cultivars to *Botrytis cinerea* based on their sensitivity to ethylene. Cultivars of seed geraniums did have different sensitivities to ET through a triple response assay (Clark et al., 2001). However, this technique is not applicable to vegetative cultivars. There is *Botrytis* susceptibility data for geraniums using detached leaf

assays and floral organs (Uchneat et al., 1999a; Uchneat et al., 1999b), but this research was looking at ploidy levels and not whole plants and applicability to commercial production. No research has been reported depicting how ethylene inhibitors affect infection and disease development on cuttings of *Pelargonium*. This research will provide information to better equip growers and suppliers to handle *Botrytis* issues during postharvest conditions to ensure high quality plant material through the shipping and storage processes.

Poinsettia

Euphorbia pulcherrima is one of the most important holiday crops. With years of cultivation, breeding and research, this crop has been transformed from its wild species. Known for its wide variety of cultivars, it is strictly propagated in the industry as unrooted cuttings. Although, chilling tolerance has been improved upon over the last couple decades (Dole, personal communication). Cuttings and liners are usually shipped during some of the hottest months of the year and face adverse conditions as with any other species produced as vegetative cuttings.

Carbohydrates

The ability of an unrooted cuttings to root is the most important aspect of a vegetative cutting. The formation of these adventitious roots has been associated with internal carbohydrate concentrations for geranium and poinsettia (Rapaka et al., 2005; Einfield, 2011). Low carbohydrate levels can be detrimental for cuttings as carbohydrates are used for adventitious root formation. In *E. pulcherrima* higher carbohydrate content in unrooted cuttings correlate with better rooting and the ability to store cuttings longer (Einfield, 2011). However, Zerche and Druege (2009) found that large amounts of nitrogen within poinsettia cuttings is better correlated with adventitious rooting than soluble carbohydrates.

Sucrose is transportable sugar while fructose and glucose are reducible sugars. Rapaka et al (2005) found sucrose to be the most prevalent sugar in unrooted cuttings of *Pelargonium* while in poinsettia glucose levels were closely related to internal nitrogen and rooting (Zerche and Druege, 2009). Zerche and Druege (2009) have found that carbohydrates decrease with increasing storage time, but little research has been done to compare cultivars of poinsettia, specifically reds with dark green leaves and whites with light green leaves. White cultivars are often thought of as less durable to many stresses than red cultivars (J. Dole, personal communication), but the correlation to carbohydrates has not been made between the two. Many studies also have looked at carbohydrates and their effects on rooting, but there is a knowledge gap on what happens throughout storage of the cuttings and whether a less destructive measurement as weight loss could predict rooting performance.

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CH. 2: Ethylene sensitivity and *Botrytis* susceptibility of *Pelargonium* cultivars

Nathan J. Jahnke[a,]*, John M. Dole[a], and H. David Shew[b]

[a]Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609, USA

[b]Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616, USA

*Corresponding author at: Department of Horticultural Science, Campus Box 7609, North Carolina State University, Raleigh, NC 27695-7609, USA. E-mail address: njjahnke@ncsu.edu.

Abstract

Nine cultivars of *Pelargonium* × *hortorum* and *Pelargonium peltatum* were tested for sensitivity to ethylene and susceptibility to *Botrytis cinerea* as unrooted cuttings. *P. peltatum* cultivars 'Great Balls of Fire Lavender' and 'Great Balls of Fire Light Lavender' were more prone to leaf yellowing and more susceptible to *B. cinerea* than *P.* × *hortorum* cultivars. The Patriot series, 'Bright Red' and 'Rose Pink', were least prone to leaf yellowing in response to 0.1 and 1.0 $\mu L \cdot L^{-1}$ ethylene after a four-day storage duration. Cultivars varied in susceptibility to *B. cinerea,* reaffirming the role of quantitative resistance to *Botrytis*. 'Tango Tango' and 'Americana Coral' were the least susceptible to *B. cinerea*. Cultivars ranked least to most sensitive by leaf yellowing three days into propagation and disease ratings four days after treatment: 'Patriot Bright Red', 'Americana Coral', 'Patriot Rose Pink', 'Tango Tango', 'Tango Dark Red', 'Americana Red', 'Great Balls of Fire Lavender', and 'Great Balls of Fire Light Lavender'. A cultivar sensitive to ethylene, seen as leaf yellowing, was not always more susceptible to *B. cinerea*. However, the cultivars least susceptible to leaf yellowing were also the

least susceptible to *B. cinerea* indicating ethylene sensitivity and susceptibility to *B. cinerea* may be correlated.

2.1 Introduction

Shoot tip cutting is one of the most cost and time efficient propagation methods used in the horticulture industry. Unrooted cuttings are placed in cardboard containers and shipped to rooting stations and growers. Shipping typically takes 2 – 4 days. During this time, cuttings can become stressed by adverse environmental conditions. High temperatures (25°C) dehydrate cuttings and deplete carbohydrate reserves, causing cuttings to wilt and leaves to turn yellow (Arteca et al., 1996). Also, dark conditions within boxes reduce the photosynthetic rates (Arteca et al., 1996). Diseases take advantage of weakened plant material and humid conditions. *Botrytis cinerea* Per.:Fr (teleomorph = *Botryotinia fuckeliana* (de Bary) Whetzel) is a common pre and postharvest pathogen, able to infect hundreds of plant species (Elad et al., 2004). Temperatures between 20 to 30°C is the perfect environment for development of Botrytis diseases (Sirjusingh and Sutton, 1996). Damaging concentrations of ethylene (ET) can accumulate in closed boxes through natural plant production and cause leaf yellowing and epinasty before and after propagation (Faust et al., 2011; Faust et al., 2006). Leaf yellowing can lead to more disease during propagation because leaf tissue is weakened and easier to infect. To mitigate this potential disease, growers take extra time and labor to remove any yellowing leaves.

Defense mechanisms and induced resistance have become topics of research as fungicide resistance has become more common among populations of *B. cinerea* (Rosslenbroich and Stuebler, 2000; Williamson et al., 2007). Ethylene, methyl jasmonate, and salicylic acid play roles in defense pathways (Diaz et al., 2002; Padney et al., 2016; Thomma et al., 1999). Thomma et al. (1999) reported that turning off ethylene sensitive genes made *Arabidopsis* plants more

susceptible to *B. cinerea*. Other research reports ethylene insensitive soybean mutants had increased susceptibility to *Septoria glycines* and *Rhizoctonia solani*, but the opposite effect was observed when challenged with isolates of *Pseudomonas syringae* and *Phytophthora sojae* (Hoffman et al., 1999).

Growers question whether ethylene sensitivity in zonal geranium (*Pelargonium* × *hortorum* L.H. Bailey) cultivars', often connected to leaf yellowing, is correlated to susceptibility to *B. cinerea*. Geranium is commonly produced as vegetative cuttings and shipped to the United States. It is one of the most common floriculture crops and prone to leaf yellowing and Botrytis blight or gray mold caused by *B. cinerea* (USDA, 2016; Faust et al., 2006). Previous research on geranium provides evidence for some quantitative resistance to *B. cinerea* among cultivars of *P.* × *hortorum* and *P. peltatum* species. However, ploidy levels and detached leaf assays were the main principles of the experiments, which does not explain the disease process on whole plants or cuttings in their respective production environments (Uchneat et al., 1999). In the greenhouse, growers use cultural, physical, and chemical means such as leaf removal, air movement, reducing leaf wetness, and fungicides to avoid and control *Botrytis* infection and disease (Elad et al., 2007; Williamson et al., 2007). There remains little a supplier can do during shipping and storage besides a pre-shipping fungicide application to protect cuttings. Postharvest disease management is especially important with approximately 1.5 billion cuttings being shipped every year (M. Miller, unpublished data). The objective of this research was to determine the ethylene sensitivity and *Botrytis* susceptibility of eight *Pelargonium* cultivars as unrooted cuttings, during simulated postharvest shipping.

2.2 Materials and Methods

2.2.1 Commercial (Dümmen Orange) and in-house

Stock plant culture, Unrooted cuttings and handling

Cuttings of 'Tango Dark Red' (TDR) and 'Rocky Mountain Violet' (RMV) were harvested from stock plants (cuttings originally obtained from Syngenta Flowers, Gilroy, Cal., USA) grown in ambient air in glass glazed greenhouse at 24°C days and 18°C nights. Plants were hand irrigated alternating between 15.0-0-12.5 and 20.6-6.7-16.6 (Jack's 15-0-15 Dark Weather and 20-10-20 General Purpose water soluble fertilizer, JR Peters Inc., Allentown, Pa., USA) at 150 $mg \cdot L^{-1}$ N. Liquid flowable lime (Limestone F^{TM} Liquid Flowable Lime, Cleary Chemicals LLC, Alsip, Ill., USA) was applied to increase substrate pH as needed. Shade cloth (Revolux XLS 15 Fire Retardant 50% shade cloth, Ludvig Svensson, Kinna, Sweden) covered the growing area near glass height of the greenhouse from mid-April to mid-September to reduce light intensity and maintain desired temperatures. Shoot tip cuttings were harvested on 26 Jan. 2017 at 10:00 AM and extra leaves were removed to leave two or three mature leaves. In addition, unrooted cuttings of 'Patriot Bright Red' (PBR) and 'Patriot Rose Pink' (PRP) were shipped from a commercial supplier (Dümmen Orange, Columbus, Ohio, USA) on 26 Jan. 2016.

Upon harvest and delivery, all cuttings were left in original packaging and placed at 4°C with moist paper towels to reduce water loss. Cuttings were removed from storage at 8:00 AM on 27 Jan. 2017, and sorted by number of leaves and stem caliper and then placed in groups of similar cuttings to make a total of 34 groups of three cuttings for PBR and PRP and 32 groups of three cuttings for TDR and RMV. Eighteen groups of PBR and PRP cuttings and sixteen groups of TDR and RMV cuttings were placed at 4°C until after the other groups had received spray treatments for testing *Botrytis* susceptibility.

Pathogen culture

Initial *B. cinerea* cultures (a field isolate from the lab of M. Benson, Department of Plant Pathology, North Carolina State University) were started from a conidia stock suspension maintained at -80°C in a solution of 0.01% Tween 80 (Sigma-Aldrich, St. Louis, Mo., USA) and 15% glycerol (Sigma-Aldrich, St. Louis, Mo., USA). Cultures were grown to sporulation on oatmeal agar (Thermo Fisher Scientific, Lenexa, Kans., USA). Subsequent cultures were started by turning a sporulating plate upside down and taping it to drop spores onto a new plate. After 10 to 14 days, Petri dishes were covered in gray sporulating mycelium (Fig. 1A).

Spore collection

Approximately 15 mL of sterilized solution, containing 0.1% Tween 80 and 15% glycerol, was poured over a sporulating plate. A sterilized, bent glass stirring rod was used to dislodge conidia by rubbing the culture for approximately 5 min. (Fig. 1B). Once the plate was completely black, the spore suspension was poured through five layers of sterilized cheesecloth to remove any hyphae. Each Petri dish was harvested from twice. A Neubauer improved hemacytometer (LW Scientific, Lawrenceville, Ga., USA) was used to quantify the number of spores/mL for the resulting suspension (Fig. 1C). Suspensions were collected and stored in 50 mL polypropylene centrifuge tubes (Thermo Fisher Scientific, Lenexa, Kans., USA) and placed at -80°C until needed for experiments.

Inoculation

Spore suspensions were removed from -80°C two hours before inoculation and tubes were allowed to thaw in a container of tap water. The suspension was diluted to 10^6 spores/mL using sterilized DI water. The suspension was loaded into a hand sprayer and a group of three cuttings was sprayed with approximately 20 mL to the point of glistening.

Ethylene application and quantification

Cuttings were either subjected to 0, 0.1 or 1.0 $\mu L \cdot L^{-1}$ ethylene overnight (approximately 22 hours). Gas-tight 210-L (v = 0.21 m^3) barrels were used to hold cuttings during treatment. The 0 $\mu L \cdot L^{-1}$ barrel contained activated charcoal and a fan for circulation to absorb any ET. Both 0.1 and 1.0 $\mu L \cdot L^{-1}$ barrels also had a fan to provide even distribution of ET. Concentrations applied were measured via a gas chromatography (Shimadzu 8A; Shimadzu Corp., Kyoto, Japan) fitted with a glass column (Porapak Q, 80/100 mesh, 183 cm × 2.6 mm; Sigma Aldrich, Inc., St. Louis, Mo., USA) running at 110°C injector, 130°C column, and 130°C detector (flame ionization) temperatures. Flow rates for the He carrier, H2 and O2 were 30, 16 and 90 $mL \cdot min^{-1}$, respectively. To determine ethylene production of cuttings placed in 900 mL glass jars (v = 946.4 cm^3), injection volume was 1 mL of headspace gas drawn via a neoprene port on the jar lid. Standards of 1.0 $\mu L \cdot L^{-1}$ ET were run before, during, and after all sampling.

Experimental design

A complete randomized design with subsampling to account for the group of 3 cuttings per rep (jar). For each cultivar, sixteen groups of three cuttings each were divided among the following treatments to test for susceptibility to *B. cinerea*: 1) unsprayed dry control; 2) sterilized deionized (DI) water (0.2 µm filter, Draco Inc, Durham, N.C., USA) control; 3) sterilized spray of 0.01% Tween 80 and 15% glycerol (spore carrier solution; 4) *Botrytis* spore suspension of 10^6 spores/mL. The DI treatment was not applied to TDR and RMV as not enough cuttings were available. After inoculation, cuttings were placed in zip seal bags (16.3 cm × 8.2 cm) with three punched holes to mimic packing for air exchange. The whole bag, containing three cuttings, was then placed into a 900 mL glass jars (v = 946.4 cm^3), (experimental unit) and sealed. Jars were held at an average temperature of 22°C and completely randomized on a lab bench under black plastic to simulate shipping conditions. There were four jars per cultivar

treatment combination. After a four-day incubation period cuttings were removed and a disease rating was recorded for each cutting based on a 0 - 5 rating scale (Fig. 2): 0, 0% disease, no symptoms; 1, <10% disease or 1 - 10 small lesions; 2, 10 - 24% leaf necrosis or shoot necrosis; 3, 25 - 50% leaf necrosis or 10% leaf necrosis with shoot necrosis; 4, 51 - 75% leaf necrosis or 25% leaf necrosis with shoot necrosis; 5, 75 - 100% leaf necrosis with or without shoot necrosis.

For each cultivar eighteen groups of three cuttings were split between the following treatments to test for ET sensitivity: 1) control of 0 $\mu L \cdot L^{-1}$ ET; 2) 0.1 $\mu L \cdot L^{-1}$ ET; 3) 1.0 $\mu L \cdot L^{-1}$ ET. The control of 0 $\mu L \cdot L^{-1}$ ET was administered by including activated charcoal in a 210-L barrel to absorb exogenous ET. Each ethylene treatment had six jars or replications of PRP and PBR and four replications of TDR and RMV with each replication having three cuttings. After 22 hours, treatment groups were put into a 900 mL glass jars ($v = 946.4\ cm^3$), with a moist paper towel and sealed with a lid and a neoprene septum. Jars were held at an average temperature of 22°C and completely randomized on a lab bench under black plastic to simulate shipping conditions. ET was sampled at 24, 48 and 96 hours. After four days after treatment (DAT) cuttings were removed and the number of yellow apices, curved petioles, yellow leaves, and abscised leaves was recorded for each jar. These numbers were converted to the leaf yellowing rating scale (Fig. 3): 0, symptoms; 1, 10% marginal yellowing; 2, 11 - 25% yellow; 3, 26- 50% yellow; 4, 51 – 75% yellow; 5, 51 - 75% yellow; 6, dead or abscised, for statistical analysis and continuity with the following experiments.

Statistical analysis

Data were analyzed using SAS (Version 9.4, SAS Institute, Inc., Cary, N.C.). Mean separation for ET production was determined using the Generalized Linear Mixed Models (GLIMMIX) and Tukey's Studentized range test procedures where $\alpha = 0.05$. Treatments were

nested within cultivar for *Botrytis* ratings because DI treatment was not applied to RMV and TDR.

2.2.2 Commercial - Dümmen Orange

Unrooted cuttings and handling

Unrooted cuttings of 'Great Balls of Fire Lavender' (GL) and 'Great Balls of Fire Light Lavender' (GLL), 'Patriot Bright Red' (PBR) and 'Patriot Rose Pink' (PRP) were received from Dümmen Orange (Columbus, Ohio, USA) production facility on 12 April 2016. All cuttings were placed at 4°C cooler with moist paper towels to prevent moisture loss. Cuttings were removed from storage at 8:00 AM on April 13th. Each cultivar was sorted by number of leaves and stem caliper and placed in groups to make sixty-three groups of three cuttings each. Twenty-seven groups of each cultivar were placed back in the cooler until after the thirty-six groups had received their respective treatments to test susceptibility to *B. cinerea*. Pathogen culture, spore collection, inoculation and ethylene application and quantification procedures were the same as previously reported.

Experimental design

Followed procedures denoted in 2.2.1, except for each cultivar thirty-six groups of three cuttings were split between the following treatments to test for susceptibility to *B. cinerea*: 1) unsprayed dry control; 2) sterilized deionized (DI) water (0.2 µm filter, Draco Inc, Durham, N.C., USA) control; 3) sterilized spray of 0.01% Tween 80 and 15% glycerol (spore carrier solution; 4) *Botrytis* spore suspension of 10^6 spores/mL. Twenty-seven groups of three cuttings were split between the following treatments to test for ET sensitivity: 1) control of 0 $\mu L \cdot L^{-1}$ ET; 2) 0.1 $\mu L \cdot L^{-1}$ ET; 3) 1.0 $\mu L \cdot L^{-1}$ ET. Cuttings from the ET sensitivity part were stuck in cell packs using a commercial peat-based substrate (Fafard® 2P Mix; Sun Gro, Agawam, Mass., USA)

after the 4 d incubation and ratings for rooting fourteen DAT using a 1 – 5 scale with 1, no roots; 2, 1-5 roots less than 2.5 cm; 3, 1-5 roots at least 2.5 cm, or 6-10 roots less than 2.5 cm; 4, 6-10 roots at least 2.5 cm, or 10+ roots less than 2.5 cm; 5, 10+ roots 2.5 cm or longer.

Statistical analysis

Data were analyzed using procedures denoted in 2.2.1. Results of this run were compared back to 2.2.1 as the second replicate for cultivars PBR and PRP.

2.2.3 Commercial - Syngenta Flowers

Unrooted cuttings and handling

Unrooted cuttings of 'Americana Coral' (AC) and 'Americana Red' (AR), 'Tango Dark Red' (TDR) and 'Tango Tango' (TT) were received from a commercial supplier (Syngenta Flowers, Mexico) on 2 Feb. 2016. All cuttings were placed at 4°C with moist paper towels to prevent moisture loss. Cuttings were removed from storage at 8:00 AM on 13 April. Cuttings of each cultivar were sorted by number of leaves and stem caliper and placed in forty-two groups of three cuttings. Eighteen groups of each cultivar were placed at 4°C until after the other twenty-four groups of cuttings had received their *Botrytis* treatments. This experiment was repeated on 10 May, 2016 with a total of fifty-four groups split between ET and *Botrytis* testing. Pathogen culture, spore collection, inoculation, germination verification, and ethylene application and quantification procedures were the same as previously reported in 2.2.1.

Germination verification

To verify that spores were not losing viability during -80°C storage, 1 mL of the suspension used for inoculation was plated onto potato dextrose agar (PDA) (Thermo Fisher Scientific, Lenexa, Kans.). After four hours at room temperature, 100 spores were counted under ×100 (Nikon TMS-F Inverted Microscope, Nikon Instruments Inc., Melville, N.Y., USA) and

spores with a definitive germination tube were counted as viable while spores without a germ tube were counted as nonviable (Fig. 1D). Germination averaged 88% for three replicates.

Experimental design

Experiments were designed as previously described in section 2.2.2, except for the following parameters. For each cultivar, the first replicate of the experiment had twenty-four groups of three cuttings split evenly among the following treatments to test for susceptibility to *B. cinerea*: 1) unsprayed dry control; 2) sterilized deionized (DI) water (0.2 µm filter, Draco Inc, Durham, N.C., USA) control; 3) sterilized spray of 0.01% Tween 80 and 15% glycerol (spore carrier solution; 4) *Botrytis* spore suspension of 10^6 spores/mL. Twenty-seven groups were used for the second replication and did not include the spore carrier solution. To test ethylene sensitivity, eighteen groups of three cuttings in the first replicate were split between the following treatments: 1) control of 0 $\mu L \cdot L^{-1}$ ET; 2) 0.1 $\mu L \cdot L^{-1}$ ET; 3) 1.0 $\mu L \cdot L^{-1}$ ET, while the second replicate had twenty-seven groups.

Statistical analysis

Procedures previously described in 2.2.1 were used. Ethylene production was not analyzed because of missing data points. A square root transformation was performed for leaf ratings 4 DAT and 7 DAT, and root ratings for the first replicate to correct normality violations seen in the residual plot. The transformation was also used on leaf ratings 7 DAT in the second replicate.

2.2.4 In-house

Stock Plant Culture, unrooted cuttings, and handling

Stock plants of PBR and PRP were started from shipped cuttings received on 26 Jan. 2016. Stock plants of AC, AR, TDR and TT were started from shipped cuttings received on 2

Feb. 2016. GL and GLL stock plants were started 12 April 2016. Stock plants were grown in ambient air in glass glazed greenhouse at 24°C days and 18°C nights. Stock plants were managed as stated in 2.2.1. Shoot tip cuttings were harvested on 31 May 2016 at 10:00 AM and extra leaves removed leaving two to three mature leaves. In the second replication of this experiment on 7 June 2016 the ivy geraniums, GL and GLL, were included. Pathogen culture, spore collection, inoculation, germination verification, and ethylene application and quantification procedures were the same as previously reported in 2.2.1 and 2.2.2.

Experimental design

Procedures followed steps in 2.2.2, except each cultivar had twelve groups of three cuttings split between the following treatments to test for *Botrytis* susceptibility: 1) unsprayed dry control; 2) sterilized deionized (DI) water (0.2 µm filter, Draco Inc, Durham, N.C., USA) control; 3) sterilized spray of 0.01% Tween 80 and 15% glycerol (spore carrier solution; 4) *Botrytis* spore suspension of 10^6 spores/mL and nine groups of three cuttings were split between the following treatments to test for ET sensitivity: 1) control of 0 $\mu L \cdot L^{-1}$ ET; 2) 0.1 $\mu L \cdot L^{-1}$ ET; 3) 1.0 $\mu L \cdot L^{-1}$ ET.

Statistical analysis

Data were analyzed using procedures denoted in 2.2.1. A square root transformation was performed on leaf ratings 7 DAT to correct normality violations seen in the residual plot.

2.2.5 Overall cultivar rankings

The responses were averaged for each cultivar across treatments as follows: leaf ratings across treatments, disease ratings across inoculated treatments, and root ratings across the untreated, control. Cultivars were then given a ranking of 1 – 8; 1 = best, 8 = worst, for each

response, leaf ratings 4 DAT and 7 DAT, *Botrytis* ratings and root ratings. The rankings were then averaged for each cultivar across responses to determine the best overall cultivar.

2.3 Results and Discussion

2.3.1 Commercial (Dümmen Orange) and in-house

Ethylene production

ET concentrations increased with time (Table 1). A three-way interaction among cultivar, ethylene concentration and time was significant ($P = 0.0166$) (Table 1). Within this interaction, PRP was the only cultivar to produce significantly higher concentrations of ethylene. PRP produced more ET than the control only at 48 h when treated with 1.0 $\mu L \cdot L^{-1}$ ET. By 96 h after treatment, PRP produced 1.818 $\mu L \cdot L^{-1}$ ET, significantly more than any other treatment, cultivar combination. PBR, TDR, and RMV were not affected by time or treatment of higher ethylene concentrations, and 96 h after treatment produced ethylene levels similar to the control at 24 h. Plants naturally produce ethylene, but are able to regulate their production through negative and positive feedback receptors that sense ethylene in the atmosphere (Xu and Zhang, 2015). PRP may not have been able to stop or slow its own production of ET, even when exposed to 1.0 $\mu L \cdot L^{-1}$ ET. PRP could have excess ET precursors or be unable to sense exogenous ethylene, because untreated PRP produced 0.685 $\mu L \cdot L^{-1}$ at 96 h, which was significantly higher than 0.223 $\mu L \cdot L^{-1}$ ET at 24 h.

Ethylene effects on leaf yellowing

A significant interaction occurred between cultivar and ET treatments ($P = 0.0125$). As ET treatment concentration increase leaf yellowing increased for all cultivars, except PBR treated with 0.1 $\mu L \cdot L^{-1}$ ET (Fig. 4). Treating cuttings with 1.0 $\mu L \cdot L^{-1}$ ET resulted in significantly higher leaf ratings of 4.0, 2.0 and 0.9 for PBR, PRP and TDR, respectively when compared to

the untreated, control. 0.1 $\mu L \cdot L^{-1}$ ET did not significantly increase leaf ratings in any cultivar compared to the control. Although it was not a significant decrease in rating, PBR had less yellowing when treated with 0.1 $\mu L \cdot L^{-1}$ compared to the control.

Across treatments, PRP had higher leaf ratings than all cultivars except PBR. The greater sensitivity PRP had to leaf yellowing matches industry observations (A. Hammer, personal communication). In addition, PRP produced the most ET and was the only cultivar significantly affected by increasing ET concentration. For RMV, as the treatment concentration of ET increased, ET production decreased and leaf rating increases were small; PBR increased in ET production and leaf rating as ET concentration increased; TDR did not increase in ET production or leaf rating until treated with 1.0 $uL \cdot L^{-1}$.

Botrytis susceptibility

A two-way interaction was significant ($P = 0.0125$) among treatments and cultivar because control treatments of cuttings left dry, sprayed with DI, or sprayed with the spore carrier solution developed *Botrytis* ratings that were sometimes significantly lower or higher than inoculated cuttings. Inoculated cuttings for all cultivars had higher ratings than their controls, except for PRP; thus, only ratings for the inoculation treatment were reported (Fig. 5). Even without treatment controls, PRP was still the most susceptible with an average *Botrytis* rating of 2.7. PBR was the second most susceptible (1.2), but was not different from TDR (0.6), which was not different from RMV (0.4). Disease severity and ranking of susceptibility was similar to ethylene production and leaf ratings. PRP usually had the highest ethylene production, highest leaf yellowing ratings and susceptibility, but PBR was not much lower. TDR and RMV were very similar in ethylene production and leaf yellowing, but RMV was less susceptible to *Botrytis* than TDR.

2.3.2 Commercial - Dümmen Orange

Ethylene effects on leaf yellowing and rooting

There was no significant interaction between ET concentration treatments and cultivar for leaf ratings after 4 d of incubation. Ivy geraniums, GLL and GL, had higher leaf ratings than both PBR and PRP (Table 2). GLL was significantly higher than PBR and PRP, while GL, PBR and PRP were not significantly different from each other. Untreated cuttings in the group had an average leaf rating of 0.9, which was significantly lower than cuttings treated with 0.1 or 1.0 $uL \cdot L^{-1}$. Once cuttings were propagated in a peat-perlite substrate and under mist for 3 days, there was a cultivar and treatment interaction for leaf ratings 7 DAT ($P = 0.0060$) (Fig. 6). Untreated cuttings did not differ in leaf ratings. PRP had the least amount of yellowing when treated with 0.1 or 1.0 $\mu L \cdot L^{-1}$ ET, but it was not different from PBR. PBR, when treated with 0.1 or 1.0 $\mu L \cdot L^{-1}$ ET, was not different from GL and GLL treated with 0.1 $uL \cdot L^{-1}$, but GL and GLL treated with 1.0 $uL \cdot L^{-1}$, were significantly higher on the leaf rating scale than all other cultivars treatment combinations. High ratings for the, GL and GLL, agrees with industry observations that these geraniums are more sensitive to ET (A. Hammer, personal communication).

After 18 d under mist in propagation, PBR had significantly higher root ratings than all other cultivars, which were not different from each other (Table 2). Treating cuttings with ET significantly reduced root ratings compared to the control. Increasing the concentration from 0.1 to 1.0 $\mu L \cdot L^{-1}$ did not reduce ratings further. Kadner and Dreuge (2004) found an application of ethylene showed a positive effect on rooting (+49% roots per cutting) when applied during storage at 20°C, but their assessment was done after 20 days in propagation in perlite and a heated root zone temperature of 20-28°C. Leaf yellowing did not seem to foreshadow rooting in any of the cultivars. Ivy geraniums, GL and GLL, had high leaf ratings while PRP was quite low.

All had lower root ratings than PBR. If more time was allowed for rooting, results may have been different because cuttings with more and healthier leaves would have been able to produce more carbohydrates for root development. Senescing and weakened leaf tissue is also an entry point for *B. cinerea*. Cutting survival and disease data was not taken on these cuttings after rooting data, which may be important as growers that stick stressed cuttings often need to input more labor to remove damaged leaves to prevent *Botrytis* infection and spread.

Botrytis susceptibility

An interaction among cultivars and treatment was significant ($P = 0.0228$). However, disease ratings for cuttings left dry, or sprayed with DI or the carrier solution of Tween 80 and glycerol were not significantly different from each other. For that reason, only disease ratings for the inoculated cuttings are presented (Fig. 7). GLL was the most susceptible with a disease rating of 2.9. PRP had a disease rating of 2.3 and was not significantly different from GLL or GL with a *Botrytis* rating of 2.1. PBR had the lowest (1.7), but was not significantly different from GL. Cultivars' susceptibility to *Botrytis* did not match sensitivity to ET when treated with 0.1 or 1.0 $uL \cdot L^{-1}$. However, when cultivars were left untreated, those that had lower leaf ratings had lower disease ratings when inoculated. While rooting was not considered in the *Botrytis* susceptibility experiment, it would be interesting to see how disease would have continued to develop during propagation. High humidity and free water during propagation could allow small lesions to grow and destroy entire cuttings. Research on survival of infected cuttings could help growers determine losses and risks involved with propagating diseased material.

2.3.3 Commercial - Syngenta Flowers

Ethylene production for experiment 1

Treating with 1.0 $\mu L \cdot L^{-1}$ ET increased ET production for every cultivar (Table 3). Treating cuttings with 0.1 $\mu L \cdot L^{-1}$ ET decreased ET concentrations compared to the control, except for AR. At every time measurement and with every treatment, AR produced the most ethylene. Over all times and treatments, TT and TDR produced the least amount of ethylene, with the lowest amount at 48 h in the 0.1 $\mu L \cdot L^{-1}$ ET treatment.

Ethylene effects on leaf yellowing and rooting

In the first experiment, leaf yellowing was recorded 4 days after treatment (DAT) and 7 DAT, which was three days into propagation. On both days there was no significant interaction between cultivar and treatment. Treating with 0.1 $\mu L \cdot L^{-1}$ significantly increased leaf ratings and 1.0 $\mu L \cdot L^{-1}$ significantly increased leaf ratings even higher as seen in Table 4. Only AR was significantly higher in leaf rating than TT, TDR, and AC, which did not differ from each other. After 3 d in propagation, TDR moved up from 3.2 to 4.9, which was not significantly different from the 5.1 rating of AR. TT and AC were significantly lower than TDR and AR with leaf ratings of 4.0 and 3.7, respectively. Treating with ET did not affect rooting of any cultivar compared to the control (Table 4). AC and AR had the highest root ratings, but AR was not significantly different from TT or TDR.

During the second experiment, cultivar was the only significant factor 4 DAT. AC had the highest leaf rating of 1.0, followed by AR (0.5) and TDR (0.5), and then TT with the least of 0.2 (Table 4). Leaf ratings were much lower in the second experiment., which is most likely due to the fact that cuttings were placed in the cooler to slow the leaf yellowing process between ET sampling at 24, 48 and 96 h. This also caused no ethylene buildup at any of these intervals. Ultimately this reduction in ET production resulted in similar leaf ratings for the 0.1 and 1.0 $\mu L \cdot L^{-1}$ ET treatments. Cultivar was the only factor significant for leaf ratings 7 DAT (3 d into

propagation). AC, TT, TDR and AR had leaf ratings of 3.3, 3.1, 2.8 and 2.5, respectively, which was completely opposite of the first experiment. Ethylene treatments did not significantly affect rooting. AC had significantly better rooting than TT, TDR and AR, but RR were much lower than in the first replicate. When comparing the two replicates of the experiment, AR went from being significantly higher in leaf rating compared to AC in experiment 1 to significantly lower in experiment 2.

Botrytis susceptibility

In experiment 1, there was no significant interaction among the controls, inoculation treatment or cultivars. Susceptibility of cultivars occurred in the following order: AR, AC, TT, and TDR with disease ratings of 2.2, 1.5, 1.2, and 1.2, respectively (Table 5). AR had significantly more damage than all other cultivars. Disease ratings were highest when inoculated with *Botrytis,* but ratings were not significantly different from DI or solution treatments. Ratings of inoculated cuttings were significantly higher than cuttings left dry, which is notable, as any excess moisture provided by the DI or carrier solution controls would increase chances of *Botrytis* infection from latent spores or growth of small lesions.

There was an interaction among treatments and cultivars in the second experiment. However, all inoculated cuttings were significantly higher than controls except AR (Table 5). Susceptibility of cultivars occurred in the following order: TDR, TT, AC and AR with ratings of 1.7, 1.6, 1.4, and 1.2, respectively. Between the two experiment replicates AR went from being the most susceptible to the least, meaning some factor such as stock plant management made these cuttings more susceptible as AR and TDR were shipped in the same container and handled the same upon arriving at NCSU.

2.3.4 In-house

Ethylene effects on leaf yellowing and rooting

Cultivar was a significant factor for leaf ratings 4 DAT (P = 0.0063). TT, PBR and AR had the highest leaf ratings, but PBR and AR were not significantly different from PRP, TDR and AC (Fig. 8). Seven DAT (3 d into propagation), cultivars responded similarly, except TDR, which had ratings significantly lower than TT. Treatments of 0.1 and 1.0 $\mu L \cdot L^{-1}$ ET caused significantly higher leaf ratings compared to the control (P = 0.0002) (Fig. 9). The higher concentration of ET did not differ from 0.1 $\mu L \cdot L^{-1}$. Root ratings were lower than when using cuttings from off-shore suppliers. 1.0 $\mu L \cdot L^{-1}$ ET did significantly increase RR by 0.1, but commercially the rating difference would not likely have been noticeable. Low root ratings may be due to low natural light levels in January and February when experiments were implemented with in-house cuttings compared to experiments implemented during April and May produced by commercial suppliers. Supplier stock plant nutrition and temperature may have also been better tailored to geranium cutting production.

Botrytis susceptibility

An interaction was present among cultivars and treatments (P<0.0001). However, inoculated cuttings had significantly higher disease ratings than all controls so just the inoculated treatment data are presented (Fig. 10). Cultivars ranked from most susceptible to least, in disease rating, as follows: GLL (4.9), PRP (4.6), GL (4.3), TDR (3.7), PBR (3.7), AR (3.4), AC (2.9), and TT (2.8). Overall, zonal geraniums were not significantly different from each other, except PRP. Ivy geraniums were most susceptible, which was similar to experiment 2.2.2 conducted with commercially produced cuttings. Cultivars from the same series were not significantly different from each other, and overall the differences in rating are small except when comparing the cultivars with the highest and lowest ratings. The disease that developed would make all

cutting of every cultivar commercially unacceptable for a grower to receive, which indicates that all cultivars are at risk of infection regardless of their resistance.

2.3.5 Overall rankings

Overall, the best performing cultivar was AC. Without rooting data, which was very low in most experiments, PBR, AC, and PRP, ranked first, second and third, respectively for resistance to yellowing and disease (Table 6). In terms of resistance to leaf yellowing the top two cultivars were PBR and PRP. The worst two cultivars were AR and GLL. The two least *Botrytis* susceptible cultivars were AC and TT. The most susceptible cultivars were the ivy geraniums, GL and GLL, with AR and PRP in 5th an 6th place, respectively. Overall, the two best *P.* × *hortorum* cultivars were AC and PBR and the two worst TDR and AR. Both *P. peltatum*, GL and GLL, overall ranked at the bottom.

Data supports the floriculture industry's observations that at least with the cultivars tested, *P. peltatum* is less durable than *P.* × *hortorum* when challenged with ethylene and *B. cinerea*. Uchneat et al. (1999a; 1999b) found varying levels of susceptibility to *B. cinerea* and resistance in geranium cultivars' floral and foliar tissue, and some ivy cultivars were more resistant and some more susceptible to *B. cinerea* than *P.* × *hortorum* cultivars. The industry has observed that cultivars with light green leaves and flower colors other than red were more sensitive to ET and *Botrytis* (H. Lang, personal communication), but this was not apparent in these studies. Uchneat et al. (1999) stated that floral and foliar resistance do not seem to be correlated, which may explain the lack of evidence for leaf and flower color matching *Botrytis* susceptibility in these experiments. Color did not seem to be a factor in seed geraniums either when Clark et al. (2001) tested for ethylene sensitivity using a triple response assay. 'Multibloom Lavender' and 'Elite White' were the least sensitive followed by 'Pinto Pink', 'Orbit Red', and

‘Elite Scarlet’. A normal triple response assay was not possible with cultivars produced vegetative shoot tip cuttings, because the technique measures roots apical hooks and hypocotyl growth from a germinating seed.

Whole unrooted cuttings may have different sensitivity and resistance than whole plants, individual leaves or flowers because cuttings lack a root system, water, sunlight, and any materials available through other parts of the stock plant during postharvest shipping and storage. Responses are also very likely to be dependent on stock plant culture and shipping environment as low carbohydrates reserves, dehydration and latent spores can all influence cutting quality (Rapaka et al., 2007; Hausbeck and Pennypacker, 1991). The findings presented here emphasize the need to monitor how environmental conditions change during shipping. If growers know what environments cuttings experience during shipping, then boxes could be manufactured to better resist temperature changes or mitigate other stresses. Real time monitoring could also give suppliers the ability to send new plant material before a grower receives a stressed or diseased shipment.

Two sources of cuttings were used for this work. Commercially produced cuttings shipped to NCSU and cuttings produced in-house at NCSU. There were some major differences in the responses between the two indicating that cultural and environmental differences influenced responses. Cuttings received from commercial suppliers were prone to leaf yellowing and rooted better. Cuttings produced at NCSU were more prone to disease. Leaf yellowing could be caused by the stress induced by shipping while higher RR could be due to management and nutrition of stock plants. Stock plants at NC State were grown according to commercial standards; however, there were periods where high temperatures and stock plant age could have reduced quality. If cuttings were stressed by the shipping process one would assume disease

would be more pronounced on shipped cuttings (Hartmann et al., 2002). However, growers were likely treating stock plants to control disease and cleanliness may have been more consistent at commercial sites than at NCSU.

The fact that almost all cuttings, regardless of treatment or source, developed some lesions from *Botrytis* infection is reason for concern. Suppliers are shipping cuttings with spores on cuttings or small lesions. Botrytis can infect living tissue and survive on dead tissue (Elad et al., 2007). It was observed that many times stipules became infected before any other organs. Stipule removal is practiced as some cuttings arrived with no stipules, but growers may want to invest the time to breed for plants with no stipules or remove a majority of stipules before packing. Based on these data, if given a long enough period of time, such as a one to two-day delay, shipments will succumb to infection regardless of cultivar. There is no known qualitative resistance or genes that confer complete resistance to *Botrytis* (Elad and Shtienberg, 1995).

While suppliers are producing high quality cuttings, those cuttings are still susceptible to *Botrytis*. Even a few small lesions or spores can be a significant issue if shipments are delayed and experience variable temperatures. Growers and suppliers either need a reliable shipping method or better *Botrytis* control such as a spray or box environment that will ensure protection regardless of shipping duration or environment. Future research should focus on methods to implement during shipping which could control or monitor environmental factors or pre-shipping treatments that could be applied to protect cuttings during the shipping time.

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Table 1. Analysis of cultivar ethylene production for Commercial (Dümmen Orange) and in-house experiment (2.2.1). Cuttings were left untreated (0 µL·L^{-1} ET), treated with 0.1 or 1.0 µL·L^{-1} ET and ethylene concentrations were measured at 24, 48 and 96 h during incubation in jars. Each rep contained 3 cuttings and means were an average of six replications for 'Patriot Bright Red' and Patriot Rose Pink', and four replications for Rocky Mountain Violet' and 'Tango Dark Red'.

		Time (h)		
Cultivar	Ethylene (uL·L^{-1})	24	48	96
'Patriot Bright Red'	0	0 d[z]	0 d	0.26 bcd
	0.1	0 d	0.01 cd	0.23 bcd
	1.0	0 d	0 d	0.43 bcd
'Patriot Rose Pink'	0	0 d	0.15 cd	0.69 cb
	0.1	0 d	0.18 cd	0.95 b
	1.0	0.22 bcd	0.50 cb	1.82 a
'Rocky Mountain Violet'	0	0 d	0.22 bcd	0.35 bcd
	0.1	0.06 d	0.12 cd	0.23 bcd
	1.0	0.1 cd	0 d	0.08 cd
'Tango Dark Red'	0	0 d	0.14 cd	0.25 bcd
	0.1	0 d	0.11 cd	0.19 bcd
	1.0	0.04 d	0.18 cd	0.29 bcd

[z]Means followed by the same letter for are not significantly different according to Tukey's Studentized range test procedure at $\alpha = 0.05$.

Table 2. Analysis of the separate cultivar and ethylene treatment effects on leaf ratings 4 days after treatment (DAT) and root ratings for Commercial - Dümmen Orange (2.2.2). Root ratings of 0 = no roots and 6 = 10 roots at least 2.5 cm, or 10+ roots less than 2.5 cm; 5, 10+ roots 2.5 cm or longer. Cultivar means were averaged across 27 replications which were each an average of 3 cuttings per rep. Treatment means were averaged across 36 replications which were each an average of 3 cuttings per rep.

Cultivar[z]/ ET treatment	Leaf rating 4 DAT	Root rating
GL	1.1 ab[y]	0.2 b
GLL	1.4 a	1.4 a
PBR	1.0 b	0.6 b
PRP	0.9 b	0.1 b
Significance	<0.0001	<0.0001
0[x]	0.9 b	0.5 a
0.1	1.1 a	0.3 b
1.0	1.3 a	0.3 b
Significance	0.0002	0.0060

[z]GL: 'Great Balls of Fire Lavender', GLL: 'Great Balls of Fire Lavender', PBR: 'Patriot Bright Red', PRP: 'Patriot Rose Pink'.

[y]Means followed by the same letter for cultivar or treatment are not significantly different according to Tukey's Studentized range test procedure at $\alpha = 0.05$.

[x]Concentration of ethylene applied in $\mu L \cdot L^{-1}$.

Table 3. Ethylene production for Commercial - Syngenta Flowers experiment 1 (2.2.3). Cuttings were left untreated (0 $\mu L \cdot L^{-1}$ ET), treated with 0.1 or 1.0 $\mu L \cdot L^{-1}$ ET and ethylene concentrations were measured at 24, 48 and 96 h during incubation in jars. Each rep contained 3 cuttings and means were an average of six replications.

		Time (h)		
Cultivar	Ethylene ($uL \cdot L^{-1}$)	24	48	96
Americana Coral	0	0.07	0.12	0.29
	0.1	0	0.04	0.27
	1.0	0	0.13	-
Americana Red	0	0.13	0.33	0.40
	0.1	0.04	0.57	0.97
	1.0	0.57	1.03	-
Tango Tango	0	0	0	0.06
	0.1	0	0	0.04
	1.0	0.06	0	0.32
Tango Dark Red	0	0	0.03	0.11
	0.1	0	0	0.07
	1.0	0.11	0.09	-

Table 4. Analysis of response of leaf ratings to cultivar and ethylene treatments for Commercial - Syngenta Flowers (2.2.3). Leaf ratings were on a scale of 0 – 6; 0, no symptoms; 6 dead or abscised. Root ratings of 0 = no roots and 6 = 10 roots at least 2.5 cm, or 10+ roots less than 2.5 cm; 5, 10+ roots 2.5 cm or longer. Cultivar and treatment means were averaged across 18 and 24 replications, respectively for experiment 1. Cultivar and treatment means were averaged across 27 and 36 replications, respectively for experiment 2. All replications were an average of 3 cuttings.

	Expt. 1			Expt. 2		
Cultivar[z]/ treatment	Leaf rating 4 DAT[y]	Leaf rating 7 DAT	Root rating	Leaf rating 4 DAT	Leaf rating 7 DAT	Root rating
AC	3.1 b[x]	3.7 b	1.2 a	1.0 a	3.3 a	0.4 a
AR	4.7 a	5.1 a	1.1 ab	0.5 b	2.5 b	0.1 b
TDR	3.2 b	4.9 a	1.0 b	0.5 b	2.8 ab	0.1 b
TT	3.3 b	4.0 b	1.0 b	0.2 c	3.1 a	0.1 b
Cultivar	<0.0001	<0.0001	0.0021	<0.0001	<0.0001	<0.0001
0[w]	2.9 c	3.5 c	-	-	-	-
0.1	3.5 b	4.5 b	-	-	-	-
1.0	4.3 a	5.3 a	-	-	-	-
Treatment	<0.0001	<0.0001	NS	NS	NS	NS

[z]AC: ‘Americana Coral’, AR: ‘Americana Red’, TDR: ‘Tango Dark Red’, TT: ‘Tango Tango’.

[y]Day after treatment.

[x]Means followed by the same letter in a column for cultivar or treatment are not significantly different according to Tukey’s Studentized range test procedure at $\alpha = 0.05$.

[w]Concentration of ethylene applied in $\mu L \cdot L^{-1}$.

Table 5. Analysis of disease ratings for Syngenta Flowers (2.2.4) experiments 1 and 2. Disease ratings were based on a 0 – 5 scale: 0 – no symptoms; 5 – 75 – 100% leaf necrosis with or without shoot necrosis. Interaction means for cuttings inoculated with *Botrytis* were averaged across 6 and 9 replications for experiments 1 and 2, respectively; cultivar and spray treatment means were averaged across 24 and 36 replications for experiments 1 and 2, respectively. The combined column contains the average across experiments 1 and 2. Each rep was an average of 3 cuttings.

Cultivar[z]/ treatment	Expt. 1	Expt. 2	Combined
	Disease rating	Disease rating	Disease rating
AC – Botrytis	1.6 b[y]	1.4 ab	1.5 ab
AR – Botrytis	2.5 a	1.2 bc	1.7 a
TDR - Botrytis	1.2 b	1.7 a	1.5 ab
TT – Botrytis	1.6 b	1.6 a	1.6 ab
Interaction	*P* = 0.5315	*P* = 0.0023	*P* = 0.1343
AC	1.4 b	1.2 a	1.3 b
AR	2.2 a	1.0 a	1.5 a
TDR	1.2 b	1.2 a	1.2 b
TT	1.2 b	1.1 a	1.1 b
Cultivar	*P* = <0.0001	*P* = 0.0462	*P* = <0.0001
Botrytis	1.8 a	1.5 a	1.6 a
DI[x]	1.5 ab	1.0 b	1.2 b
Dry	1.4 b	1.0 b	1.1 b
Solution	1.5 ab	-	-
Treatment	*P* = 0.0446	*P* = <0.0001	*P* = <0.0001

[z]GL: ‘Great Balls of Fire Lavender’, GLL: ‘Great Balls of Fire Lavender’, PBR: ‘Patriot Bright Red’, PRP: ‘Patriot Rose Pink’.

[y]Means followed by the same letter in a column for interaction, cultivar, or treatment are not significantly different according to Tukey’s Studentized range test procedure at $\alpha = 0.05$.

[x]DI: Sterilized distilled water.

Table 6. Cultivar rankings based on the means for each cultivar across treatments as follows: leaf ratings across treatments, disease ratings across inoculated treatments, and root ratings across the untreated, control. Cultivars were then given a ranking of 1 – 8; 1 = best, 8 = worst, for each response, leaf ratings 4 d after treatment, leaf ratings 7 d after treatment, disease rating and root rating. The rankings were then averaged for each cultivar across responses to determine the best overall cultivar.

Cultivar	LR4 DAT[z]	LR7 DAT[y]	DR[w]	Avg[v]	RR[u]	Avg w/RR
'Americana Coral'	5	3	1	3	1	2.5
Americana Red'	7	6	5	6	3	5.3
'Great Balls of Fire Lavender'	6	8	7	7	5	6.5
'Great Balls of Fire Light Lavender'	8	7	8	7.7	2	6.3
'Patriot Bright Red'	1	1	3	1.7	6	2.8
'Patriot Rose Pink'	2	2	6	3.3	8	4.5
'Tango Dark Red'	3	4	4	3.7	7	4.5
'Tango Tango'	4	5	2	3.7	4	3.8

[z]LR4 DAT: Leaf rating 4 d after treatment

[y]LR7 DAT: Leaf rating 7 d after treatment (3 d into propagation)

[w]DR: Disease rating

[v]Avg: Average ranking from LR4 DAT, LR7 DAT and DR

[u]RR: Root rating

Figures

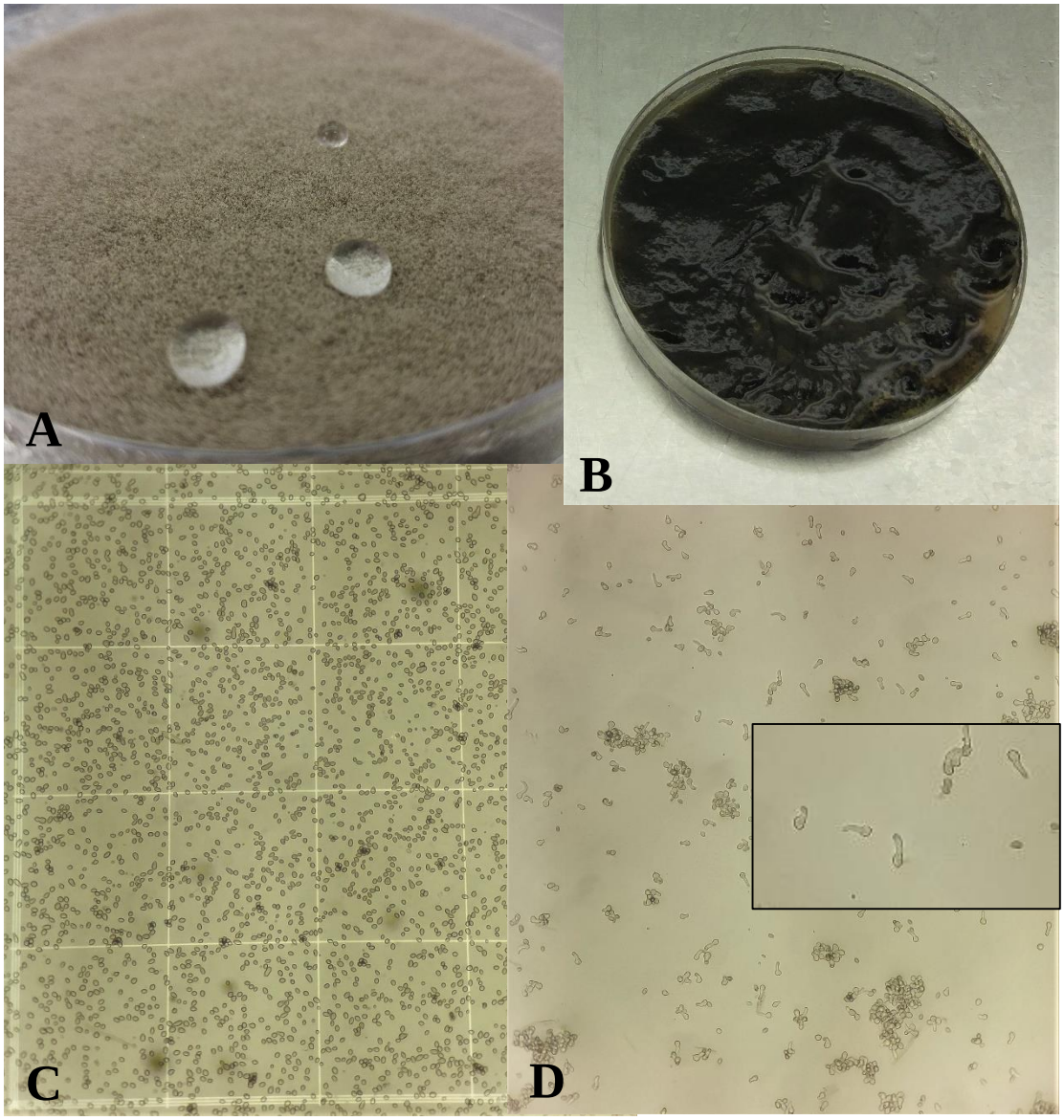

Fig. 1A-D. A. Sporulating plate covered in gray conidiophores after 14 d of incubation. B. *Botrytis* culture after rubbing with glass stirring rod with 10 mL of 0.01% Tween 80 and 15% glycerol. C. Sample of collected spores on a hemocytometer. D. Germination of conidia after four hours on potato dextrose agar at room temperature.

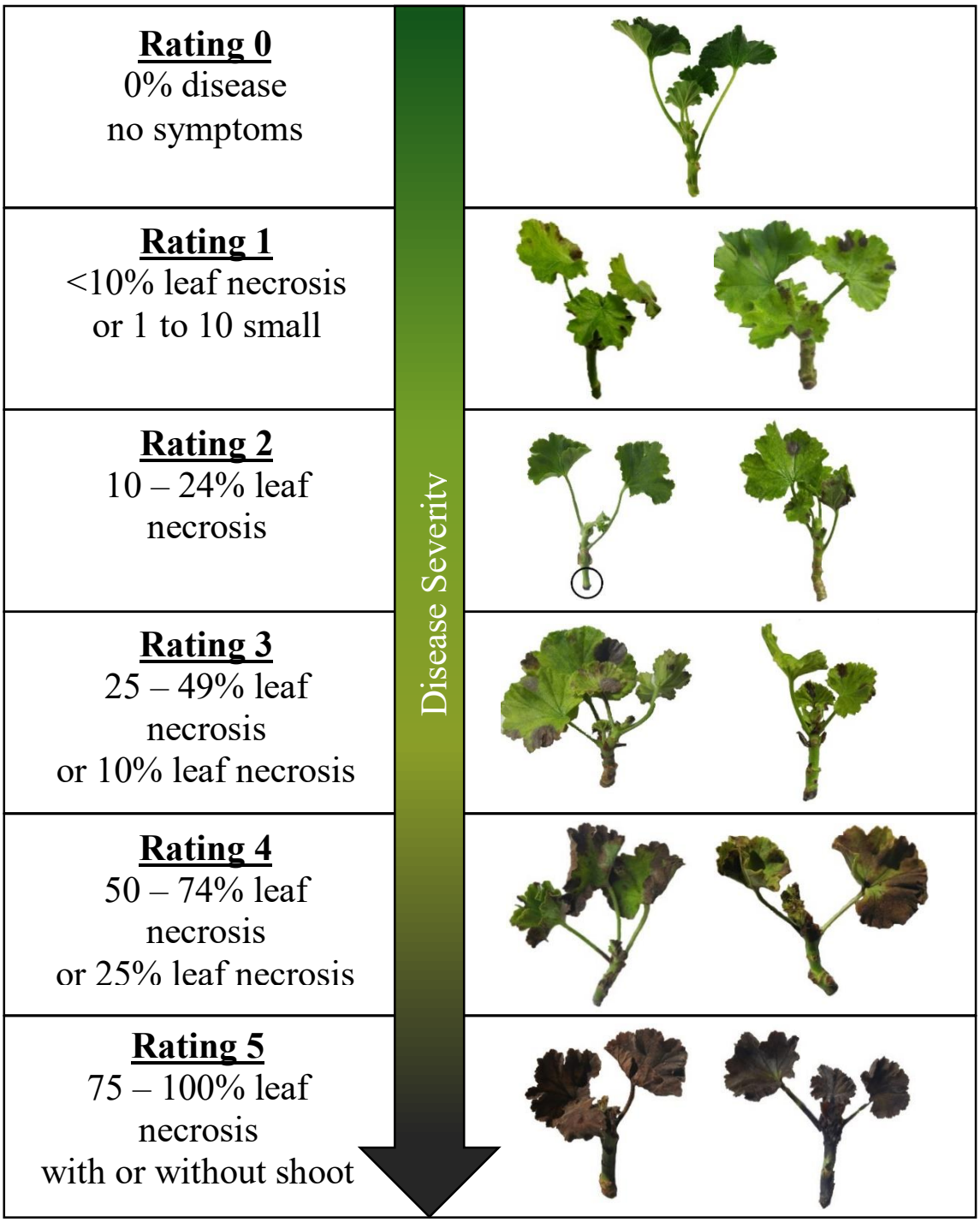

Fig. 2. Disease rating scale from 0 to 6 for unrooted cuttings of *Pelargonium* based on necrotic leaf area and shoot necrosis.

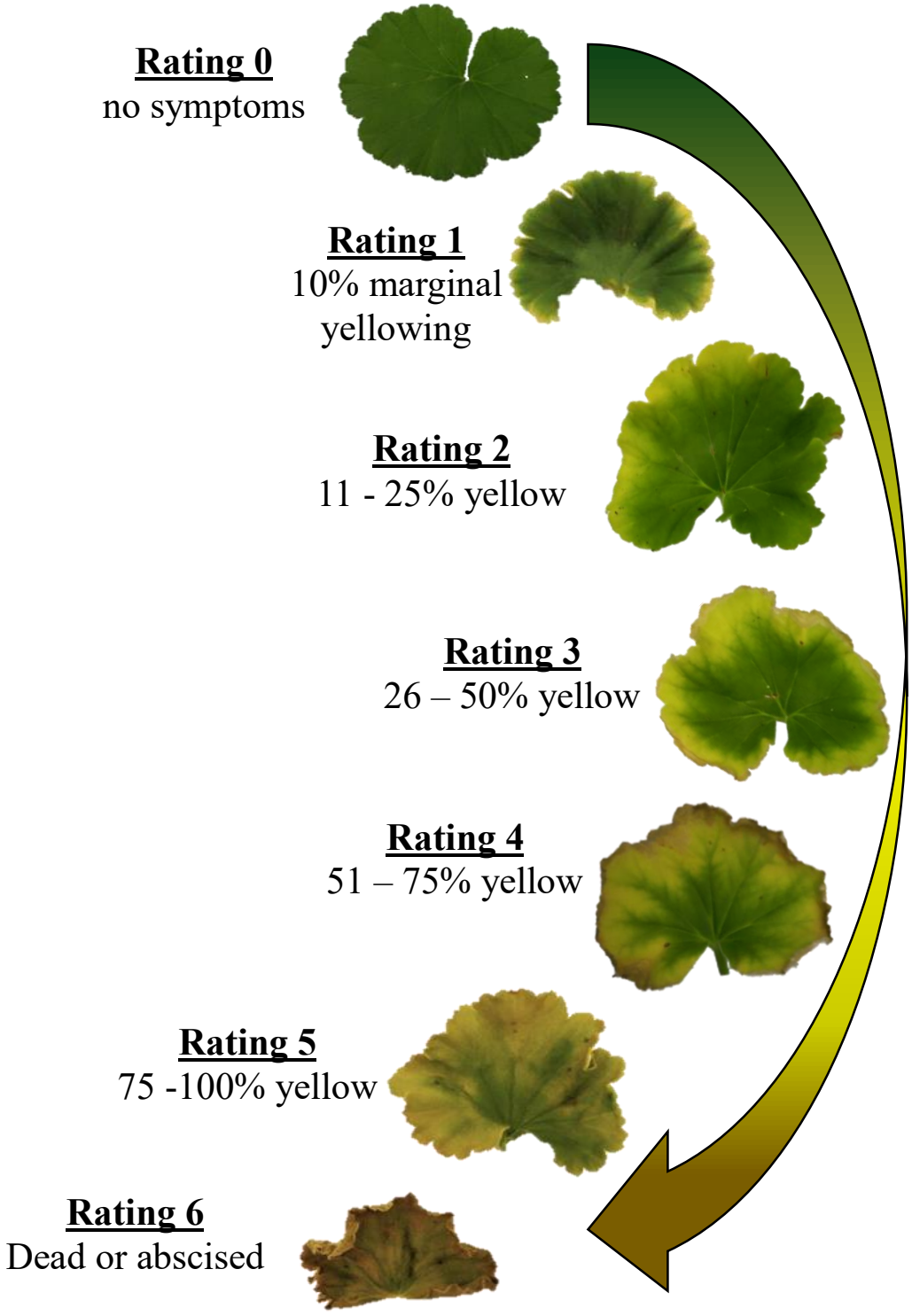

Fig. 3. Leaf yellowing ratings scale from 0 to 6 for unrooted cuttings of *Pelargonium* based on leaf yellowing.

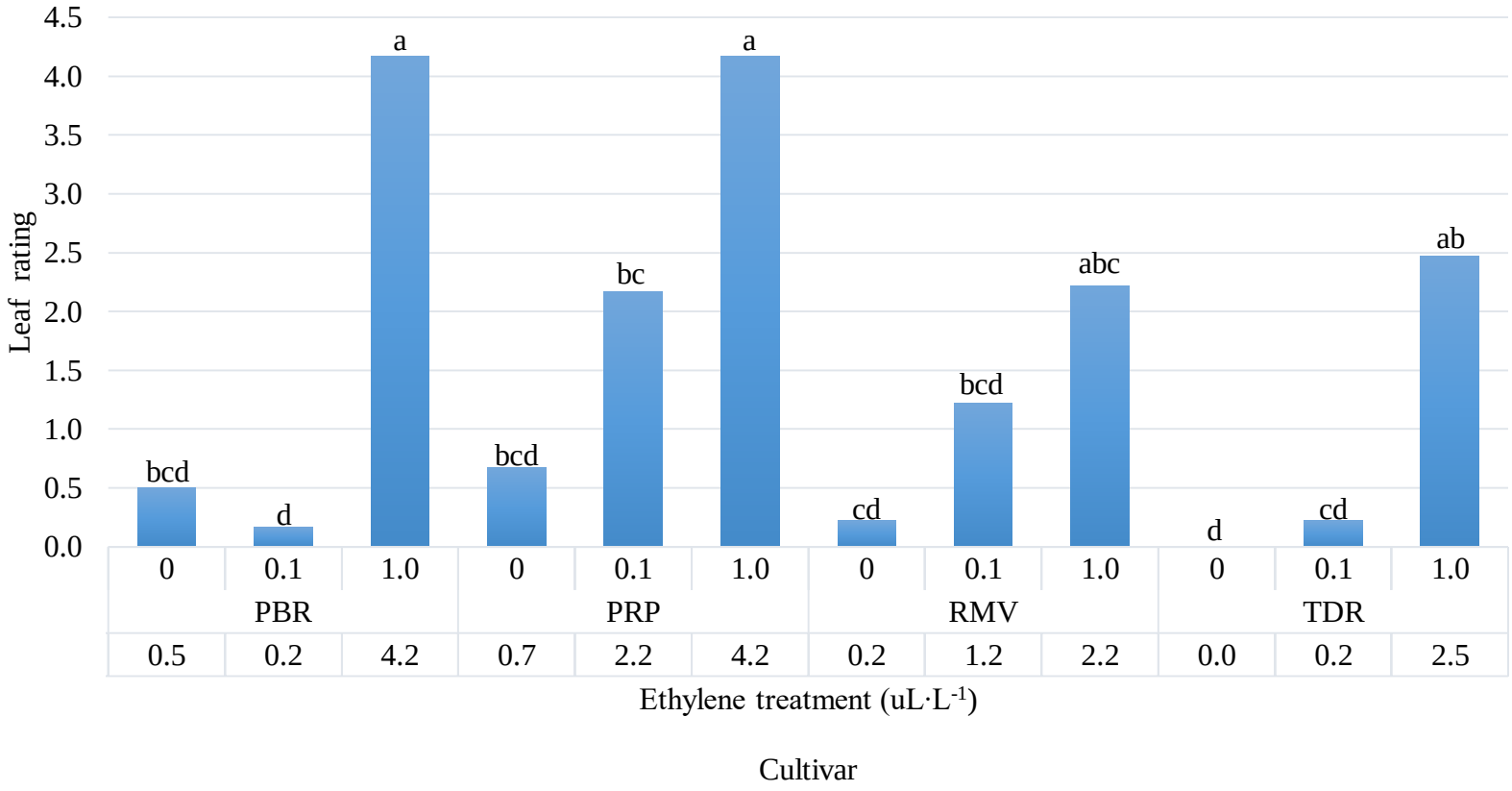

Fig. 4. Analysis of leaf ratings for Commercial (Dümmen Orange) and in-house (2.2.1), four days after treatment, of 'Patriot Bright Red' (PBR), 'Patriot Rose Pink' (PRP), 'Rocky Mountain Violet' (RMV), and 'Tango Dark Red' (TDR) in response for ethylene concentrations of 0, 0.1, and 1.0 $\mu L \cdot L^{-1}$. Columns with the same letter are not significantly different according to Tukey's Studentized range test procedure at $\alpha = 0.05$. Calculated from the means of 6 replications per treatment for PBR and PRP and 4 replications for RMV and TDR ($P = 0.0125$).

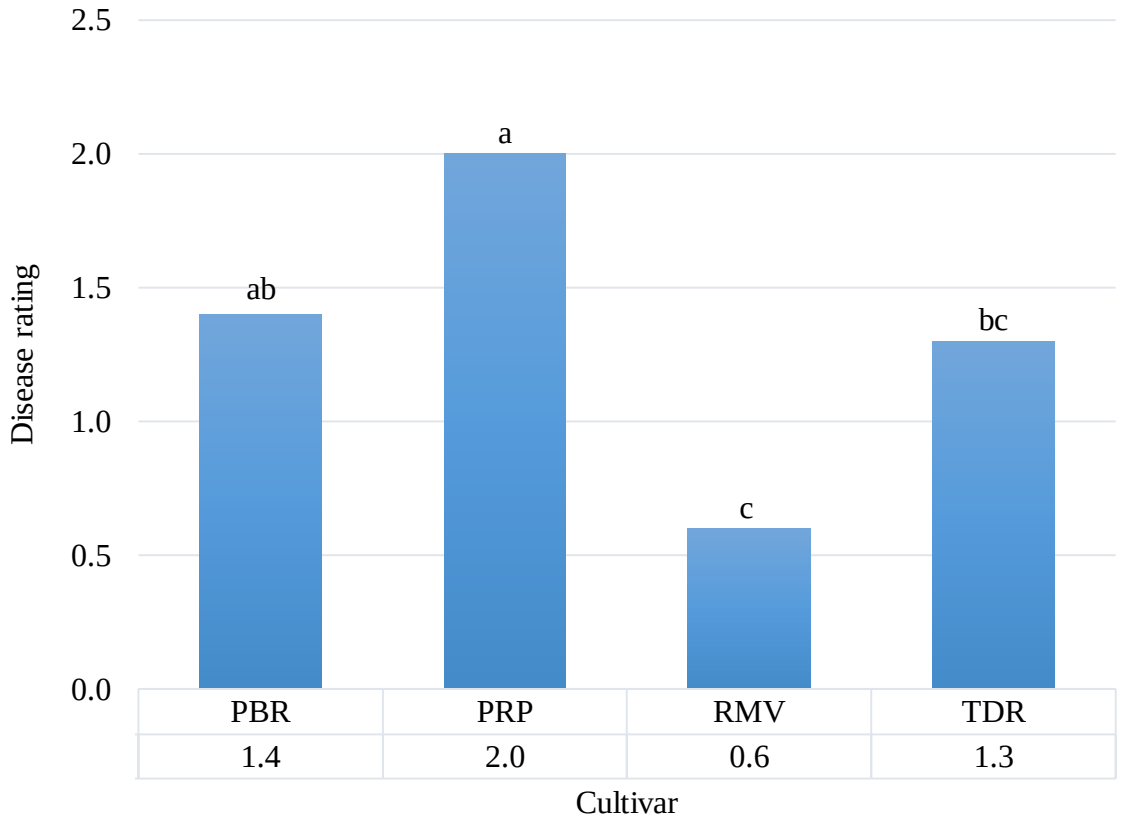

Fig. 5. Analysis of disease ratings caused by *B. cinerea* for Commercial (Dümmen Orange) and in-house (2.2.1), of 'Patriot Bright Red' (PBR), 'Patriot Rose Pink' (PRP), 'Rocky Mountain Violet' (RMV), and 'Tango Dark Red' (TDR) in response to being inoculated with 10^6 spores/mL. Columns with the same letter are not significantly different according to Tukey's Studentized range test procedure at $\alpha = 0.05$. Calculated from the means of 6 replications per treatment for PBR and PRP and 4 replications for RMV and TDR ($P = 0.0125$).

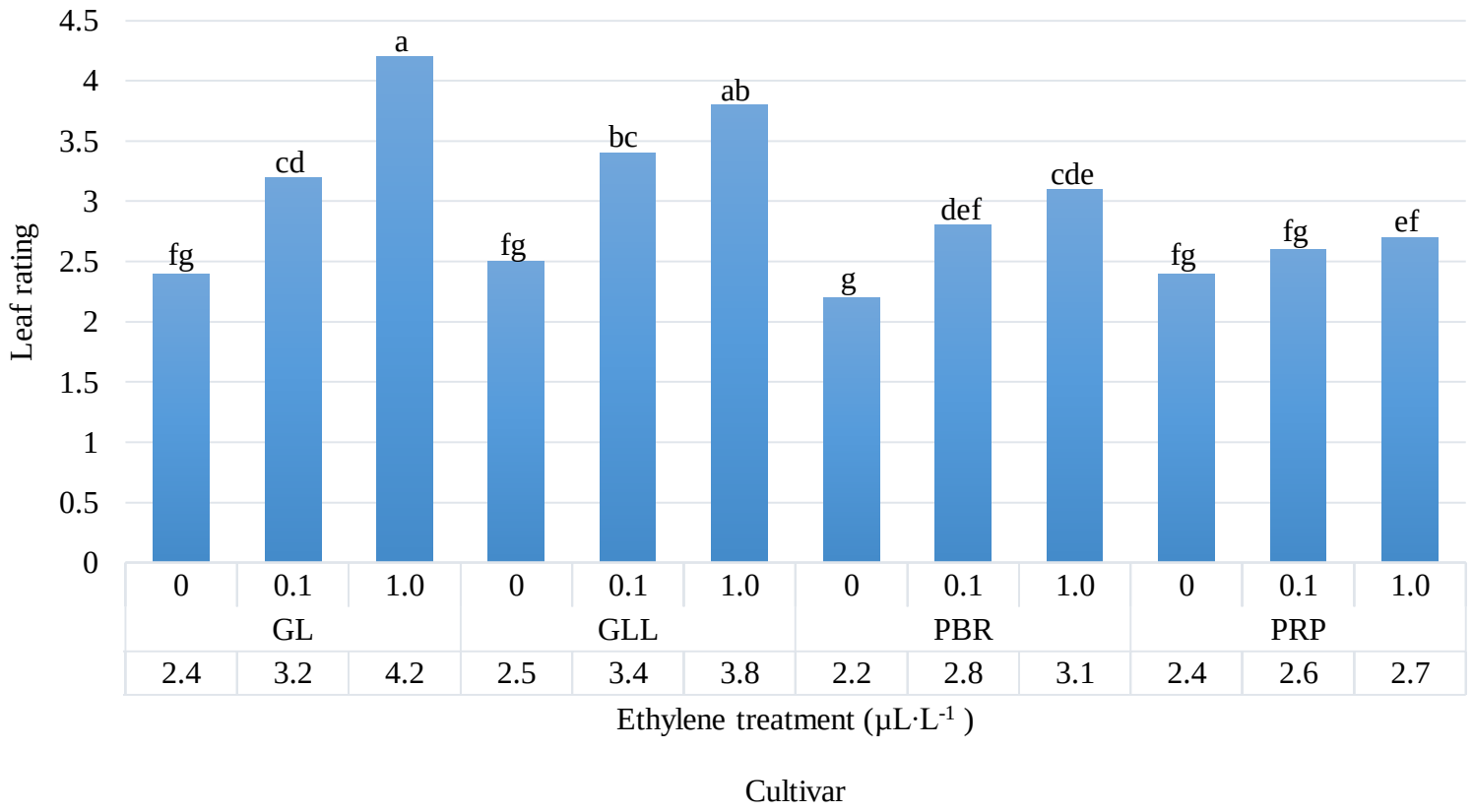

Fig. 6. Analysis of leaf ratings for Commercial - Dümmen Orange (2.2.2), seven days after treatment, of 'Great Balls of Fire Lavender' (GL), 'Great Balls of Fire Light Lavender' (GLL), 'Patriot Bright Red' (PBR), and 'Patriot Rose Pink' (PRP) in response for ethylene concentrations of 0, 0.1, and 1.0 uL·L^{-1}. Columns with the same letter are not significantly different according to Tukey's Studentized range test at $\alpha = 0.05$ ($P = 0.0055$).

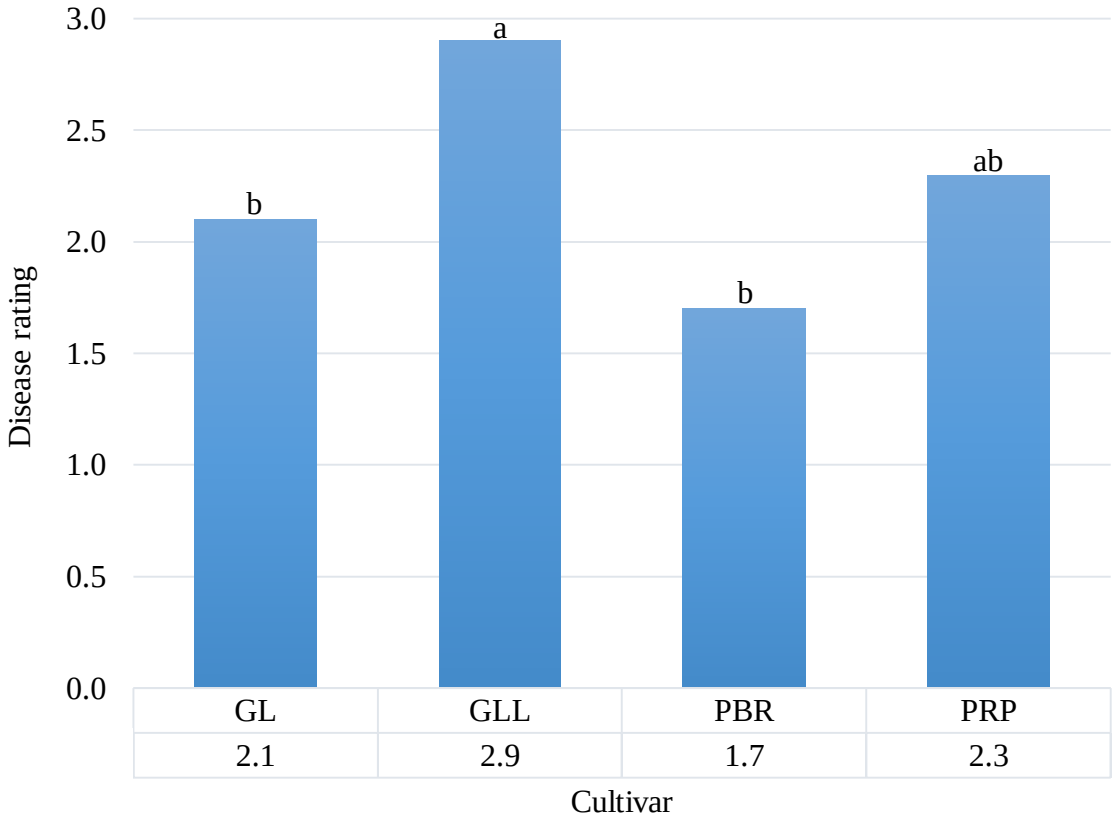

Fig. 7. Analysis of disease ratings caused by *B. cinerea* for Dümmen Orange (2.2.2) of 'Great Balls of Fire Lavender' (GL), 'Great Balls of Fire Light Lavender' (GLL), 'Patriot Bright Red' (PBR), and 'Patriot Rose Pink' (PRP) in response to being inoculated with 10^6 spores/mL. Columns with the same letter are not significantly different according to Tukey's Studentized range test at $\alpha = 0.05$, when looking at the interaction between all cultivars and treatments ($P = 0.0228$). Controls were not presented as they were significantly different than the inoculated cuttings, except on PBR.

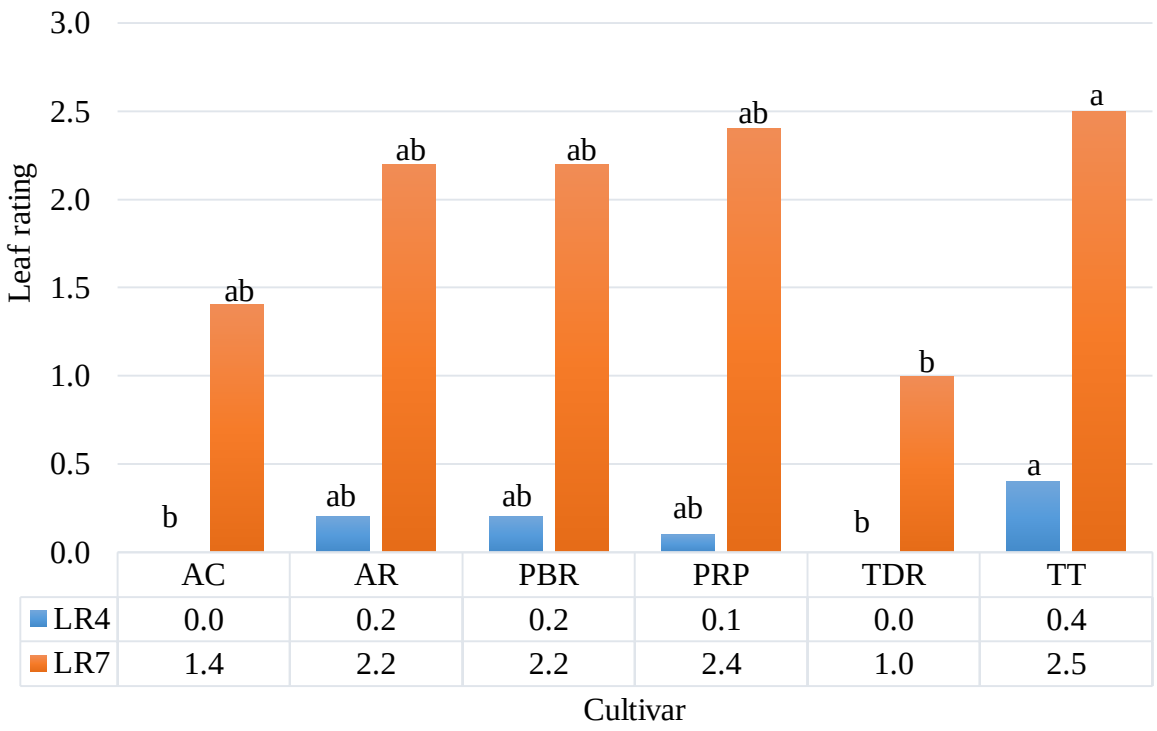

Fig. 8. Analysis of leaf ratings for In-house (2.2.4) 4 d after treatment (LR4) and leaf 7 d after treatment (LR7) of cultivars: ‘Americana Coral’ (AC), ‘Americana Red’ (AR), ‘Patriot Bright Red’ (PBR), ‘Patriot Rose Pink’ (PRP), ‘Tango Dark Red’ (TDR), and ‘Tango Tango’ (TT). LR4 columns with the same letter are not significantly different according to Tukey’s Studentized range test at $\alpha = 0.05$ ($P = 0.0063$) and LR7 columns with the same letter are not significantly different according to Tukey’s Studentized range test at $\alpha = 0.05$ ($P = 0.0145$).

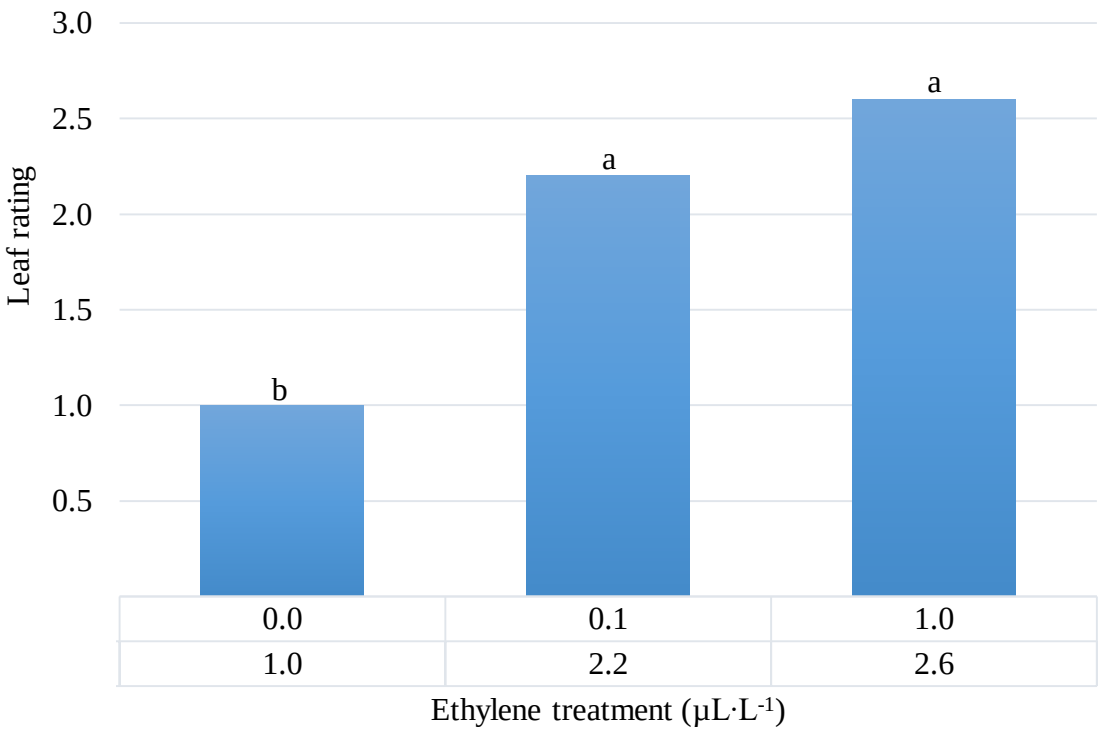

Fig. 9. Analysis of leaf ratings for In-house (2.2.4) across cultivars when treated with 0, 0.1, or 1.0 µL·L^{-1} ET. Columns with the same letter are not significantly different according to Tukey's Studentized range test at $\alpha = 0.05$ ($P = 0.0002$).

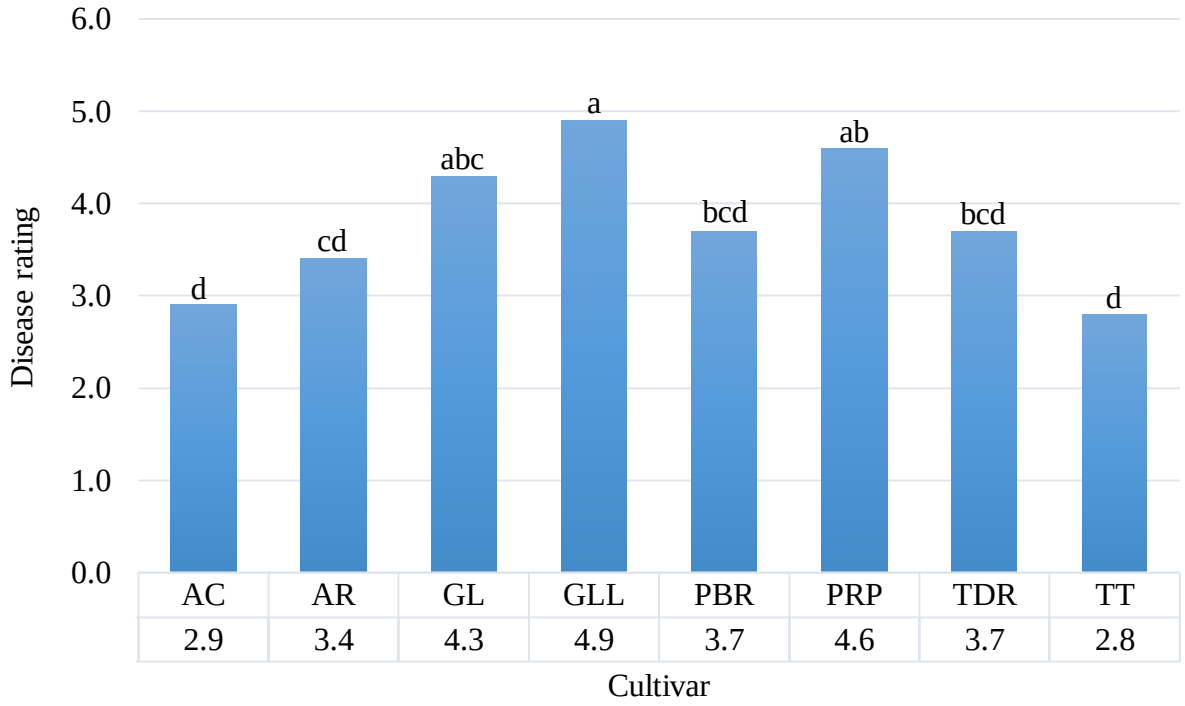

Fig. 10. Analysis of disease ratings caused by *B. cinerea* for In-house (2.2.4) of cultivars: 'Americana Coral' (AC), 'Americana Red' (AR), 'Great Balls of Fire Lavender' (GL), 'Great Balls of Fire Light Lavender' (GLL), 'Patriot Bright Red' (PBR), 'Patriot Rose Pink' (PRP), 'Tango Dark Red' (TDR), and 'Tango Tango' (TT). Columns with the same letter are not significantly different according to Tukey's Studentized range test at $\alpha = 0.05$ ($P<0.0001$).

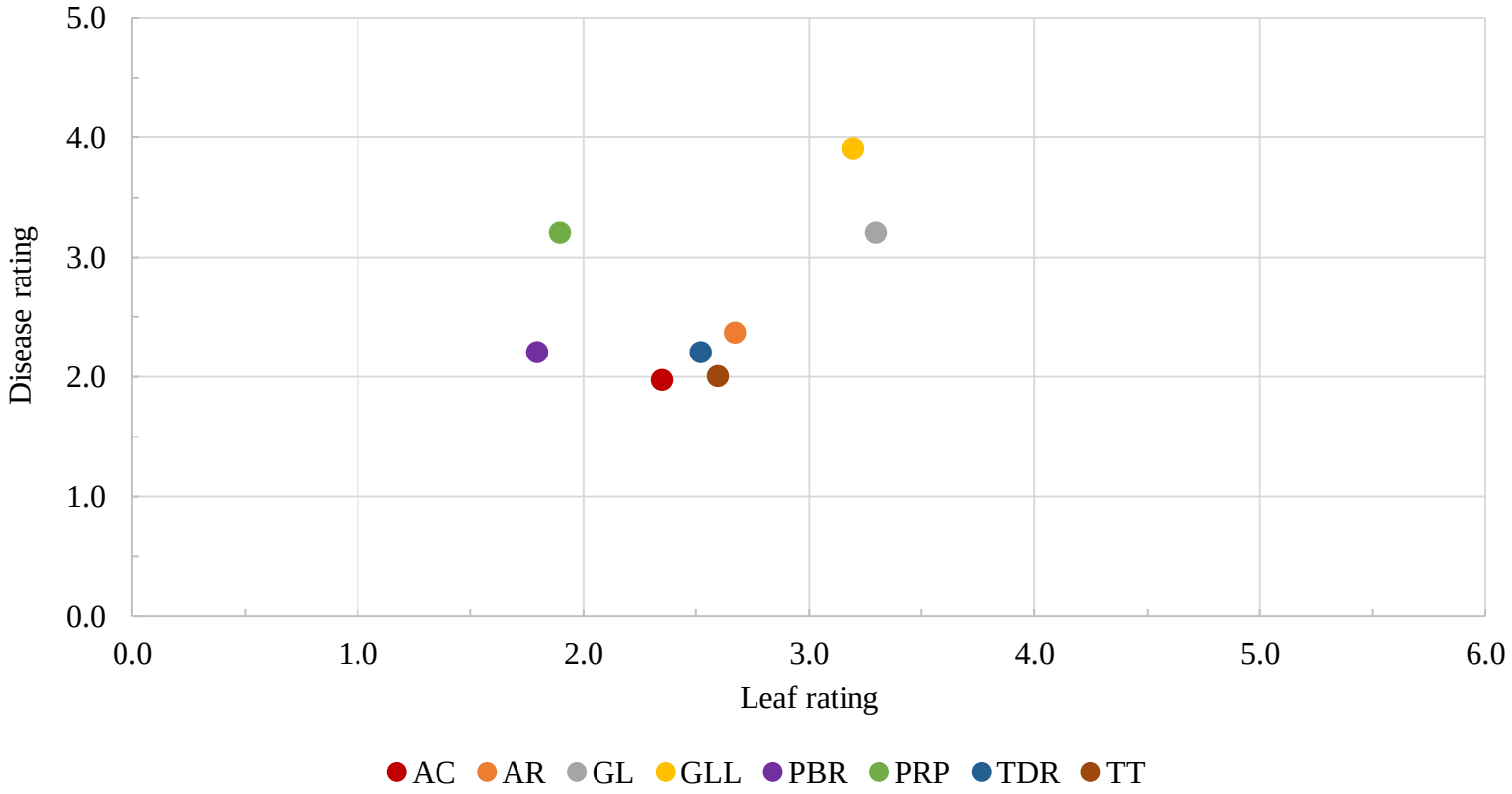

Fig. 11. Scatter plot showing the relationship between cultivars' leaf ratings (averaged across experiments for leaf ratings 7 d after treatment) and disease ratings in response to *B. cinerea*. Cultivars shown are 'Americana Coral' (AC), 'Americana Red' (AR), 'Great Balls of Fire Lavender' (GL), 'Great Balls of Fire Light Lavender' (GLL), 'Patriot Bright Red' (PBR), 'Patriot Rose Pink' (PRP), 'Tango Dark Red' (TDR), and 'Tango Tango' (TT).

CH. 3: Effects of ethylene and 1-methylcyclopropene on disease severity

Nathan J. Jahnke[a,]*, John M. Dole[a], and H. David Shew[b]

[a]Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609, USA

[b]Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695-7609, USA

*Corresponding author at: Department of Horticultural Science, Campus Box 7609, North Carolina State University, Raleigh, NC 27695-7609, USA. E-mail address: njjahnke@ncsu.edu.

Abstract

Four cultivars of *Pelargonium* × *hortorum* were used to quantify ethylene production, leaf yellowing and susceptibility to *Botrytis cinerea* when unrooted cuttings were treated with ethylene (ET) or 1-methylcycopropene (1-MCP). Treating cuttings with 1-MCP resulted in a significant increase in ethylene production over a 96 h incubation period. Cultivars did not differ in ET production when treated with 1 $\mu L \cdot L^{-1}$ ET, but when treated with 1-MCP 'Patriot Rose Pink' and 'Patriot Bright Red' produced concentrations of 1.76 to 2.41 $\mu L \cdot mL^{-1}$ while 'Americana Red' only produced 1.20 to 1.56 $\mu L \cdot mL^{-1}$ ET. TDR produced ethylene levels much higher than any other cultivars when treated with 1-MCP. Interestingly, ET pretreatments did not increase leaf yellowing compared to controls, but applications of 1-MCP reduced leaf yellowing compared to controls. A pre-treatment of 1.0 $\mu L \cdot L^{-1}$ ET increased disease ratings for all cultivars, while 1-MCP produced ratings equal to the control. 'Patriot Rose Pink' was the most susceptible cultivar while 'Americana Red' and 'Tango Dark Red' were the least susceptible when inoculated with a concentration of 10^5 and 10^6 spores/mL of *B. cinerea*. Disease ratings on cuttings kept dry were low after the 4-d incubation period, but the development of small lesions

on uninoculated cuttings indicated that cuttings are shipped with latent infections by *B. cinerea*. These small lesions can go unnoticed, and have potential to develop given enough time and favorable environmental conditions. There is not a clear relationship between ethylene production, leaf yellowing and susceptibility to *B. cinerea* for the cultivars tested. Quantitative cultivar resistance and 1-MCP was not effective at limiting *disease* development in this experiment, and is not recommended to control *Botrytis* diseases when shipping unrooted cuttings. Control of cutting susceptibility to *B. cinerea* and yellowing remains heavily reliant on stock plant culture and shipping conditions.

3.1 Introduction

Shoot tip cuttings are cost and time efficient means used by the horticulture industry to propagate specific plant varieties on a mass scale. Geranium (*Pelargonium* × *hortorum* L.H. Bailey) is one of the most popular floriculture crops and is prone to leaf yellowing and Botrytis blight during shipping, making healthy transplant and production challenging (USDA, 2016; Faust et al., 2006). Usually, growers control *B. cinerea* through an integrated approach using cultural, physical, and chemical means such as leaf removal, air movement, reducing leaf wetness, and fungicides (Elad et al., 2007; Williamson et al., 2007). However, these methods are difficult to implement when unrooted cuttings are grown and shipped to rooting stations and growers in cardboard containers, which takes 2 – 3 days. During this time temperature can vary, and relative humidity is high. Cuttings most likely have some latent infections that have the potential to expand under the right conditions. If shipments are delayed cuttings can be rendered unusable due to disease, which delays production schedules due to filing claims and waiting for new material. Ethylene (ET) concentrations can also increase to detrimental levels in boxes as plants produce it throughout development causing increased yellowing before and after

propagation (Faust et al., 2011; Gibson et al., 2000). Leaf yellowing can lead to more disease development during propagation because leaf tissue is weakened and easier to infect (Elad et al., 2004). To mitigate disease caused by *B. cinerea*, growers take extra time and labor to remove any yellowing leaves.

Defense mechanisms and induced resistance have become topics of disease research as fungicide resistance has become more common in populations of *B. cinerea* (Rosslenbroich and Stuebler, 2000; Williamson et al., 2007). Ethylene along with methyl jasmonate and salicylic acid play roles in defense pathways (Diaz et al., 2002; Thomma et al., 1999). Thomma et al. (1999) reported that turning off ethylene sensitive genes made *Arabidopsis* plants more susceptible to *B. cinerea*. Other research reports ethylene insensitive mutants of soybean (*Glycine max*) had increased susceptibility to *Septoria glycines* and *Rhizoctonia solani* but the opposite was observed when challenged with *Pseudomonas syringae* and *Phytophthora sojae* (Hoffman et al., 1999). Biologically, growers question whether ethylene sensitivity of geranium cultivars', often connected with leaf yellowing, is correlated with susceptibility to *B. cinerea*.

Anti-ethylene agents such as silver thiosulfate and 1-methylcyclopropene (1-MCP) are commonly used to prevent ET effects on a variety of floriculture crops and horticulture produce Blankenship and Dole, 2003). Because ET-sensitive mutants have not reacted the same way to different fungal pathogens, there is potential for 1-MCP to also influence *Botrytis* disease levels on geraniums (Hoffman et al., 1999; Thomma et al., 19999) 1-MCP has been shown to increase lesion expansion on tomato plants (Diaz et al., 2002). 1-MCP could decrease damage by keeping tissue physically healthy and green or increase damage because defense pathways reliant on ethylene and its precursors would be unable to made or function.

Previous research on geranium gave evidence that there is some quantitative resistance to *Botrytis*, but ploidy levels and detached leaf assays were the main principles of the experiments (Uchneat et al., 1999). Management practices have been heavily researched for greenhouse grown plants and fruit production, but there remains little a floriculture supplier can do during shipping and storage besides a pre-shipping fungicide application. Postharvest disease management is especially important with approximately 1.5 billion cuttings being shipped every year (M. Miller, unpublished data). The objective of this research was to determine how pretreatments of ET or 1-MCP affected susceptibility of unrooted cuttings of four *Pelargonium* × *hortorum* cultivars to *B. cinerea*.

3.2 Materials and Methods

Unrooted cuttings and handling

Cuttings of 'Americana Red' (AR), 'Patriot Bright Red' (PBR), 'Patriot Rose Pink' (PRP), and 'Tango Dark Red' (TDR) were received on 28 Nov. 2016 from commercial suppliers (Dümmen Orange, Columbus, Ohio, USA; Syngenta Flowers, Mexico) and placed at 4°C with moist paper towels to reduce moisture loss. On 29 Nov. cuttings were removed from the cooler at 8:00 AM and sorted by number of leaves and stem caliper. 45 groups of nine cuttings each. A second replication of this study was performed on 10 Jan. 2017.

A small sample of cuttings were harvested at 8:00 AM on 26 Nov. 2016 and 8 Jan. 2017, cuttings with 2 – 3 mature leaves of AR and TDR from stock plants started on 26 Jan. 2016 and from PBR and PRP from stock plants started on 2 Feb. 2016. Stock plants were hand watered alternating between 15.0-0-12.5 and 20.6-6.7-16.6 (Jack's 15-0-15 Dark Weather and 20-10-20 General Purpose water soluble fertilizer, JR Peters Inc., Allentown, Pa., USA) at 150 $mg \cdot L^{-1}$ N. Liquid flowable lime (Limestone F^{TM} Liquid Flowable Lime, Cleary Chemicals LLC, Alsip, Ill.)

was applied to increase substrate pH when needed. Shade cloth (Revolux XLS 15 Fire Retardant 50% shade cloth, Ludvig Svensson, Kinna, Sweden) covered the growing area near glass height of the greenhouse from mid-Apr. to mid-Sept. to reduce light intensity and air temperature. Cuttings were stored at 4°C until the arrival of cuttings received from suppliers. At 8:00 AM cuttings were removed and sorted by leaf number and stem caliper. Five groups of nine were then made using the sorted cuttings. These groups were only inoculated with the spore suspension of *B. cinerea* to compare the susceptibility of shipped in cuttings to cuttings produced at North Carolina State University (NCSU).

Pathogen culture

The isolate of *B. cinerea* (was obtained from M. Benson, Department of Plant Pathology, North Carolina State University. Cultures were started from a conidia stock suspension maintained at -80°C in a solution of 0.01% Tween 80 (Sigma-Aldrich, St. Louis, Mo., USA) and 15% glycerol (Sigma-Aldrich, St. Louis, Mo., USA). Cultures were grown to sporulation on oatmeal agar (Thermo Fisher Scientific, Lenexa, Kans., USA). Subsequent cultures were started by turning a sporulating plate upside down and taping it to drop spores onto a new plate. After 10 to 14 days, Petri dishes were covered in gray sporulating mycelium.

Spore collection

Approximately 15 mL of sterilized solution, containing 0.1% Tween 80 and 15% glycerol, was poured over a sporulating plate. A sterilized, bent glass stirring rod was used to dislodge conidia by rubbing the culture for approximately 5 min. Once the plate was completely black, the spore suspension was poured through five layers of sterilized cheesecloth to remove any hyphae. Each Petri dish was harvested from twice. A Neubauer improved hemacytometer (LW Scientific, Lawrenceville, Ga., USA) was used to quantify the number of spores/mL for the

resulting suspension. Suspensions were collected and stored in 50 mL polypropylene centrifuge tubes (Thermo Fisher Scientific, Lenexa, Kans., USA). Collected spore suspensions were stored at -80°C until used for inoculation.

Inoculation

Spore suspensions were removed from -80°C two hours before inoculation and tubes were allowed to thaw in a container of tap water. The suspension was diluted to 10^6 spores/mL using sterilized DI. The suspension was loaded into a hand sprayer and a group of three cuttings was thoroughly sprayed with approximately 20 mL to the point of glistening with spore suspension.

Germination verification

Viability of stored conidia was verified by plating 1 mL of suspension used for inoculation was fresh dishes of PDA (Thermo Fisher Scientific, Lenexa, Kans., USA). After four hours at room temperature, 100 spores were counted under ×100 (Nikon TMS-F Inverted Microscope, Nikon Instruments Inc., Melville, N.Y., USA). Spores with a definitive germination tube were counted as viable while spores without a germ tube were counted at nonviable giving an average 68% germination rate of spores used for inoculation.

Gas applications and ET sampling

Cuttings were subjected to 0, 700 $nL \cdot L^{-1}$ 1-MCP, or 1.0 $\mu L \cdot L^{-1}$ ET overnight (approximately 22 hours) in 210-L gas-tight barrels (v = 0.21 m^3). The barrel used to hold the untreated controls contained activated charcoal to remove ET and a fan for circulation. Both 1-MCP and ET barrels also had a fan to provide even distribution of ET. Concentrations applied were measured via gas chromatography (Shimadzu 8A; Shimadzu Corp., Kyoto, Japan) fitted with a glass column (Porapak Q, 80/100 mesh, 183 cm × 2.6 mm; Sigma Aldrich, Inc., St. Louis,

Mo., USA) running at 110°C injector, 130°C column, and 130°C detector (flame ionization) temperatures. Flow rates for the He carrier, H2 and O2 were 30, 16 and 90 $mL \cdot min^{-1}$, respectively. Injection volume was 1 mL of headspace gas drawn via a neoprene port on the jar lid. Standards of 1.0 $\mu L \cdot L^{-1}$ were run before, during, and after all sampling.

1-MCP was prepared by mixing 400 mg Ethylbloc (Floralife, Walterboro, S.C.) with 50 mL DI water heated to 30°C to provide a concentration of 700 $nL \cdot L^{-1}$. The beaker containing 1-MCP was then placed into its respective 210-L barrel and sealed. A fan was used to circulate the air inside the container.

Experimental design

The experiment was replicated twice, once on 28 Nov. 2016 and again on 11 Jan. 2017. There were a total of nine cuttings per replication (jar) with 5 replications per treatment combination. Treatments were arranged in a 4 × 3 × 3 factorial completely randomized design. Before the gas treatment phase groups of nine cuttings were paced in separate zip seal bags (16.3 cm × 8.2 cm) with three punched holes to mimic packing for air exchange of commercial shipments. Fifteen bags of each cultivar were placed in each barrel with either no treatment, 700 $nL \cdot L^{-1}$ 1-MCP or 1.0 $\mu L \cdot L^{-1}$ ET for 22 hours. After treatment, five groups of each cultivar from each gas treatment were either kept dry, sprayed with the spore carrier solution of 0.01% Tween 80 and 15% glycerol (contained no *Botrytis*), or sprayed with the *Botrytis* spore suspension for inoculation. Groups were then put back into bags and placed in 900 mL glass jars (v = 946.4 cm^3), with a moist paper towel. Jars were sealed and lids with a rubber septum were used for ET sampling. Jars were completely randomized on a lab bench top and covered with black plastic to simulate shipping conditions.

ET was measured at 24, 48 and 96 h. After the four-day incubation period cuttings were removed and each cutting was given a disease rating based on a 0 – 5 scale (Fig. 1): 0, 0% disease, no symptoms; 1, <10% disease or 1 - 10 small lesions; 2, 10 - 24% leaf necrosis or shoot necrosis; 3, 25 - 50% leaf necrosis or 10% leaf necrosis with shoot necrosis; 4, 51 - 75% leaf necrosis or 25% leaf necrosis with shoot necrosis; 5, 75 - 100% leaf necrosis with or without shoot necrosis. Each cutting was also rated for leaf yellowing using a 0 – 6 scale (Fig. 2) with 0, symptoms; 1, 10% marginal yellowing; 2, 11 - 25% yellow; 3, 26- 50% yellow; 4, 51 – 75% yellow; 5, 51 - 75% yellow; 6, dead or abscised. Run 1 used a concentration of 10^5 spores/mL while replicate 2 used 10^6 spores/mL, similar to the concentrations used in chapters 2 and 4.

Statistical analysis

Data were analyzed using SAS (Version 9.4, SAS Institute, Inc., Cary, N.C.). Mean separation for ET production, leaf ratings and disease ratings were determined using the Generalized Linear Mixed Models (GLIMMIX) and Tukey's Studentized range test procedures where $\alpha = 0.05$. Rep was nested within the interaction of cultivar, gas treatment and spray treatment. It was treated as the subject for the random residual for ET production and the subject for the random intercept for leaf and disease ratings to account for subsampling within jars. ET production measurement times of 24, 48 and 96 h were analyzed separately.

3.3 Results & Discussion

Ethylene production in response to Botrytis, ET and 1-MCP

Ethylene concentrations increased over the 96 h of incubation. After 24 h of incubation, PRP produced the most ET while TDR produced the least. A three-way interaction was significant among the cultivars, gas treatments and spray treatments ($P = 0.0029$). Cuttings treated with 1-MCP responded with more ET production compared to the ET treatment and

control (Fig. 3). Cuttings pre-treated with 1-MCP and then inoculated with *Botrytis* produced the most ethylene production in every cultivar, but only PBR and PRP had significantly higher levels when comparing treatments within the respected cultivar. AR and TDR were not significantly impacted by gas treatments. Treating cuttings with ET did not significantly increase or decrease ethylene production compared to the control. Cultivars also did not differ in ethylene production when untreated.

After 48 h, ET concentrations were similar to the 24 h readings and the spray treatment did not differ across cultivars. Cuttings treated with 1-MCP and then inoculated with *Botrytis*, produced an average of 1.82 $\mu L \cdot L^{-1}$ ethylene which was significantly higher compared to all other treatments (Fig. 4A). 1-MCP caused more ET production in all cultivars (Fig. 5A). PRP produced the most ET, followed by PBR, AR and TDR when treated with 1-MCP, but cultivars did not differ in ET production in response to any other treatments. TDR was the least responsive to the gas treatments as it was after 24 h.

At the final sampling, 96 h, overall trends stayed similar to concentrations at 48 h (Fig. 4B). Inoculated cuttings pre-treated with 1-MCP produced 2.42 $\mu L \cdot L^{-1}$ ET. Without a spray of the spore suspension of *B. cinerea*, 1-MCP only caused 1.08 $\mu L \cdot L^{-1}$ ET, which was not different from the other treatments. TDR was the least responsive, producing 0.64 $\mu L \cdot L^{-1}$ ET when treated with 1-MCP (Fig. 5B). TDR ET concentrations were significantly different from PBR and PRP, producing 0.64, 2.41 and 2.41 $\mu L \cdot L^{-1}$ ET, respectively. However, 0.64 $\mu L \cdot L^{-1}$ ET was not different from the 1.56 $\mu L \cdot L^{-1}$ ET produced by AR. Cuttings produced at NCSU had similar trends with PRP producing the most ET, followed by PBR and TDR, but AR produced the least amount of ET (data not shown).

Replicate 2

Cuttings treated with the control spray of the spore carrier solution were measured for ET production in this replication and at each time interval there was no difference between any of the sprays (Fig. 6A-C). Cultivars produced similar amounts of ET when left untreated or treated with 1.0 $\mu L \cdot L^{-1}$ ET. 1-MCP caused cuttings of each cultivar to produce more ethylene than ET treated or control cuttings like replicate 1. Aside from TDR, PBR and PRP produced more ET than AR when treated with 1-MCP at each time point. TDR produced a significant amount of ET at each time point when treated with 1-MCP, which made the concentrations measured for the other cultivars and treatments insignificant in relation to each other. It is unknown as to why TDR behaved differently to 1-MCP between replications of the experiment. Untreated and ET treated cuttings produced ET concentrations similar to the other cultivars.

The small sample of cuttings produced at NCSU responded similarly to cuttings received from suppliers, with PBR producing the most ethylene, followed by PRP, AR and lastly TDR (data not shown). Cuttings received from suppliers were all treated the same upon arrival at NCSU. Differences in stock plant or postharvest handling are the most likely candidates causing the differences of this replicate compared to the first replicate. Cuttings could have been stored before shipment or stock plants experienced some type of stress. These results indicate that even though cutting suppliers are producing good quality cuttings, the overall quality is not consistent between cultivars, which can make growers' jobs more difficult when multiple cultivars are all placed under the same conditions and expected to root by a specific day.

In conclusion, cultivars reacted similarly to 1-MCP by producing more ET and increasing the production as time increased. 1-MCP is known to cause increased ET production on many crops such as banana (*Musa* sp.) and geranium cuttings (Blankenship and Dole, 2003; Golding et al., 1998; Kadner and Druege, 2004). Aside from TDR, PBR and PRP produced more ET. ET

production in plants is controlled by negative and positive feedback receptors that sense ethylene in the atmosphere (Xu and Zhang, 2015). If this feedback is inhibited, then plants continue to produce ET as seen in the response of all of the cultivars tested and treated with 1-MCP. After 48 h, cuttings in these studies produced an average of 0.37 $\mu L \cdot L^{-1,}$ which is around the average time cuttings stay in shipping boxes. Many floriculture species are sensitive to concentrations as low as 0.1 $\mu L \cdot L^{-1}$, while 0.5 $\mu L \cdot L^{-1}$ has been shown to decrease geranium leaf chroma and chlorophyll content (Mutui et al., 2015). If shipping lasts four days due to a delay, ethylene concentrations could rise as high as 0.5 – 1.0 $\mu L \cdot L^{-1}$ ET. This amount of time in transit and concentration of ET could cause some marginal leaf yellowing and petiole curling.

Leaf yellowing and ethylene sensitivity

In replicate 1, the spray treatment significantly interacted with the gas pretreatment (P = 0.0023). 1-MCP was able to significantly reduce leaf ratings for the dry and solution controls from 2.26 and 3.30 to 1.51 and 1.41, respectively (Fig. 7). 1-MCP did not reduce leaf ratings for cuttings inoculated with *B. cinerea*, as leaf ratings were already low compared to the control and ethylene treatments. 1-MCP may not have been able to reduce leaf yellowing any further. Interestingly, the ET pretreatment did not significantly increase LR for any spray treatment as it is commonly known to cause leaf yellowing (Faust et al., 2011; Gibson et al., 2000). Cuttings pretreated with ET did not have significantly higher leaf ratings when compared to the untreated controls (Fig. 8A). When left untreated, PRP was significantly more susceptible to leaf yellowing than AR and TDR, but not PBR (P = 0.0445). 1-MCP was able to significantly reduce leaf ratings for AR, PBR and PRP by 1.2, 0.9 and 0.9, respectively, but only 0.5 for TDR. TDR did not show any differences between gas treatments.

Leaf ratings were higher in replicate 2, but cultivars followed the same trends (Fig. 8B). TDR leaf ratings were again barely responsive to gas treatments, although it showed the same slight increase when treated with ET and decrease when treated with 1-MCP as seen in replicate 1. Cultivars did not differ in leaf ratings when left untreated or treated with ET. Compared to the untreated control, 1-MCP reduced leaf ratings for AR by 1.6, 1.1 for PBR and 0.8 for PRP. TDR was unaffected by gas treatments, and 1-MCP was not able to reduce leaf ratings for TDR compared to the control. Although it was not significant, the ET increased LR in all cultivars except PBR. TDR was again unaffected by spray treatments, while the spore carrier solution had significantly higher LR than the untreated controls in PBR and PRP (Fig. 9). The Tween 80 or glycerol within the control solution may have degraded leaves' waxy cuticle pre-disposing them to yellowing. TDR may not have experienced the increased yellowing because it naturally had high values of yellowing.

There may be a slight connection to a cultivar's ethylene production and leaf yellowing. When looking at the untreated controls cultivars that produced lower amounts of ethylene by 96 h, for example AR and TDR, both had lower leaf ratings. PBR and PRP usually produced more ethylene than AR and TDR, but did not have higher LR in replicate 2. Because of the inconsistency of the response between replications, it appears that cultivar responses are partially determined by shipping conditions and stock plant culture. Across both replicates, 1-MCP was most effective in reducing LR in AR, but still effective on PBR and PRP. Kadner and Druege (2004) also found 1-MCP to be partially effective in reducing leaf yellowing of geraniums.

Botrytis susceptibility

In both replicates, cuttings pretreated with ET had significantly higher ratings than the control and 1-MCP treated cuttings (Fig. 10). 1-MCP caused cuttings to have lower ratings than

the control, but the difference was not significant. The resulting differences in disease ratings among treatments were not clearly visible or differentiable using the rating scale developed for these experiments. Counting the number of lesions or measuring lesion sizes may be better for quantifying resistance. The measured differences were also not likely a significant concern for growers, because having any *Botrytis* on plant material is potential for an outbreak and loss. The differences seen may be important for cultivars that show high ethylene sensitivity, because a pretreatment of ET increased disease ratings in this study. An application of ET after inoculation may simulate shipping and delayed shipments better as cuttings would naturally go into the shipping process with latent *Botrytis* and then experience elevated concentrations of ET. 1-MCP did not have a strong enough effect to prevent *Botrytis* development on a commercial scale. If cuttings were stored for longer than four days that might change, but it is unlikely that cuttings would maintain other quality traits need for successful propagation if shipments should take longer than 4 days. In addition, the beneficial effects against *Botrytis* may not be justifiable as 1-MCP can sometimes have adverse effects on rooting and increased ET production (Serek et al., 1998; Blankenship and Dole, 2003). ET may have made cuttings more susceptible to *B. cinerea*. As a hormone, ET promotes maturation and weakens tissue. The necrotrophic pathogen, *B. cinerea*, could then easily infect when plant defenses are poor. Sirjusingh and Sutton (1996) reported that naturally, old (>10 weeks) and young (<4 weeks) geranium leaves are more susceptible to infection. Cuttings usually contain 2 – 4 leaves, 2 of which are usually mature meaning they are around 4 weeks old. In these experiments, immature apical leaves were often observed to yellow more and become infected before mature leaves.

Cultivar genetics and regulation of defense pathways are areas where ethylene and 1-MCP could interact with *Botrytis* susceptibility. However, these data do not support ethylene's

role in defense induction as with tomato (Diaz et al. 2002), because disease ratings increased with a pre-treatment of ET. It does not mean ET does not play a role in defense pathways. Making some plants ET insensitive through genetic modification has made them more susceptible to *B. cinerea* (Thomma et al., 1999; Zhao et al., 2012). Ethylene increased disease in these experiments, but silencing genes may provide similar or better resistance than seen with 1-MCP. Concentrations lower than 1.0 $\mu L \cdot L^{-1}$ ET may be more effective on the defense pathway than high concentrations which can cause damage to leaf tissue.

Inoculating with *B. cinerea* did not significantly impact disease ratings for AR and TDR in both replicates (Fig. 11). This means that these two cultivars were able to resist the extra damage that should have developed by the addition of more inoculum. TDR developed higher disease ratings in replicate 2 than replicate 1 (Fig. 11B), but because the inoculation treatment did not increase disease ratings, this cultivar would still be noted to have some resistance. Stock plants may not have been as clean or cuttings may have been stored before shipping making them more susceptible to *Botrytis*. In replicate 1, PRP was the most susceptible with a rating of 1.2 (Fig. 11A). Excluding the solution treated TDR in replicate 2, PRP and PBR were the most susceptible with ratings of 1.79 and 2.19, respectively. Overall, AR was the least susceptible.

It could be argued that cuttings of TDR and AR were healthier and less stressed than PBR and PRP and that suppliers caused the difference seen in the data. The Patriot series came from Syngenta Flowers while AR and TDR came from Dümmen Orange. It is also possible production conditions differed over time between cultivars from the same company as seen in TDR and AR, between the two replicates. TDR had much higher ratings in the second replicate, while AR had lower ratings. Disease that developed from inoculum present on dry cuttings was close to levels produced by inoculation. Botrytis is known to lie dormant until conditions are perfect for growth

and development, even after surface sterilization (Verhoeff, 1974; Elad, 1988). A concentration of 10^6 spores/mL (*Botrytis* treatment) produced higher ratings in experiment 2 compared to the 10^5 concentration used in replicate 1, but damage on the dry cuttings was also higher. A higher concentration of spores or ratings cuttings over time until death may have better represented susceptibility to *B. cinerea* and its development over time. This research rated disease caused by *B. cinerea* after 96 h of incubation to describe *Botrytis* development in terms of a delayed shipment and along enough time to allow disease development.

The control, spore carrier "solution", caused some variation in disease ratings. The solution caused higher disease ratings in PBR in replicate 1 and TDR in replicate 2. Overall, the solution control usually caused higher disease ratings than the dry control, but not higher than inoculated cuttings, which was its purpose as a control with no spores. The spore carrier solution as a control most likely caused higher disease ratings compared to the dry control and sometimes the *Botrytis* inoculation treatment, because the concentration of Tween 80 and glycerol was not diluted as it was in the suspension of *B. cinerea* spores used for inoculation. The higher concentration of Tween 80 and glycerol caused a longer duration of leaf wetness. It is unknown why the solution treatment was not consistent among the replicates and cultivar as it was the same concentration in each replicate. The solution treatment was also inconsistent for ET production and LR as it would sometimes cause more ET production and higher leaf ratings than the control and inoculation treatments, and sometimes less.

Botrytis and leaf yellowing susceptibility correlation

The resulting data from these experiments form an argument that cultivar susceptibility to *B. cinerea* does not seem to be related to a cultivar's specific ET production or LR, based on yellowing. However, it is clear that when cultivars were exposed to ET before being inoculated,

disease was more severe. This would infer that if a cultivar is more sensitive to the effects of ethylene then it could be more susceptible. Leaf yellowing may not be the best response to measure to determine geranium's ethylene sensitivity. Triple response assays on seed geraniums have proven to be effective on measuring ethylene sensitivity (Hoffman et al., 1999), but this is not an option for cultivars produced as vegetative cuttings. Genomic tools such as measuring gene activity may be better suited to determine ethylene's role in defense pathway and susceptibility in geranium. 1-MCP is helpful in decreasing leaf yellowing, but that effect is not enough to justify its use to control *Botrytis* diseases as it does not have a significant impact on disease ratings and it can negatively affect rooting.

The amount of disease that developed on dry cuttings is cause for concern. If cuttings are delayed longer than 4 days there could be substantial disease development, which could be magnified by the temperatures and humidity cuttings experience in transit. This research did not look at the outcome of planting diseased cuttings over time, but determining how much latent infection is present or level of loss that occurs when planting diseased plants would be beneficial for growers and suppliers to gauge their effectiveness of control of *B. cinerea*. If growers plant cuttings without noticing small lesions, it could result in disease outbreaks, variable rooting, survival, and performance later down the production line.

There are some clear differences in *Botrytis* susceptibility and resistance between cultivars. Whether that resistance can be tapped into and increased is unknown and warrants further research as fungicide resistance continues to appear in *Botrytis* populations (Rosslenbroich and Stuebler, 2000; Elad, 2004). The determining factor in *Botrytis* development in this study was the inoculum that developed on untreated cuttings. Suppliers and growers need to keep stock and cuttings *Botrytis* free before shipping. There is also a need for some type of

protectant that would be effective for the duration of shipping, whether those cuttings experience variable temperatures or remain in a box for 2 days.

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Figures

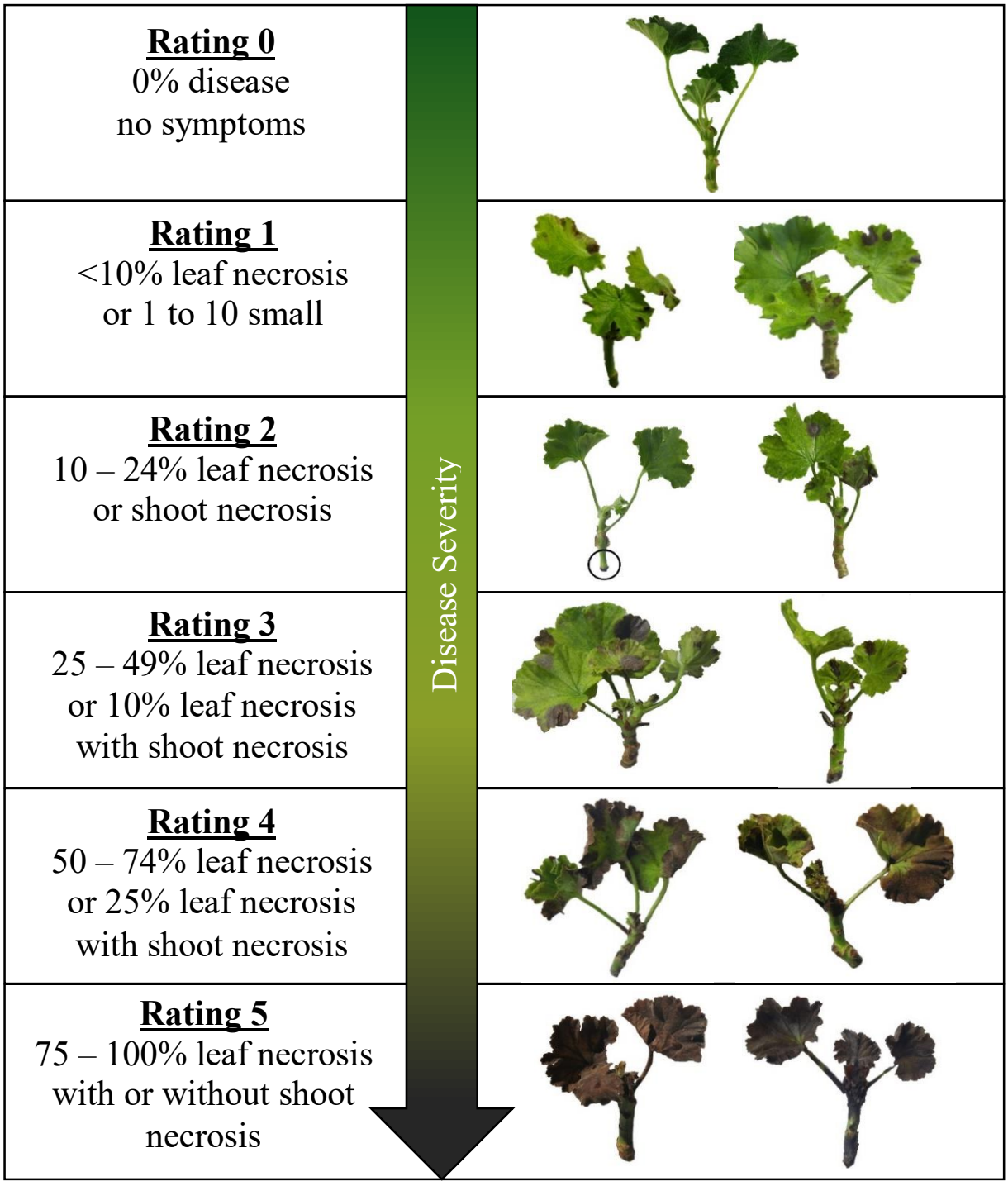


Fig. 1. Disease rating scale from 0 to 6 for unrooted cuttings of *Pelargonium* based on necrotic leaf area and shoot necrosis.

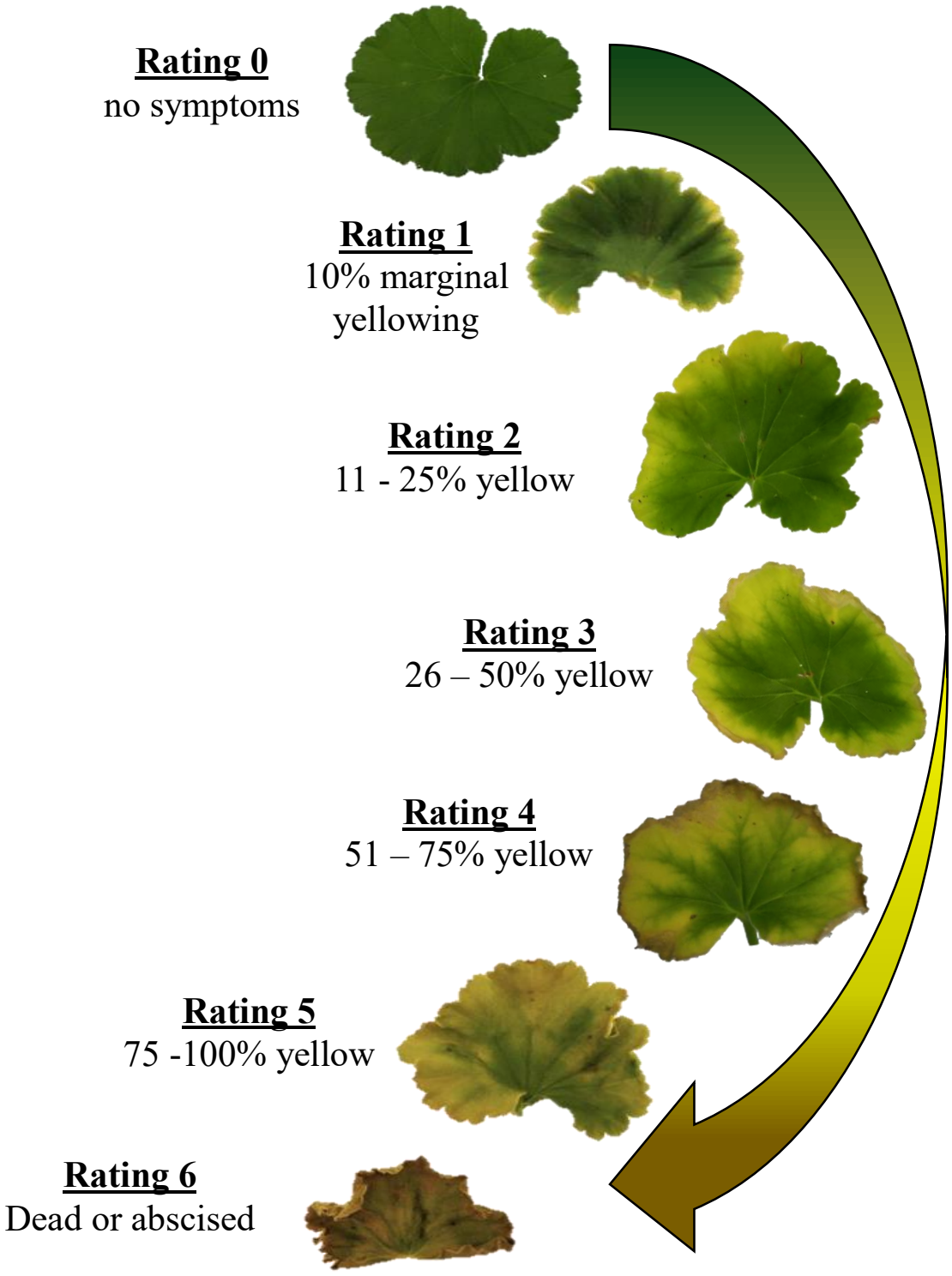
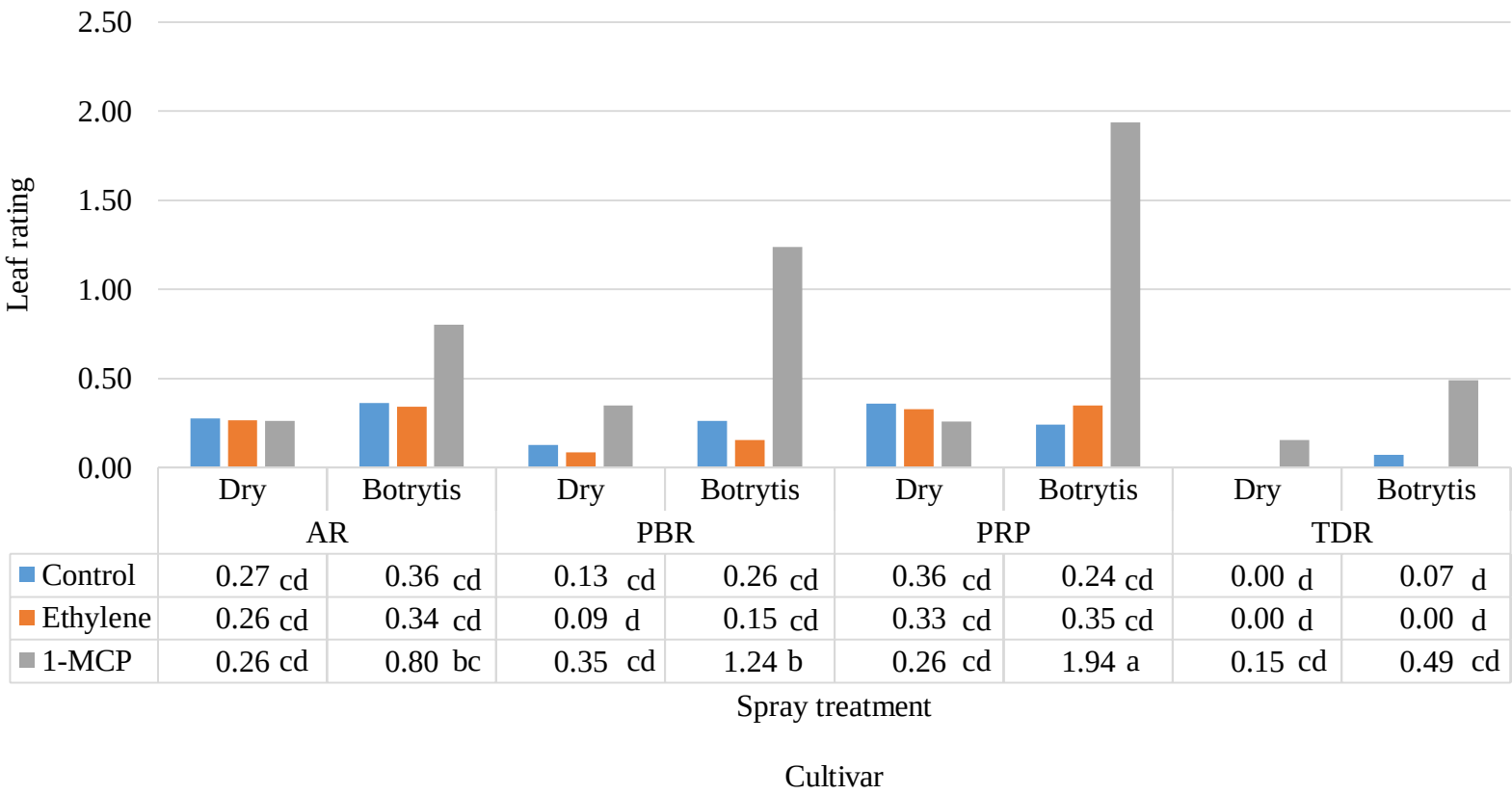


Fig. 2. Leaf yellowing ratings scale from 0 to 6 for unrooted cuttings of *Pelargonium* based on leaf yellowing.



	AR		PBR		PRP		TDR	
	Dry	Botrytis	Dry	Botrytis	Dry	Botrytis	Dry	Botrytis
Control	0.27 cd	0.36 cd	0.13 cd	0.26 cd	0.36 cd	0.24 cd	0.00 d	0.07 d
Ethylene	0.26 cd	0.34 cd	0.09 d	0.15 cd	0.33 cd	0.35 cd	0.00 d	0.00 d
1-MCP	0.26 cd	0.80 bc	0.35 cd	1.24 b	0.26 cd	1.94 a	0.15 cd	0.49 cd

Fig. 3. Replicate 1 ethylene production ($\mu L \cdot L^{-1}$) after 24 h incubation off of cultivars 'Americana Red' (AR), 'Patriot Bright Red' (PBR), 'Patriot Rose Pink' (PRP), and 'Tango Dark Red' (TDR) with no treatment (control) or treated with 1.0 $\mu L \cdot L^{-1}$ ethylene, or 700 $nL \cdot L^{-1}$ 1-methylcyclopropene (1-MCP), and then either kept dry or inoculated with *Botrytis* spray of 10^5 spores/mL. Means with the same letter are not significantly different according to Tukey's Studentized range test at $\alpha = 0.05$ ($P = 0.0029$).

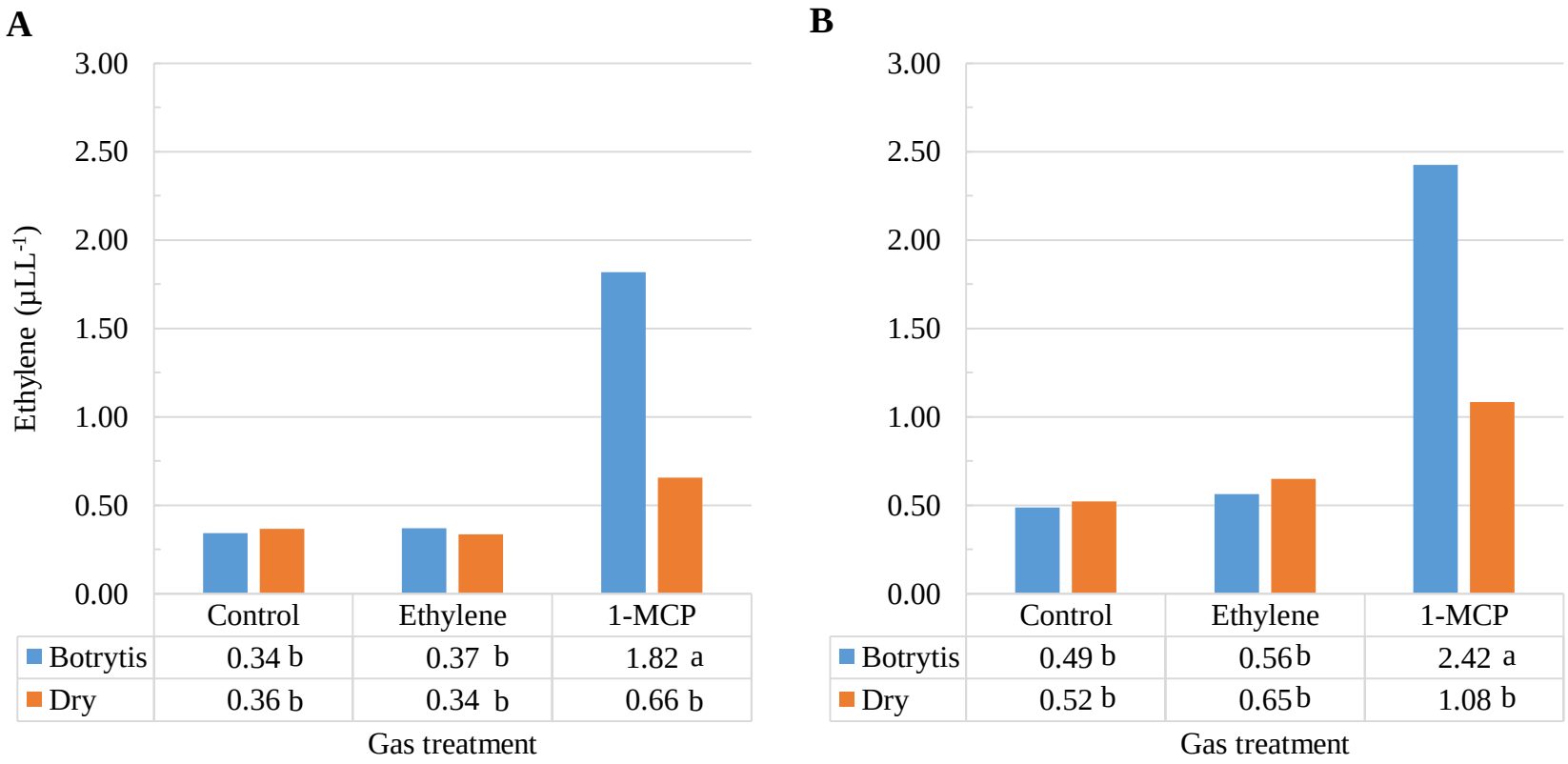


Fig. 4A-B. Replicate 1 ethylene production ($\mu L \cdot L^{-1}$) after 48 h (A) and 96 h (B) of incubation off of cuttings with no pre-treatment (control) or pre-treated with 1.0 $\mu L \cdot L^{-1}$ ethylene, or 700 $nL \cdot L^{-1}$ 1-methylcyclopropene (1-MCP) and then either kept dry or inoculated with a *Botrytis* spray of 10^5 spores/mL. Means with the same letter for each time are not significantly different according to Tukey's Studentized range test at $\alpha = 0.05$ ($P<0.0001$).

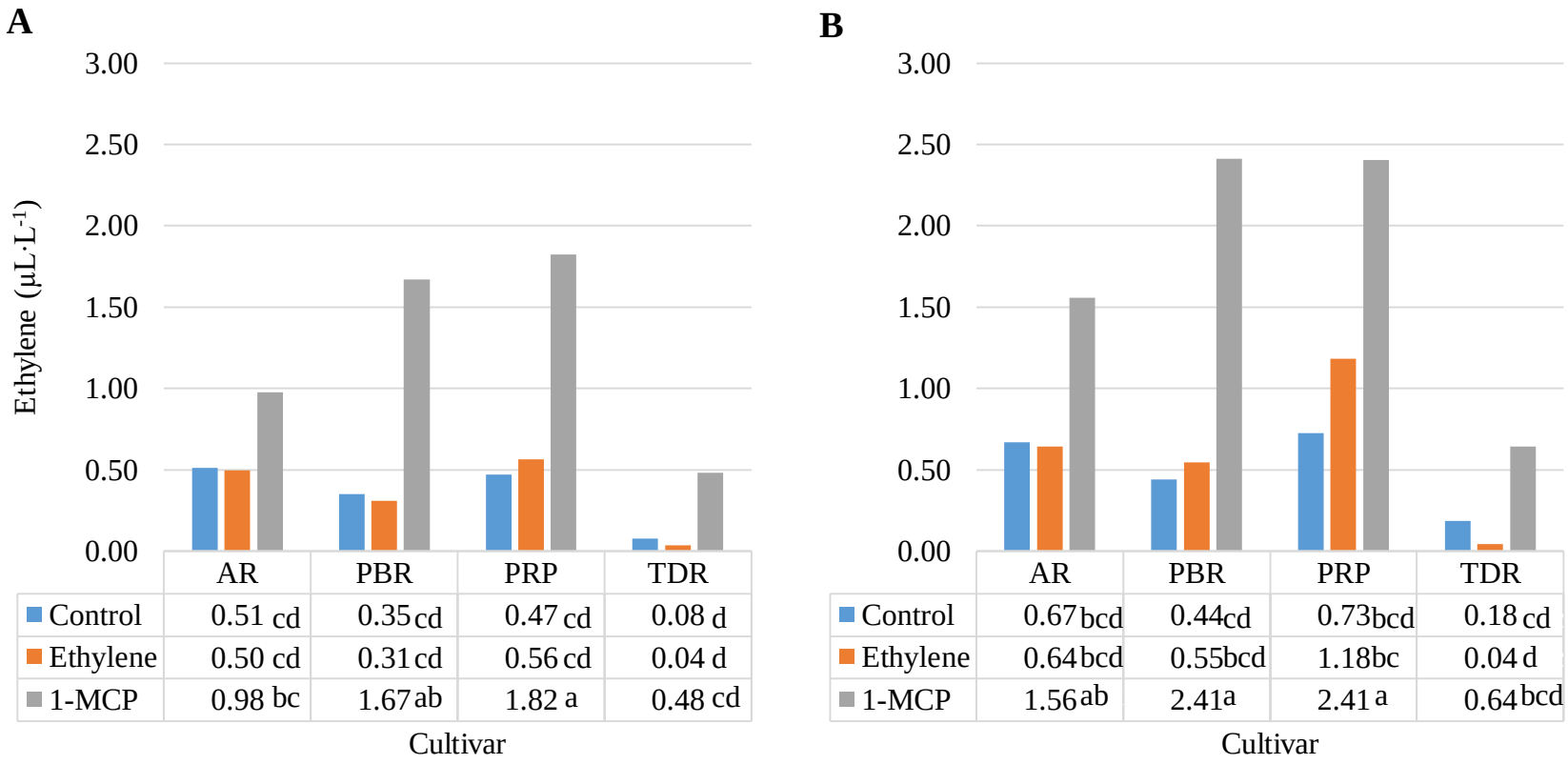
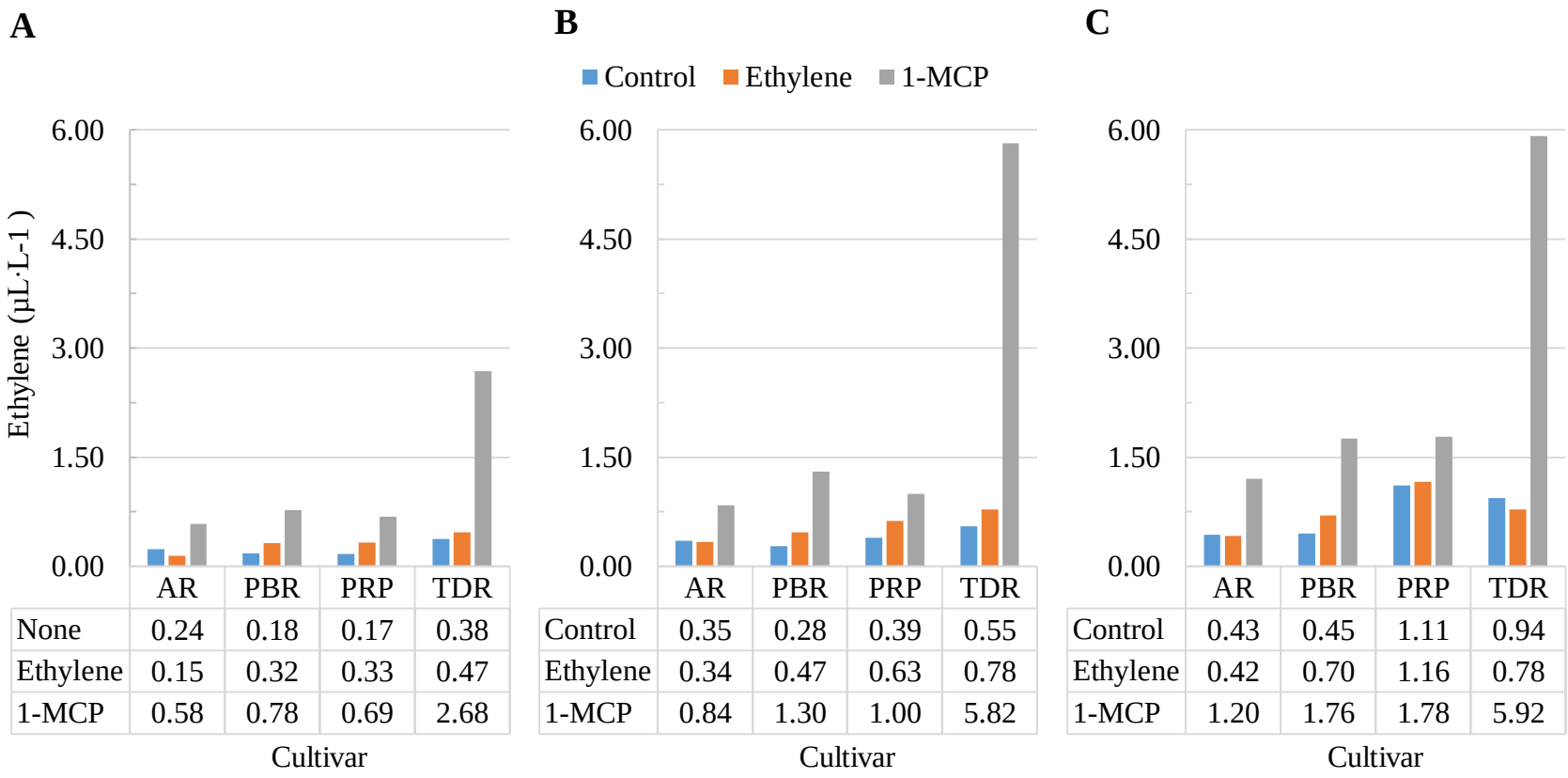


Fig. 5A-B. Replicate 1 ethylene production (µL·L^{-1}) of cultivars 'Americana Red' (AR), 'Patriot Bright Red' (PBR), 'Patriot Rose Pink' (PRP), and 'Tango Dark Red' (TDR) after a 48 h (A) and 96 h (B) incubation off of cuttings with no pre-treatment (control) or treated with 1.0 µL·L^{-1} ethylene, or 700 nL·L^{-1} 1-methylcyclopropene (1-MCP). Means with the same letter for each time are not significantly different according to Tukey's Studentized range test at $\alpha = 0.05$ ($P = 0.0071$ and 0.0264 for 48 and 96 h, respectively).



	AR	PBR	PRP	TDR
None	0.24	0.18	0.17	0.38
Ethylene	0.15	0.32	0.33	0.47
1-MCP	0.58	0.78	0.69	2.68

	AR	PBR	PRP	TDR
Control	0.35	0.28	0.39	0.55
Ethylene	0.34	0.47	0.63	0.78
1-MCP	0.84	1.30	1.00	5.82

	AR	PBR	PRP	TDR
Control	0.43	0.45	1.11	0.94
Ethylene	0.42	0.70	1.16	0.78
1-MCP	1.20	1.76	1.78	5.92

Fig. 6A-C. Replicate 2 ethylene production (µL·L^{-1}) of cultivars 'Americana Red' (AR), 'Patriot Bright Red (PBR), 'Patriot Rose Pink' (PRP), and 'Tango Dark Red' (TDR) after 24 (A), 48 (B), and 96 h (C) of incubation off of cuttings with no treatment (control) or treated with 1.0 µL·L^{-1} ethylene, or 700 nL·L^{-1} 1-methylcyclopropene (1-MCP). Means with the same letter for each time are not significantly different according to Tukey's Studentized range test at $\alpha = 0.05$ ($P = 0.0007$, <0.0001, and 0.0002 for 24, 48 and 96 h, respectively).

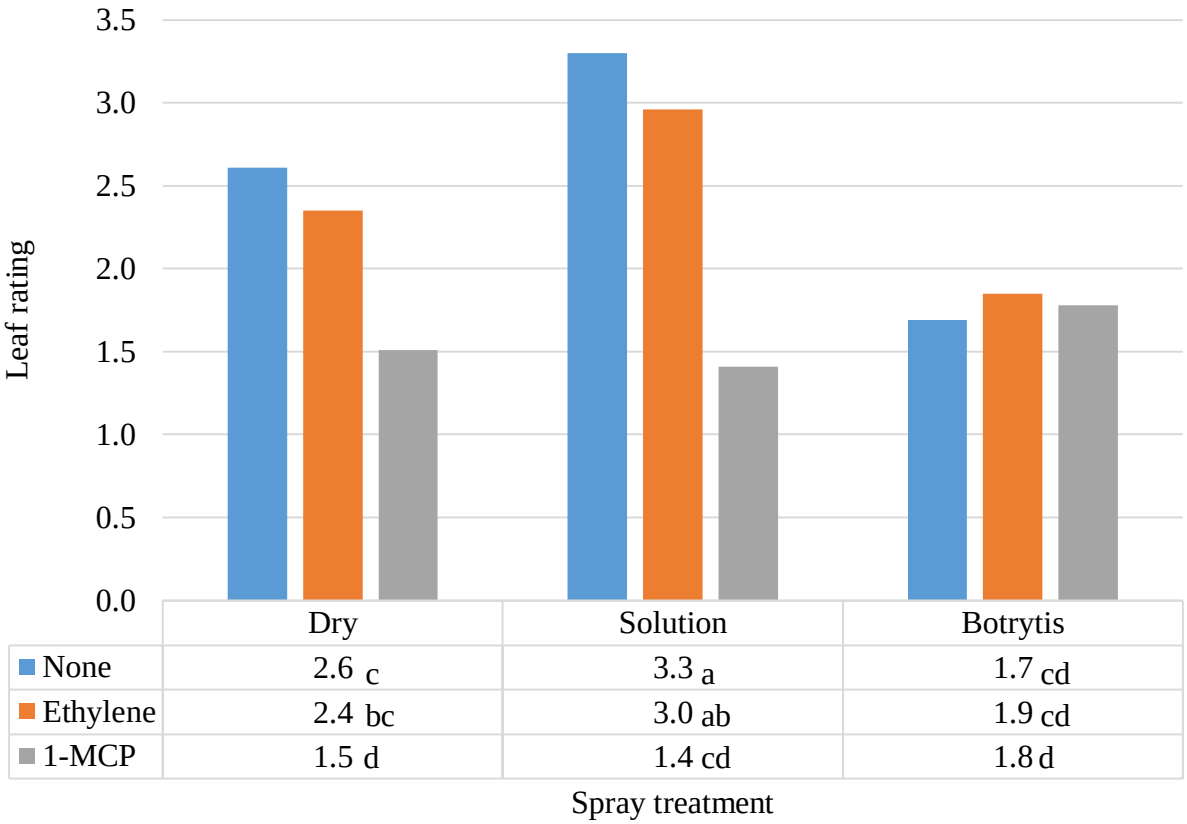


Fig. 7. Replicate 1 leaf ratings for cuttings with no pre-treatment (control) or treated with 1.0 $\mu L \cdot L^{-1}$ ethylene (ET), or 700 $nL \cdot L^{-1}$ 1-methylcyclopropene (1-MCP) then either kept dry or inoculated with *Botrytis* spray of 10^5 spores/mL. Means with the same letter are not significantly different according to Tukey's Studentized range test at $\alpha = 0.05$ ($P = 0.0023$).

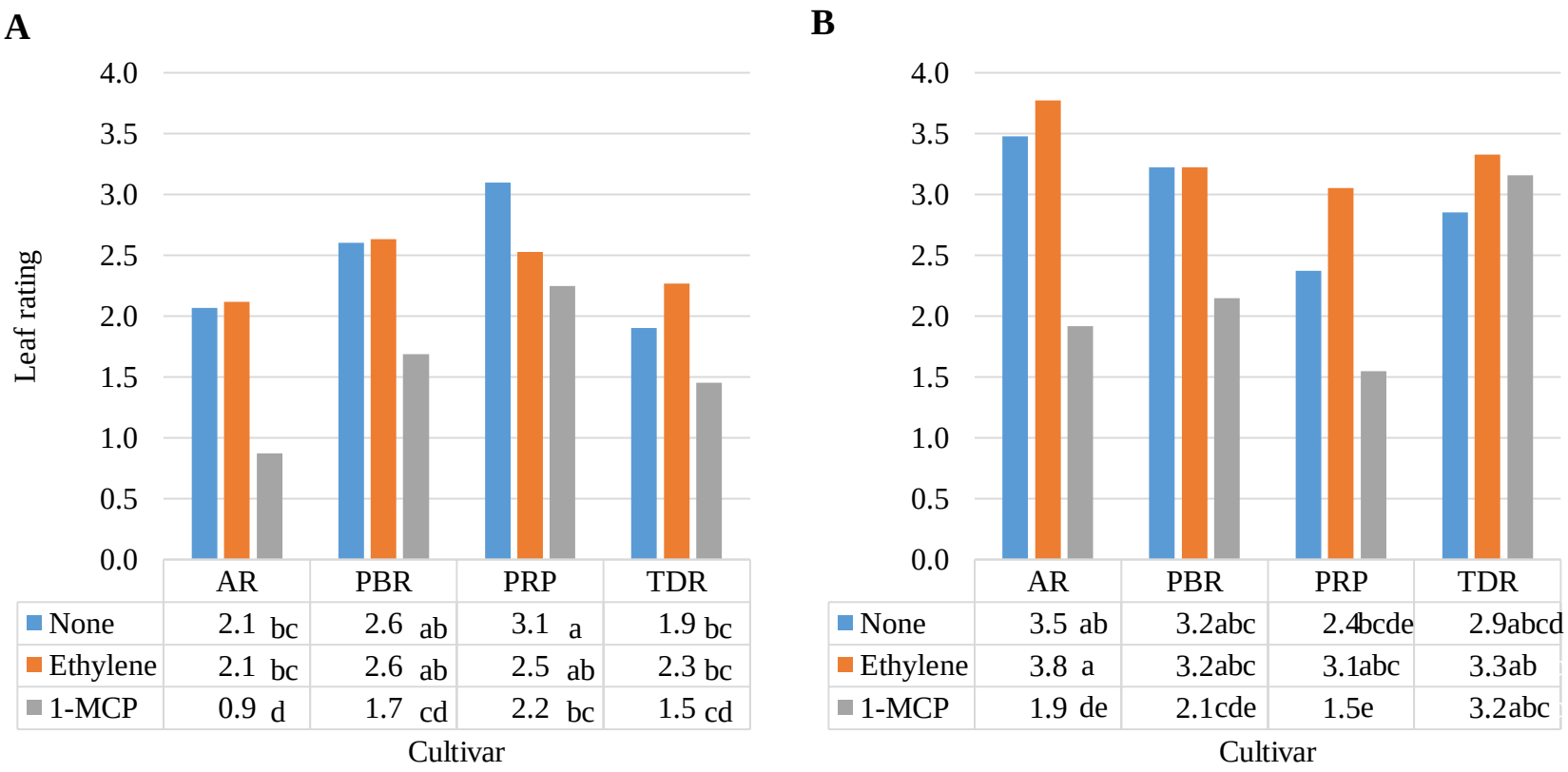


Fig. 8A-B. Replicates 1 (A) and 2 (B) leaf ratings of cutting of cultivars 'Americana Red' (AR), 'Patriot Bright Red' (PBR), 'Patriot Rose Pink' (PRP), and 'Tango Dark Red' (TDR) with no treatment (control) or treated with 1.0 $\mu L \cdot L^{-1}$ ethylene, or 700 $nL \cdot L^{-1}$ 1-methylcyclopropene (1-MCP). Means with the same letter for each replicate are not significantly different according to Tukey's Studentized range test at $\alpha = 0.05$ ($P = 0.0445$ or 0.0008 for replicate 1 and 2, respectively).

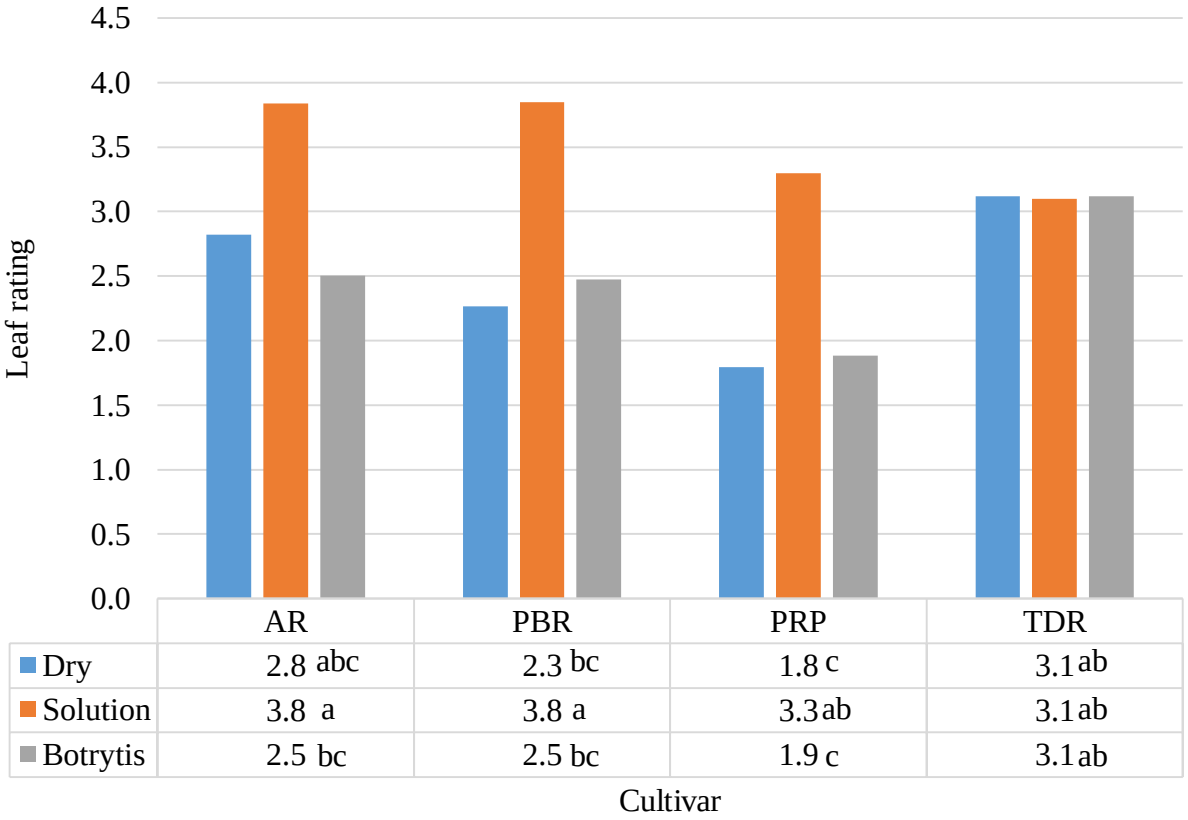


Fig. 9. Replicate 2 leaf ratings for cuttings of cultivars ‘Americana Red’ (AR), ‘Patriot Bright Red’ (PBR), ‘Patriot Rose Pink’ (PRP), and ‘Tango Dark Red’ (TDR) either kept dry, sprayed with the control spore carrier “solution” or inoculated with a *Botrytis* spray of 10^6 spores/mL. Means with the same letter for each time are not significantly different according to Tukey’s Studentized range test at $\alpha = 0.05$ ($P = 0.0020$).

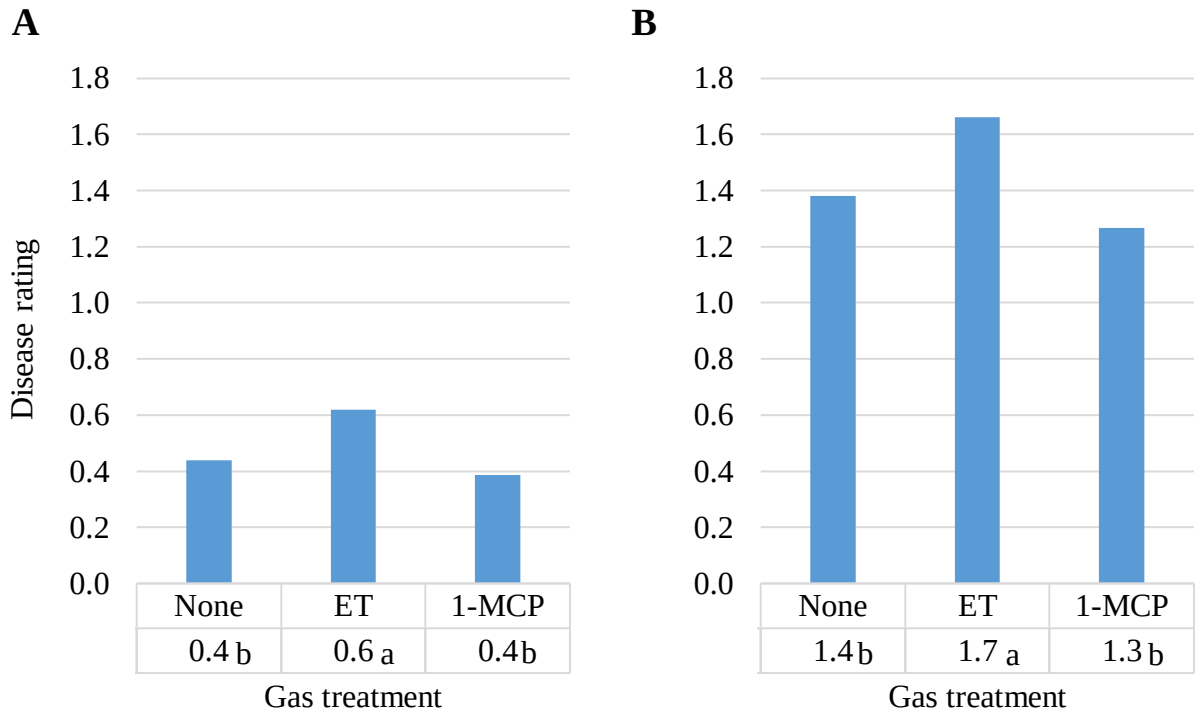


Fig. 10A-B. Replicates 1 (A) and 2 (B) disease ratings caused by *B. cinerea* for cuttings with no treatment (control) or treated with 1.0 $\mu L \cdot L^{-1}$ ethylene (ET), or 700 $nL \cdot L^{-1}$ 1-methylcyclopropene (1-MCP). Means with the same letter for each replication are not significantly different according to Tukey's Studentized range test at $\alpha = 0.05$ ($P = 0.0013$, <0.0001, respectively).

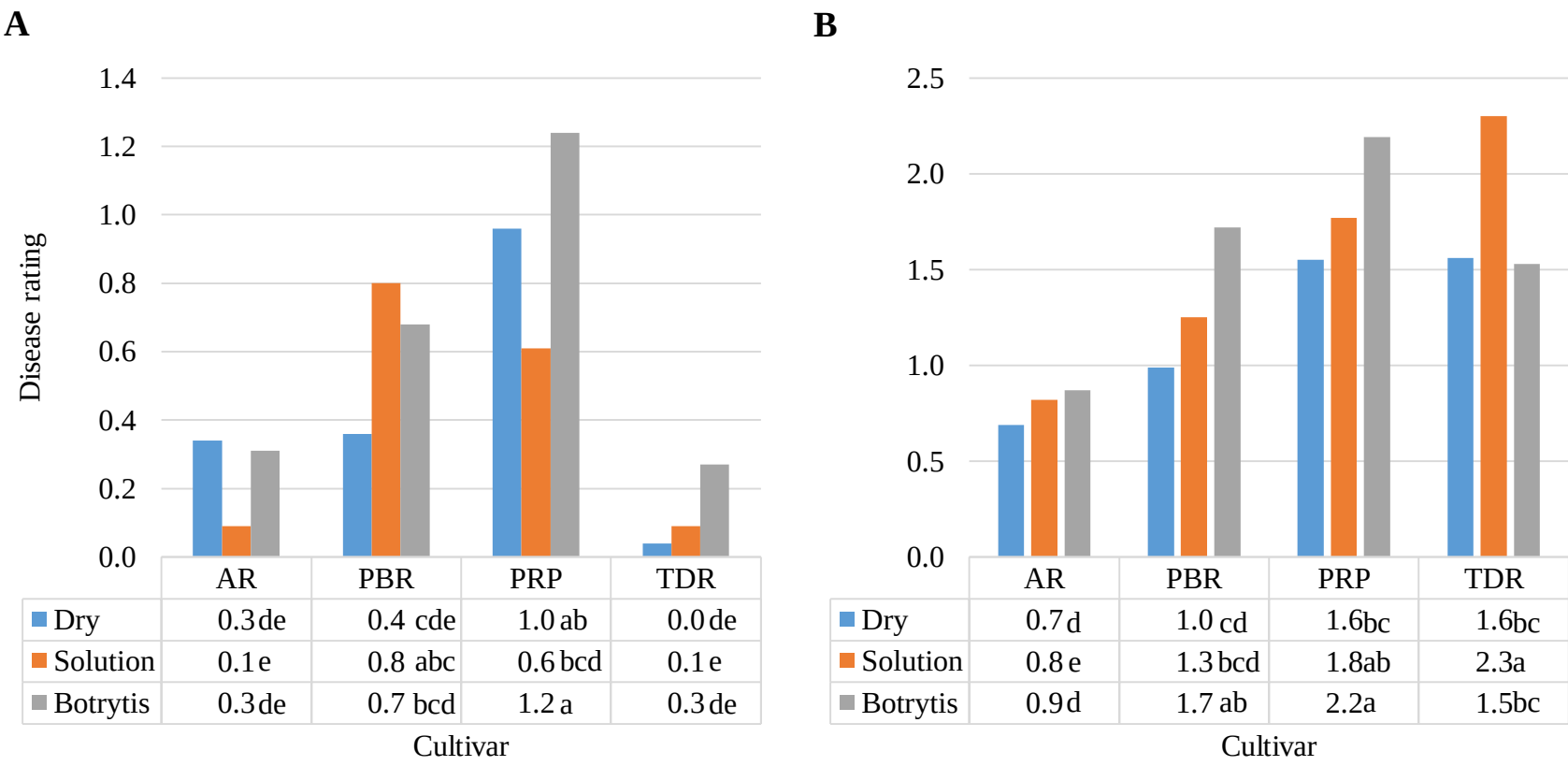


Fig. 11A-B. Replicates 1 (A) and 2 (B) disease ratings caused by *B. cinerea* for cuttings of cultivars 'Americana Red' (AR), 'Patriot Bright Red' (PBR), 'Patriot Rose Pink' (PRP), and 'Tango Dark Red' (TDR) either kept dry, sprayed with the control spore carrier solution, or inoculated with *B. Cinerea* spore suspension of 10^5 and 10^6 spores/mL for A and B, respectively. Means with the same letter for each replicate are not significantly different according to Tukey's Studentized range test at $\alpha = 0.05$ ($P = 0.0003$, <0.0001, respectively).

CH. 4: Leaf yellowing and *Botrytis* susceptibility of *Pelargonium* liners

Nathan J. Jahnke[a,*], John M. Dole[a], and H. David Shew[b]

[a]Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609, USA

[b]Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695-7609, USA

*Corresponding author at: Department of Horticultural Science, Campus Box 7609, North Carolina State University, Raleigh, NC 27695-7609, USA. E-mail address: njjahnke@ncsu.edu.

Abstract

Liners of *Pelargonium* × *hortorum*, 'Americana Red' (AR), 'Patriot Bright Red' (PBR), 'Patriot Rose Pink' (PRP), and 'Tango Dark Red' (TDR), were subjected to one of two shipping temperature regimes following inoculation with one of two spore concentrations of *Botrytis cinerea*. Based on disease ratings, TDR was more susceptible to *B. cinerea* than AR and PRP, but not PBR. Inoculated liners were significantly more damaged than untreated, dry liners until day 6. Inoculating liners with spore concentrations of 10^4 and 10^6 spores/mL resulted in similar levels of disease throughout the storage duration for all cultivars. Inoculated liners developed necrotic lesions covering 10 – 24% of their leaf area by 8 d in storage. Dry, control liners did not have noticeable disease until day 6, which is promising for suppliers if shipments get delayed. Plants sprayed with only the spore carrier solution, Tween 80 and glycerol, had significantly higher disease ratings starting at day 4 compared to the dry-control. The prolonged leaf wetness present on plants that received the spore carrier solution could have been a factor in disease development. Subjecting cuttings to a simulated shipping temperature regime, with temperatures varying between 10 and 30°C, did not impact disease ratings compared to a constant temperature

of 15°C. However, leaf yellowing was significantly worse for all cultivars at 2, 4 and 6 days when liners were stored in the simulated shipping regime compared to liners stored at the constant temperature of 15°C. PRP developed the most leaf yellowing out of the four cultivars. Commercial liner shipments do not usually take longer than 48 hours to reach customers, which is notable because many of the disease and yellowing symptoms seen in this research did not reach significant levels until after 2 days of simulated shipping. However, if shipments are delayed or face variable temperatures *Pelargonium* liners could develop lesions and marginal leaf yellowing.

4.1 Introduction

Liners are the rooted product of unrooted cuttings. Rooting of shoot tip cuttings varies among floriculture crop species and is either completed by a rooting station or a grower who grows the cutting to a finished product (Gibson and Dole, 2006). Rooting stations mass produce liners of many floriculture crops and ship them across the country almost year round. Working with a live, temperature sensitive product can make year round production and shipping problematic. Quality liners need optimum nutrition and light during production to ensure quality through the shipping process and quick establishment once planted (Hartmann et al., 2002). Plants are shipped in cardboard boxes through normal consumer methods, such as FedEx or UPS, unless a grower has access to climate controlled transportation. This means liners can be exposed to extreme fluctuations of temperature and humidity during transit.

High temperatures increase respiration causing leaf yellowing (Faust, 2011). Dark conditions within boxes also reduce the photosynthetic rates and carbohydrate reserves resulting in leaf yellowing (Arteca et al., 1996). Pathogens can take advantage of weakened plant material and humid conditions to gain entry into host tissues and initiate diseases. Temperatures between

20 to 30°C are perfect for *Botrytis cinerea* Per.:Fr [teleomorph = *Botryotinia fuckeliana* (de Bary) Whetzel], a common necrotrophic pathogen which can infect hundreds of plant species (Elad et al., 2004). Geranium (*Pelargonium* × *hortorum* L.H. Bailey) is one of the most popular floriculture crops and is prone to leaf yellowing and *Botrytis* blight making shipping and production challenging (USDA, 2016; Gibson and Dole, 2006).

During production, management practices such as removing yellow leaves, increasing air circulation, reducing leaf wetness, and applying fungicides are used to control *Botrytis* diseases (Elad et al., 2004; Williamson et al., 2007). However, growers do not have these options during the shipping process because shipments remain boxed and unopened until delivery. The objectives of this research were to 1) determine temperature and humidity conditions of commercial liner shipments throughout the growing season, 2) evaluate the susceptibility of geranium liners to leaf yellowing and *Botrytis* development during simulated shipping, and 3) determine if varying temperature during shipping effects leaf yellowing and development of *Botrytis* diseases on geranium liners.

4.2 Materials and Methods

Liner rooting and culture

Unrooted cuttings of 'Americana Red' (AR), 'Patriot Bright Red' (PBR), 'Patriot Rose Pink' (PRP) and 'Tango Dark Red' (TDR) were received from commercial suppliers (Dümmen Orange, Columbus, Ohio, USA; Syngenta Flowers, Gilroy, Calif., USA) on 28 Nov. 2016. Liners for a second replication were started from cuttings received on 10 Jan. 2017. Cuttings were inserted into peat-perlite-vermiculite plugs lined with a cellulose non-woven net (26 count cell strips Oasis® Fertiss® Plant Propagation System, Plug Size: 3 cm × 4 cm – Round, Smithers-Oasis, Kent, Ohio, USA). Cuttings were placed in a glass glazed greenhouse at 24°C days and

18°C nights with natural photoperiod providing a daily light integral of 5 – 10 mol·m^{-2}·d^{-1}. An automatic mist system operated every 8 min for 6 s. After 24 h, mist interval was extended to once every 10 min. Once roots initiated, liners were irrigated with clear water for 1 week. Afterwards, liners were hand watered, alternating between 15.0-0-12.5 and 20.6-6.7-16.6 (Jack's 15-0-15 Dark Weather and 20-10-20 General Purpose water soluble fertilizer, JR Peters Inc., Allentown, Pa., USA) at 150 mg·L^{-1} until experiment initiation. Total propagation time was 5 weeks. Flowers and yellow or dead leaves were removed before experiment initiation.

Pathogen culture

Botrytis cinerea (obtained from M. Benson, Department of Plant Pathology, North Carolina State University) cultures were initiated from conidia stock maintained at -80°C in a solution of 0.01% Tween 80 (Sigma-Aldrich, St. Louis, Mo., USA) and 15% glycerol (Sigma-Aldrich, St. Louis, Mo., USA). Culture were grown to sporulation in 15 × 90 mm polystyrene Petri dishes containing 20 ml oatmeal agar medium (Thermo Fisher Scientific, Lenexa, Kans., USA) prepared per manufacturer's directions. Culture dishes were not individually sealed, but placed into closed, clear plastic tubs with wet paper towels. Tubs were placed on a laboratory bench at room temperature under natural day length. New cultures were started by turning a sporulating plate upside down over a new plate and tapping it to drop spores onto fresh medium.

Spore collection

Petri dishes were typically covered by dense, dark gray, sporulating mycelium 14 d after culture initiation. Spores were harvested by pouring approximately 15 mL of sterilized solution containing 0.1% Tween 80 and 15% glycerol, into the sporulating plate. A bent, glass stirring rod was used to dislodge conidia by rubbing the culture for approximately 5 min before pouring the spore suspension through five layers of sterilized cheesecloth to remove hyphae. The process

was repeated twice on each Petri dish. A Neubauer Improved hemacytometer (LW Scientific, Lawrenceville, Ga., USA) was used to quantify the number of spores/mL for the resulting suspension. Suspensions were stored in 50 mL polypropylene centrifuge tubes (Thermo Fisher Scientific, Lenexa, Kansas, USA), and placed at -80°C.

Inoculation

Tubes of spore suspensions were removed from -80°C storage 2 h before cutting inoculation and allowed to thaw in tap water at room temperature. The spore suspension concentration was adjusted to 10^4 from 10^6 spores/mL by diluting with sterilized DI water. Suspensions were loaded into a hand sprayer. While agitating, the spore suspension was sprayed onto all sides and on top of 24 liners while in the 26 ct trays using approximately 45 mL of the spore suspension.

Confirmation of spore concentration and germination

Spore concentration used for inoculation was verified via hemocytometer. Spore viability was determined by placing 1.0 mL of spore suspension onto potato dextrose agar (Thermo Fisher Scientific, Lenexa, Kansas, USA). After 4 h incubation at room temperature, 100 spores were counted under 100× magnification (Nikon TMS-F Inverted Microscope, Nikon Instruments Inc., Melville, N.Y., USA). Spores with a definitive germination tube (at least twice the diameter of the spore) were counted as viable, while spores without a germ tube were counted as nonviable. Spore viability in inoculation suspensions averaged 68%.

Logging temperature and humidity of liner shipments

Data loggers (RHT10, Extech Instruments, Waltham, MA, USA) were sent to Lucas Greenhouses in Monroeville, NJ to record temperature and relative humidity of liner shipments. Data loggers were placed within geranium liner shipments to Washington (26 April 2016),

Wisconsin (11 Oct. 2016), North Carolina (11 Oct. 2016) and Oregon (6 Dec., 2016). Once shipments were received by the grower, data loggers and a form, detailing the time of arrival and quality, were sent back to North Carolina State University. Temperature and relative humidity trends during shipping were used to define suitable temperatures for simulated shipment treatments.

Experimental design

After 5 weeks of propagation, eight trays of twenty-four rooted liners were selected of each cultivar. Trays were watered and foliage was allowed to dry before treatment application. Two trays of each cultivar were randomly assigned to one of the four following treatments: 1) non-inoculated, dry, control; 2) sprayed with a solution of 0.01% Tween 80 (Sigma-Aldrich, St. Louis, Mo., USA) and 15% glycerol; 3) sprayed with same solution as #2 with 2×10^4 spores/mL of *B. cinerea* added; 4) sprayed with same solution as #2 plus 2×10^6 spores/mL of *B. cinerea* added. Spray treatments were applied by spraying each side and top of a tray using approximately 50 mL.

Liners were placed into boxes for the imposition of simulated shipping temperature and constant temperature regimes. The simulated temperature regime was derived from documented liner shipments, as described above, and included a base temperature of 10°C from which temperature was varied by an electric heater over a 24 h cycle. From 8:00 AM to 10:00 AM the ambient air was heated, increasing the box temperatures to approximately 25 - 30°C. At 10:00 AM heat was turned off and the temperature was allowed to decrease until 12:00 p.m. Temperature was increased over the next 6 h and remained between 25 - 30°C. The heater was turned off for another 6 h, back on again for 6 h, and finally off until 8:00 AM when the 24 h cycled would restart. The constant temperature regime was an average temperature of 15°C.

Each box held eight trays of liners, split into two compartments (Fig. 1). Each compartment held two trays, each a different treatment. Two compartments were placed into a single box (80.01 cm × 54.61 cm × 33.66 cm, Lucas Greenhouses, Monroeville, N.J., USA).

Six liners (replications) were removed from each cultivar by treatment combination at 2, 4, 6, and 8 d after the start of temperature treatments. At each sampling time each liner was given a disease rating and leaf yellowing rating. A 0 – 5 rating scale was used for the disease ratings (Fig. 2): 0, 0% disease, no symptoms; 1, <10% disease or 1 - 10 small lesions; 2, 10 - 24% leaf necrosis or shoot necrosis; 3, 25 - 50% leaf necrosis or 10% leaf necrosis with shoot necrosis; 4, 51 - 75% leaf necrosis or 25% leaf necrosis with shoot necrosis; 5, 75 - 100% leaf necrosis with or without shoot necrosis). A 0 – 6 scale was used for the leaf yellowing ratings (Fig. 3): 0, symptoms; 1, 10% marginal yellowing; 2, 11 - 25% yellow; 3, 26- 50% yellow; 4, 51 – 75% yellow; 5, 51 - 75% yellow; 6, dead or abscised.

Statistical analysis

Data were analyzed using SAS (Version 9.4, SAS Institute, Inc., Cary, N.C., USA). Mean separation was determined using MIXED and Tukey's Studentized procedures at $\alpha = 0.05$. In the split-plot design shipping temperature regime was the whole-plot effect tested with cultivar, sampling day, and treatment as the sub plot effects. Median values were used for the six replications for each shipping duration, cultivar and sampling day combination.

4.3 Results & Discussion

Logging temperature and humidity of liner shipments

Shipments of liners usually reached growers in less than 36 h (Fig. 4). Liners were packed between 7 and 11:00 a.m. Temperatures increased initially or started high while boxes were waiting to be picked up by a delivery service. Following pickup, temperatures decreased for

about two hours followed by an increase for about six hours in the evening to approximately 18 - 21°C. Night and morning temperatures decreased about 2.8 – 8.3°C. Until delivery, temperatures rose by about 5.6 – 8.3°C degrees and fluctuated. Some boxes experienced high temperatures after being delivered, indicating customers may not be placing boxes in adequately cooled locations. Growers did not identify any quality issues after unpacking, except for the April shipment, where some leaf yellowing was present on some of the liners, most likely caused by the higher temperatures experienced by this shipment. Kadner (unpublished) had found that average temperature of geranium shipments was 12°C, but this was not seen in liner shipments. Average temperature, maximum and minimum temperatures for all shipments were 17, 28, and 4°C, respectively, which were extremely variable.

Most growers did not report any damage or leaf yellowing meaning the short shipping durations prevented stress. However, the extreme temperature ranges liners experienced is cause for concern if any delays would occur in transit. It is recommended that shipping and storage temperatures be kept at 2 - 10°C (35 - 50°F) (Gibson and Dole, 2006; Faust et al., 2011). This temperature reduces respiration and ethylene production and is low enough to prevent sporulation and growth of *Botrytis* (Sirjusingh and Sutton, 1996; Elad et al., 2004). High temperatures seen in shipments promotes leaf yellowing during propagation. This requires more labor to remove yellow leaves and reduce the possibility of *Botrytis* diseases. Rooting stations try to avoid shipping when temperatures exceed or drop below recommended ranges to ensure delivery of quality products.

Botrytis susceptibility and leaf yellowing

Surprisingly, throughout the storage duration, disease did not differ between the constant shipping temperature of 15°C and the simulated temperature regime with temperatures ranging

from 10 - 30°C. There was a significant two-way interaction (P<0.0001) for the BR response between shipping duration (0, 2, 4, 6, and 8 d) and treatment [dry, carrier solution, *B. cinerea* 10^4 (B4), and *B. cinerea* 10^6 (B6)] (Fig. 5). With no shipping (day 0) there was no disease, but as time increased to 2 days the dry control was significantly lower levels of disease than the carrier solution control and the inoculated treatments B4 and B6. Disease ratings at day two were just under 1 meaning there was less than 10% disease coverage or a few small lesions on a liner. Since most shipments will not experience this level of disease pressure or be in transit for more than 36 hours, *Botrytis* should not be a problem. After day two, the control of the solution carrier had significantly higher ratings than the other treatments until day eight. This was most likely caused by the prolonged leaf wetness. Liners treated with the carrier solution were noticeably wetter than the other treatments by day four. Even though relative humidity was often over 80% for both temperature regimes, leaves were mostly dry by day two or four for the dry control and *Botrytis* treatments.

The two different spore suspension concentrations of *Botrytis* resulted in similar disease ratings over the shipping durations. They were, however, significantly higher than the dry control until day six. The fact that the dry control was not significantly different from the inoculated treatments by day 8 means that naturally occurring levels of inoculum are enough to damage liners in the proper conditions and if given enough time. Cultivars were also significantly different for disease ratings (P = 0.0161), although ratings were very close and physically indistinguishable between 0.79 and 1.00 (Fig. 5). Cultivars did not react differently to the different temperature regimes provided or over time.

There was a significant interaction between shipping temperature regimes and duration (P<0.0001) for leaf yellowing ratings. Both factors significantly increased leaf yellowing ratings

over time, but at day four, the simulated shipping regime caused significantly higher leaf ratings until day eight where regimes did not differ (Fig. 6). Although the preferred shipping and storage ranges are 2 - 5°C (35 - 41°F) an average constant temp of 15°C is enough to significantly prevent leaf yellowing in two days (Faust et al. 2011). It is important to note that the large swings in temperature can impact leaf yellowing, and based on the logging of shipments, it is known that liners can experience these large swings. PRP was more susceptible to leaf yellowing than AR, PBR and TDR, with ratings of 1.59, 1.26, 1.34 and 1.35, respectively (Fig. 5). Disease development on PRP matches with industry observations that it is a less durable cultivar during shipping (A. Hammer, personal communication). Cultivars differed in habit and leaf area which means respiration rates, dehydration and potential area for infection were factors that affected responses. AR, PBR and TDR had larger leaf areas and were overall larger, while PRP was shorter and had smaller leaves. PRP was consistently a slower grower and produced a smaller liner in all experiments.

Conditions for disease development occur during commercial liner shipments. However, conditions were not sustained long enough for significant disease development, because shipping lasted only a short time. Suppliers should continue to keep clean stock, allow air movement and reduce leaf wetness to ensure quality plant material before shipping. In contrast, leaf yellowing is a problem. Even though shipping times were short, any leaf yellowing reduced visual quality and could add to the labor needed to remove leaves to prevent disease development. Products such as 1-methylcyclopropene (1-MCP) may be an option as a pre-shipping treatment to ensure green leaf tissue (Cameron and Reid, 2001), but 1-MCP loses effectiveness at higher temperatures (≥20°C) and has had adverse effects on rooting of cuttings (Kadner and Druege, 2004). This is problematic when liners experience a wide range of temperatures in transit and are expected to

fill containers quickly after planting. A combination of benzyladenine and gibberellic acid has been used to counteract yellowing in unrooted cuttings. It may be a better option for growers to use on the already rooted liner product, because that combination has adverse effects rooting of cuttings (Faust et al., 2011). If shipments take two days or less, leaf yellowing should be kept to a minimum.

Cardboard material has been the majority of floriculture postharvest packaging. These containers retain humidity, ethylene, and have no protection from temperature fluctuation. Modified atmosphere packaging has been used in variety of horticulture products to increase shelf life, but species react differently to oxygen levels and ethylene concentration can increase to damaging levels (Crawford, 2013). As temperatures and weather become less predictable there should be methods available to ensure quality throughout shipping such as boxes that resist temperature changes such as coating boxes in wax, using Styrofoam containers or a pre-shipping solution to spray on cuttings. Future research needs to focus on protection during shipping so that quality can be maintained.

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Figures

Fig. 1. Treated *Pelargonium* liners in shipping box with two compartments, each containing two 26 cell trays.

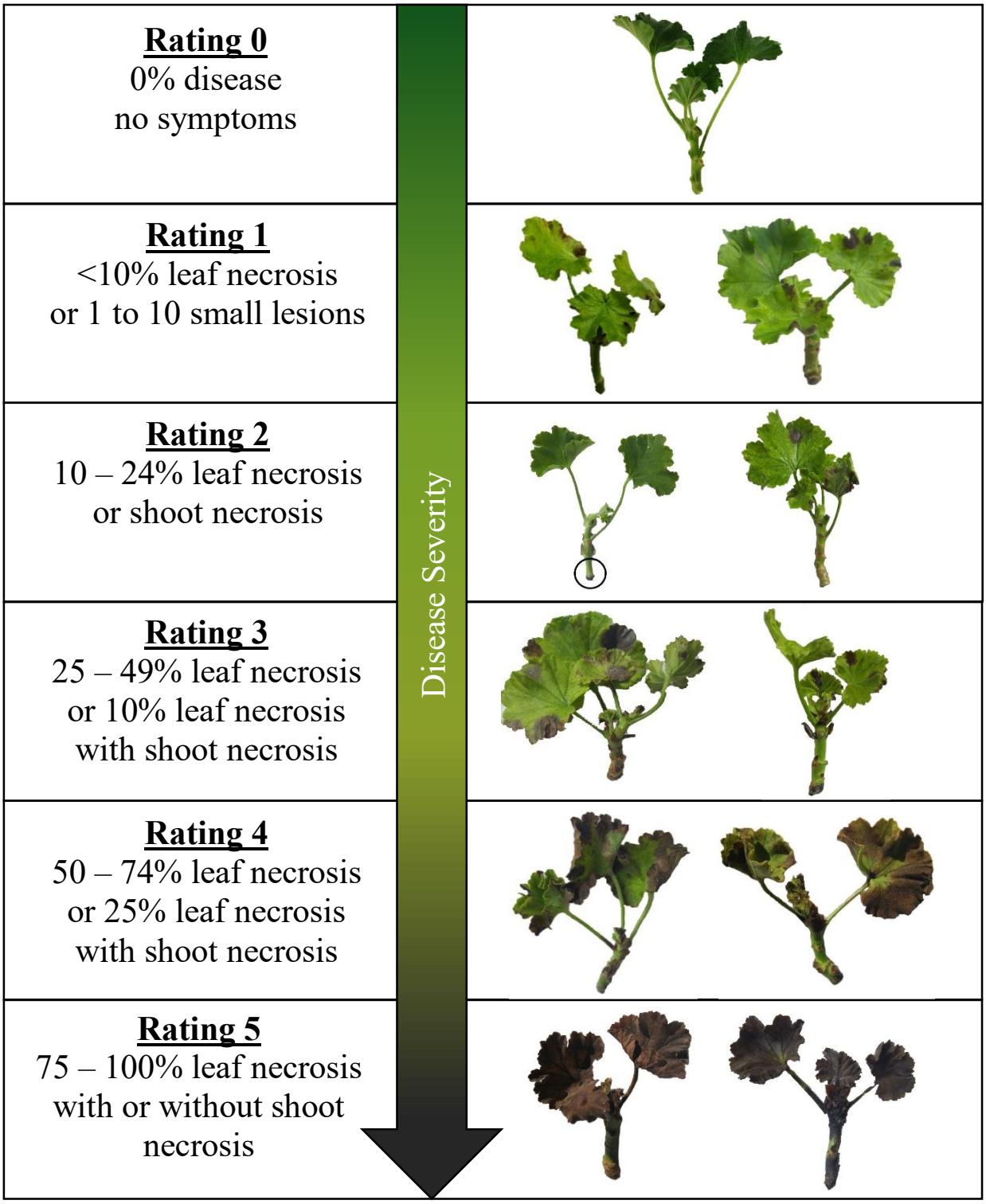


Fig. 2. Disease rating scale from 0 to 6 for unrooted cuttings of *Pelargonium* based on necrotic leaf area and shoot necrosis.

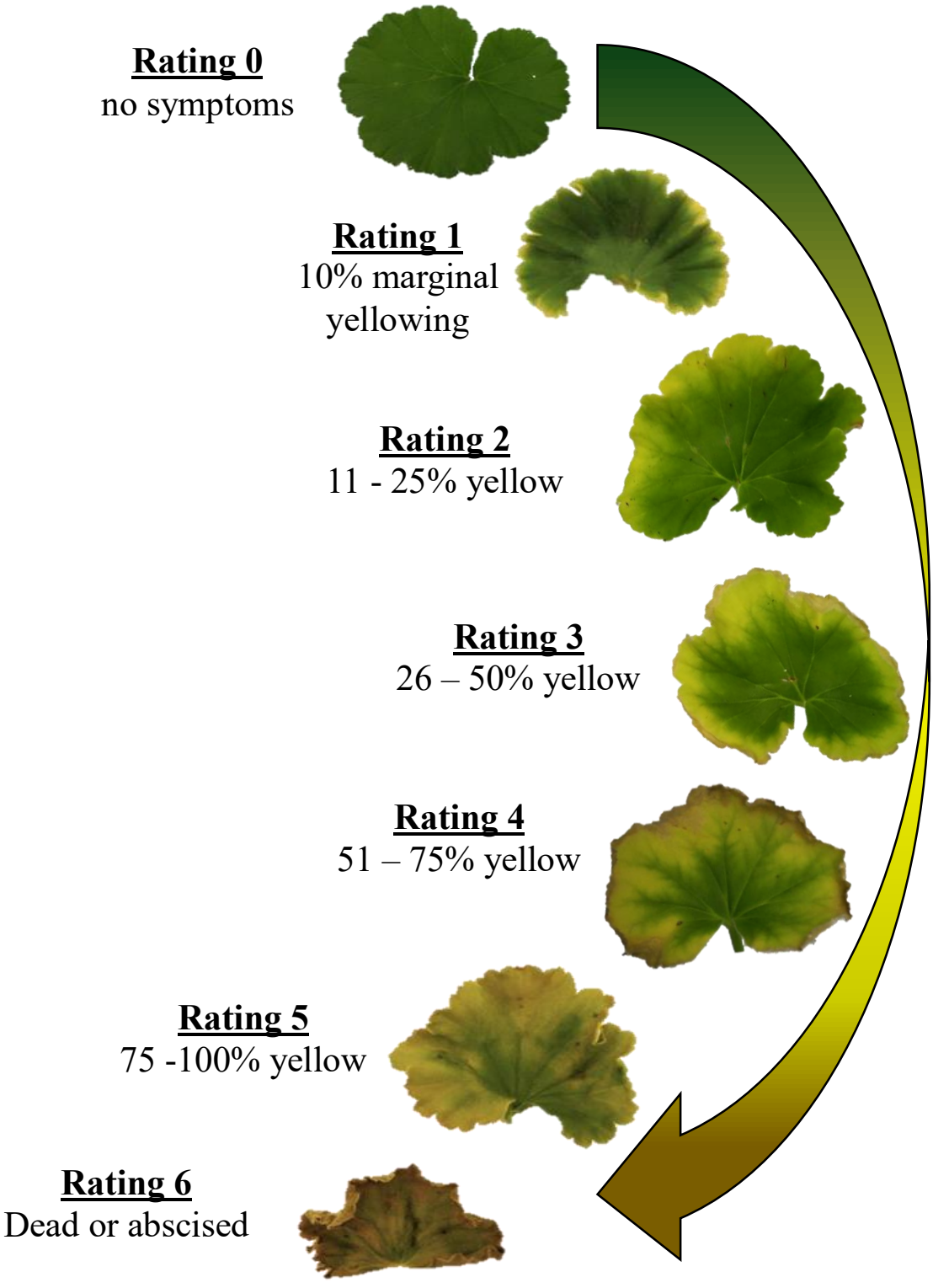


Fig. 3. Leaf yellowing ratings scale from 0 to 6 for unrooted cuttings of *Pelargonium* based on leaf yellowing

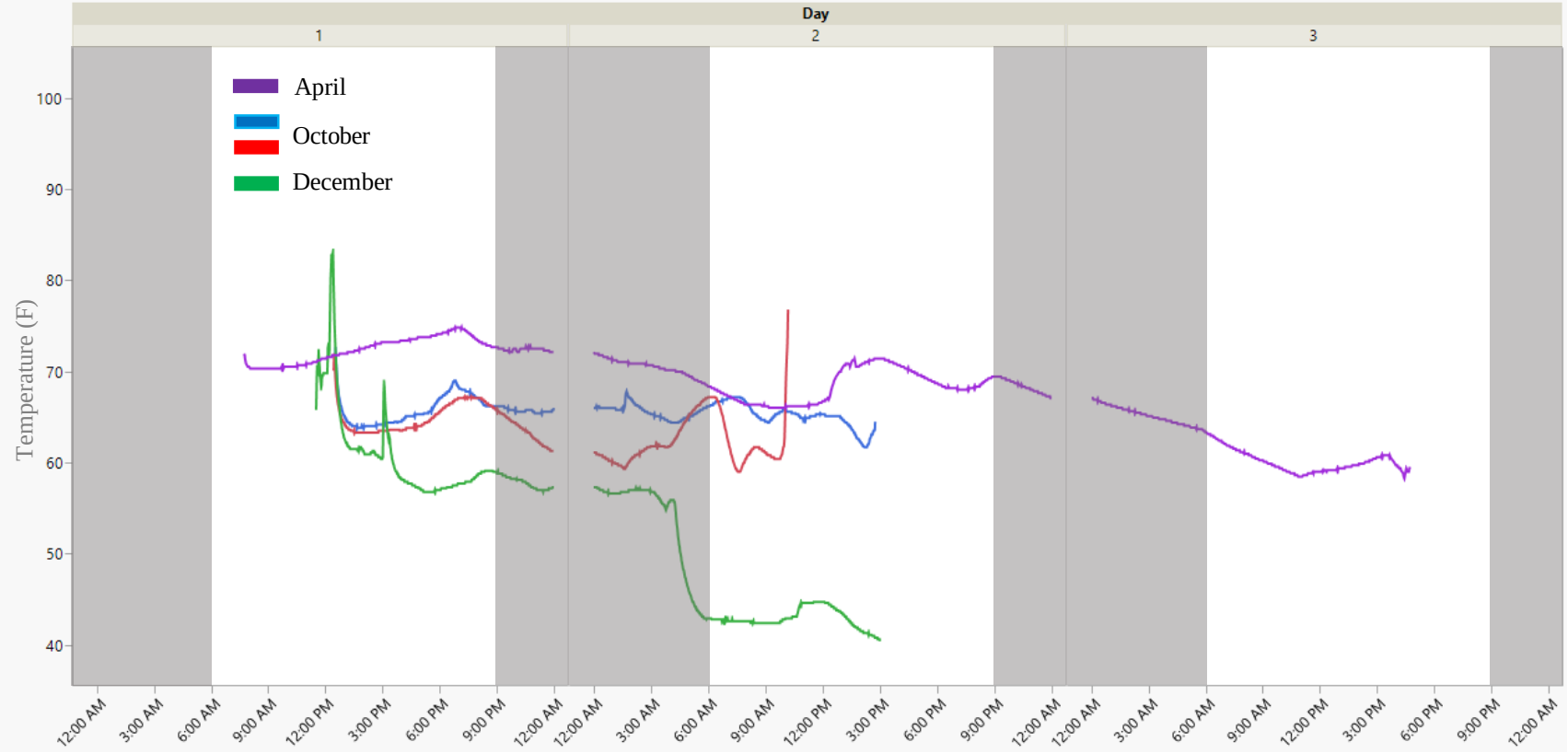
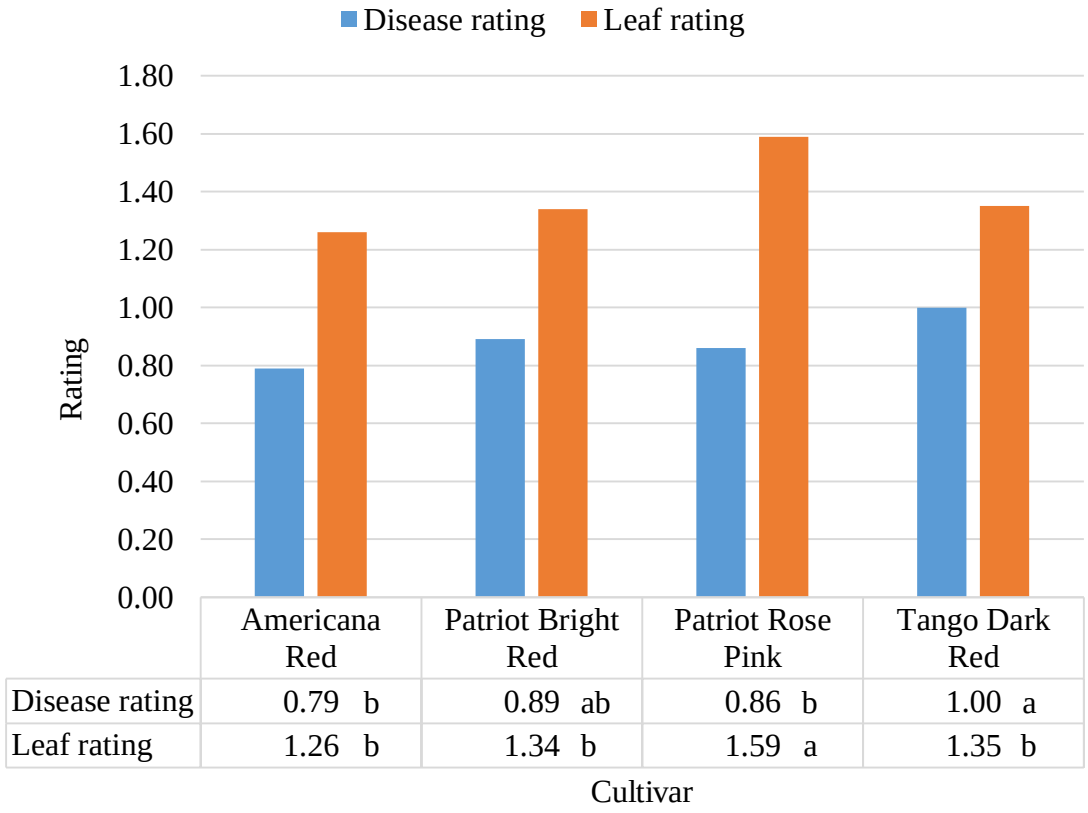
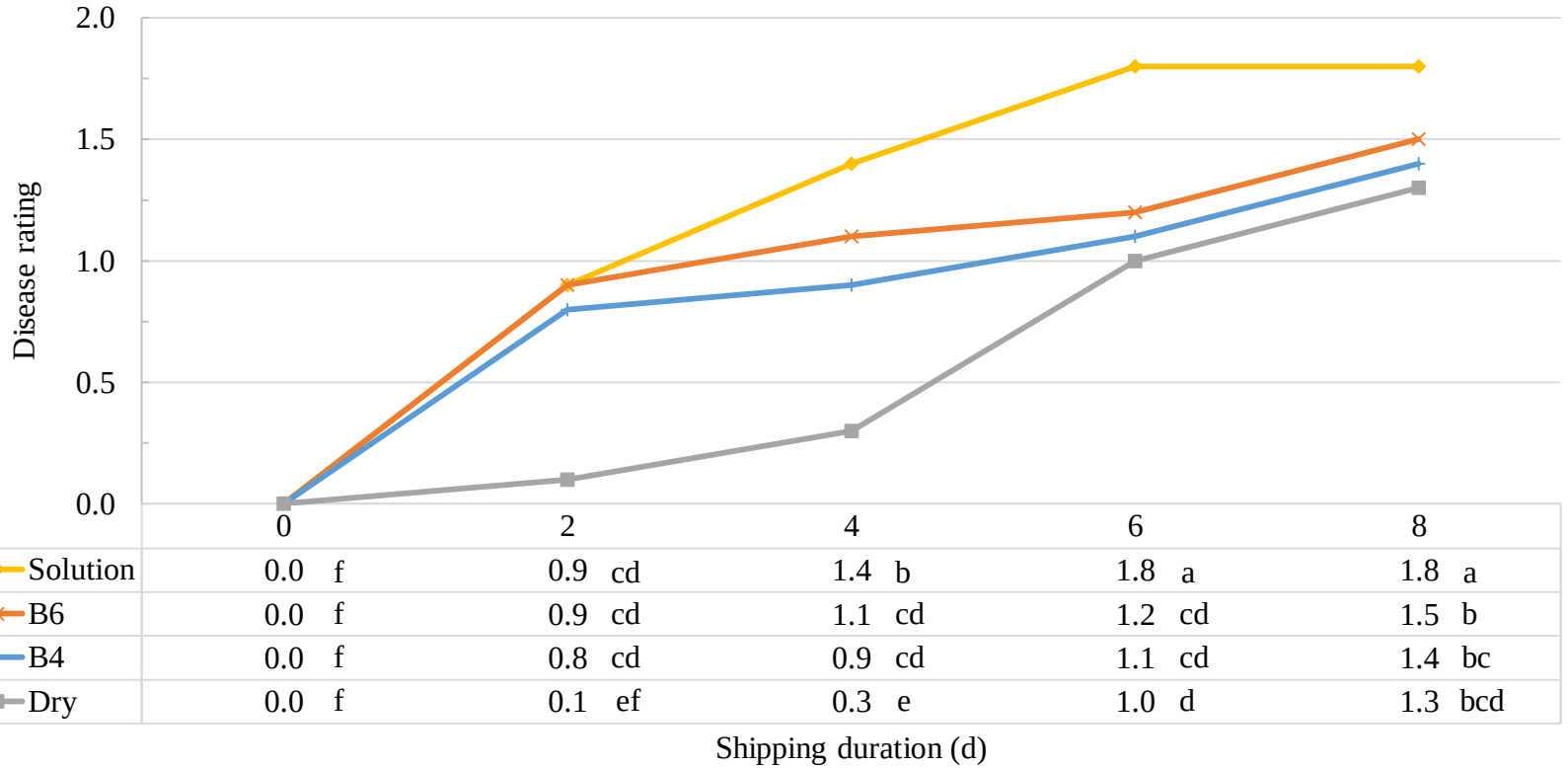


Fig. 4. Temperature logs of *Pelargonium* liner shipments by the months of which data loggers were sent from Lucas Greenhouses in New Jersey to five locations, North Carolina, Oregon, Washington, and Wisconsin. Shipments spanned two days from packing to receiving, except for the April shipment which spanned three days. Shaded regions depict an approximate natural dark period from 9:00 p.m. to 6:00 a.m.



	Americana Red	Patriot Bright Red	Patriot Rose Pink	Tango Dark Red
Disease rating	0.79 b	0.89 ab	0.86 b	1.00 a
Leaf rating	1.26 b	1.34 b	1.59 a	1.35 b

Fig. 5. Disease ratings (P = 0.0161) and leaf ratings (P<0.0001) for cultivar across shipping temperature regimes (constant, 15°C or simulated, variable temperature) sprays treatments (dry, spore carrier solution, and inoculated with *B. cinerea* at 10^4 or 10^6 spores/mL), and shipping duration (0, 2, 4, 6, 8 d). Disease ratings were based on a severity scale (0 to 5; 0 no disease and 5 dead). Leaf ratings were based on a scale (0 to 6; 0 completely green and 6 completely brown or abscised). For each cultivar, means were an average of 40 median values, each one determined from a group of 6 replications for a shipping regime, spray and time combination. Means followed by the same letter for each response are not significantly different according to Tukey's Studentized range test procedure at $\alpha = 0.05$.



	0	2	4	6	8
Solution	0.0 f	0.9 cd	1.4 b	1.8 a	1.8 a
B6	0.0 f	0.9 cd	1.1 cd	1.2 cd	1.5 b
B4	0.0 f	0.8 cd	0.9 cd	1.1 cd	1.4 bc
Dry	0.0 f	0.1 ef	0.3 e	1.0 d	1.3 bcd

Fig. 6. Disease ratings for the interaction of shipping duration (d) and spray treatment (P<0.0001): control (Dry), *Botrytis* spore suspension 10^4 spores/mL (B4), *Botrytis* spore suspension 10^6 spores/mL (B6), spore carrier solution of 0.01% Tween 80 and 15% glycerol (solution). Disease ratings were based on a severity scale (0 to 5; 0 no disease and 5 dead). Means followed by the same letter are not significantly different according to Tukey's Studentized range test procedure at $\alpha = 0.05$.

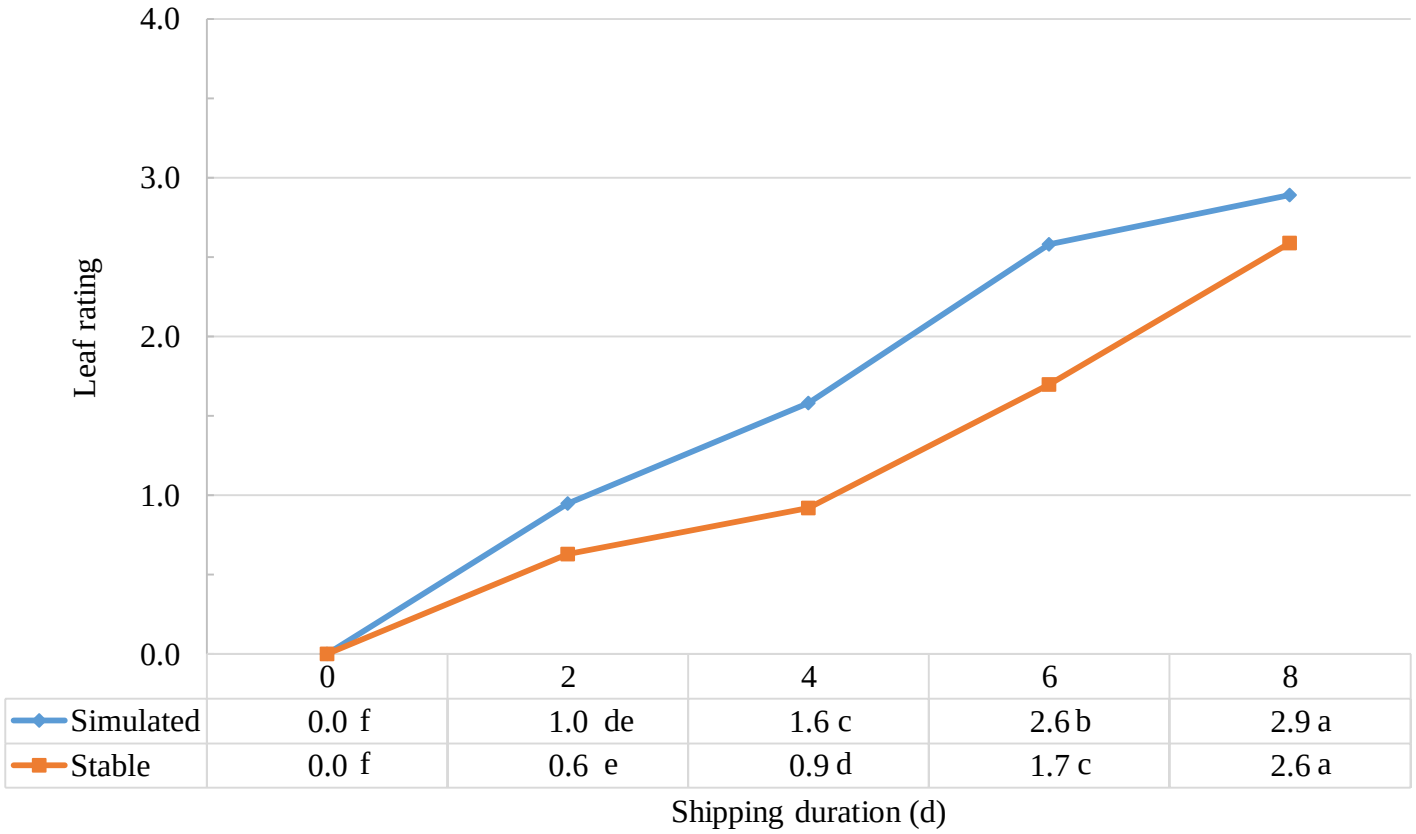


Fig. 7. Leaf ratings for the interaction of shipping duration (d) and shipping method (P<0.0001): base temperature of 10°C for 2 h, 2 h increasing temperature to approximately 25 - 30°C, 2 h decreasing temp to base, 6 h increasing heat to 25 - 30°C, 6 h decreasing temperature to base, 6 h heating and then repeated (simulated) and 15°C (constant). Means followed by the same letter are not significantly different according to Tukey's Studentized range test procedure at $\alpha = 0.05$.

CH. 5: The role of carbohydrates during storage and its effects on propagation of poinsettia

Nathan J. Jahnke[a,]*, John M. Dole[a], and Hamid Ashrafi[a]

[a]Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609, USA

*Corresponding author at: Department of Horticultural Science, Campus Box 7609, North Carolina State University, Raleigh, NC 27695-7609, USA. E-mail address: njjahnke@ncsu.edu.

Abstract

Unrooted cuttings are prone to environmental stress during shipping and storage, which can cause dehydration and carbohydrate depletion, potentially delaying rooting and extending crop schedules. *Euphorbia pulcherrima* (poinsettia) is especially prone to environmental stresses during shipping as unrooted cuttings and liners are shipped during hotter parts of the year. Unrooted cuttings of poinsettia 'Prestige Red' (PR) and 'White Star' (WS) were stored at 10°C for 0, 2, 4, 6 or 8 days. Fresh weight loss averaged across cultivars was 8% when cuttings were stored for 8 days. Shoot turgidity, rated on a scale of 1 - 5 with 5 being fully turgid, decreased an average of 1.7 after 8 days in storage. After 24 hours in propagation under mist, all cuttings returned to a turgidity rating of 5. After 4 weeks of propagation, root ratings for PR decreased from 2.2 with no storage to 1.2 after 8 days in storage on a scale of 1 – 5 with 1 having no roots and 5 having >10 roots, 2.5 cm or longer. Root ratings for WS increased from 2.1 with no storage to 2.7 after 2 days in storage, but decreased to 1.9 after 8 days in storage. Overall, WS had significantly higher root ratings than PR. Initial fructose and glucose concentrations were not significantly different between cultivars, while sucrose content was greater in PR (4.48 $mg \cdot g^{-1}$ dry weight) than WS (3.27 $mg \cdot g^{-1}$ dry weight). As storage duration increased, concentrations of fructose, glucose, and sucrose in WS decreased by 13, 29, and 38%, respectively, and in PR by

22, 54, and 63%, respectively. Multivariate correlation analysis showed glucose ($r = 0.4824$, $P \leq 0.01$) followed by reducible sugars, fructose + glucose, ($r = 0.4222$, $P \leq 0.05$), to be the best predictors of root rating. While white poinsettia cultivars have often been thought to be less durable than red cultivars in postharvest environments, PR was more negatively impacted by storage than WS, which may have been caused by its loss of carbohydrates over the 8-day storage duration.

5.1 Introduction

Shipping vegetative, unrooted cuttings from off-shore locations is one of the main methods by which propagation material gets to horticulture industry in the United States. Over 1.5 billion cuttings are imported into North America every year (M. Miller, unpublished). The time it takes for those cuttings to get from a supplier to a customer is crucial and usually occurs in two to three days (Faust et al, 2017). However, not all shipments arrive in this timeframe, leaving valuable plant material in adverse environmental conditions. A stressed cutting may not root well or even be worth propagating as finishing time can become variable and slower than originally scheduled. Growers rely on quality cuttings to meet production schedules, which requires cuttings to be free of disease, turgid for quick inserting into media, and of optimum nutrition to produce roots. Carbohydrates are energy sources for adventitious root formation (Einfield, 2011; da Costa et al., 2013). Lower concentrations of carbohydrates in geranium and poinsettia have been shown to decrease rooting potential (Rapaka et al., 2005; Faust et al., 2017).

Euphorbia pulcherrima (poinsettia) has undergone decades of breeding introducing a variety of colors, hybrids, and response groups so growers can select the best cultivar that fits their needs. Previous work on poinsettias has shown that carbohydrate status and rooting potential to be influenced by stock plant stage before harvesting cuttings (Einfield, 2011; Zerche

and Druege, 2009). White cultivars have often been thought to be less durable than red as a potted plant. The goal of this study was to determine if carbohydrates and rooting ability differed between 'Prestige Red' and 'White Star' and if carbohydrates were correlated with rooting ability. Understanding cultivar differences and carbohydrates rooting ability will help the industry better understand how cuttings respond to shipping stress and to explain cultivar variation.

5.2 Materials and Methods

Stock plant culture

Cuttings of 'Prestige Red' and 'White Star' were harvested on 6 June 2016 from stock plants grown at North Carolina State University with ambient air in glass glazed greenhouse at 24°C days and 18°C nights. Cuttings were rooted in peat-perlite substrate (Fafard® 2P Mix; Sun Gro, Agawam, MA, USA) and then potted into 20-cm standard pots in a peat-perlite substrate (Fafard® 4P Mix; Sun Gro, Agawam, MA, USA). Plants were maintained in a vegetative state with a 4-h night interruption from September to March. Stock plants were hand irrigated alternating between Jack's 15-0-12.5 Dark Weather and 20-7-16 water soluble fertilizer (N-P-K; 150 $mg \cdot mL^{-1}$ N; JR Peters Inc., Allentown, PA)

Carbohydrate extraction

Cutting fresh weight (g) was recorded after storage (ASFW) and cuttings were immediately frozen in liquid nitrogen and placed in a whirlpak (Thermo Fisher Scientific, Lenexa, KS, USA). Cuttings were placed in -80°C until lyophilization. After freeze drying, cuttings were ground into a fine powder using a mortar and pestle. Dry weights (DW) were then recorded, and 50 mg of tissue was placed in a 15 mL centrifuge tube. Mannitol (1 $mg \cdot mL^{-1}$) was added to the sample as an internal standard. Soluble sugars were extracted using 80% EtOH.

Three mL of EtOH was added to each sample, vortexed for five minutes and heated in an 80°C water bath for 5 min, followed by centrifuging at $3{,}000_{ng}$ at 4°C for 5 min. The sample was then decanted into a new 15 mL tube. This process was repeated two more times to attain 9 mL of liquid. Extracts were then dried *in vacuo* (ISS110 Integrated SpeedVac, Savant Instruments, Inc., Holbrook, NY, USA) for 24 h at 24°C. Once dried, samples were reconstituted with 1 mL of deionized (DI) water (0.2µm filter, Draco Inc, Durham, NC, USA), vortexed and moved to a 1.5 mL microcentrifuge tube (Thermo Fisher Scientific, Lenexa, KS, USA). Samples were centrifuged at 10,000 rpm for five min., decanted to a new tube and centrifuged again for 5 min. Samples were again decanted, but into a 2 mL microcentrifuge tube with a 0.22 µm nylon filter (Corning, New York, USA). Samples were centrifuged until all liquid passed through the filter.

Carbohydrate quantification

Filtered extract (750 µL) was aliquoted to a 2 mL autosampler vials (Thermo Fisher Scientific, Lenexa, KS, USA). Soluble carbohydrates (sucrose, glucose, and fructose) were separated using a high performance liquid chromatograph (LaChrom Elite, Hitachi, Pleasanton, CA, USA) equipped with a refractive index detector, a 50 mm × 4.6 mm BP-100 Ca^{++} guard column, and a 200 mm × 7.8 mm BP-100 Ca^{++} carbohydrate column (Benson Polymeric Inc, Reno, Nevada, USA). Flow rate was set at 0.4 $mL \cdot min^{-1}$ with an oven temperature of 60°C. Area under the corresponding carbohydrate peaks was calculated using Elite EZChrom software (Agilent Technologies, Inc., Pleasanton, CA, USA). Areas were used to calculate totals for each soluble carbohydrate using formulas obtained by calibration curves of the respective standards. The carbohydrate levels were then calculated to represent sucrose, glucose and fructose in mg per gram of DW ($mg \cdot g^{-1}$). A second technical replication was run for all samples.

Experimental design

The experiment was replicated three times in a complete randomized design on 9 Sept., 27 Sept., and 18 Oct. 2016. Cuttings were harvested from 8:00 a.m. to 9:00 a.m. and processed to leave 2 – 3 mature leaves with a stem caliper between 4 – 6 mm. Cuttings were sorted by leaf number and stem caliper and then dispersed into five groups of twelve cuttings per cultivar. Fresh weights (g) were recorded and then groups of twelve cuttings were placed in separate zip seal bags (16.3 cm × 8.2 cm) with three holes for air circulation. All ten bags were placed in a cardboard shipping box at 10°C.

One group of each cultivar was randomly selected and removed from storage every 2 d for 8 d. First, cuttings were rated on a scale of 1 – 5: 1, dead; 2, poor, extremely wilted; 3, average, slight wilt; 4, good, turgid; 5, excellent, fully turgid. Then, ASFW (g) was measured. Six of the twelve cuttings were randomly selected for carbohydrate analysis, while the other six were directly stuck into pre-moistened 26 count plug strips containing a peat-perlite-vermiculite mix (Oasis® Fertiss® Plant Propagation System, Plug Size: 3 cm × 4 cm – Round, Smithers-Oasis, Kent, OH, USA). Trays were placed under mist, and after 24 h, turgidity was rated again. At the end of four weeks, rooting was rated using a scale of 1 – 5: 1, no roots; 2, 1-5 roots less than 2.5 cm; 3, 1-5 roots at least 2.5 cm, or 6-10 roots less than 2.5 cm; 4, 6-10 roots at least 2.5 cm, or 10+ roots less than 2.5 cm; 5, 10+ roots 2.5 cm or longer.

Statistical analysis

Data were analyzed using SAS (Version 9.4, SAS Institute, Inc., Cary, NC). Mean separation was determined using Generalized Linear Model (GLM) and Tukey's Studentized procedures at $\alpha = 0.05$

5.3 Results & Discussion

Analysis of weight and turgidity loss

Cultivars did not differ in weight loss. As storage duration increased cuttings lost significantly more weight, except when stored for 2 or 4 d where cuttings lost 71.96 and 91.82 $mg \cdot g^{-1}$ FW respectively (Table 1). By the end of 8 d in storage, cuttings averaged a loss of 151.69 $mg \cdot g^{-1}$ FW or 8% of their total weight, corresponding with an average loss of 1.7 in shoot rating based on appearance of the cutting turgidity. Weight loss and shoot rating were highly correlated and significant ($r = 0.9795$, $P<0.001$) (Table 2). There was no significant correlation between rooting and weight loss or shoot rating loss. All cuttings recovered to a rating of 5, fully turgid, after 24 hours under mist during propagation, which could explain this lack of correlation.

The fact that cuttings recover regardless of any degree of wilting is promising that stressed cuttings can still be used. However, growers should still be cautious when using stressed cuttings, because they may affect crop schedules if development is delayed. Water stress has been connected to root formation (Svenson, 1995). It also commonly known in the industry that cuttings wilt during propagation if leaf area is too large. In addition, wilted cuttings can reduce efficiency of workers and make automated sticking difficult. “Recovery” treatments of water, carbohydrates and endogenous growth regulators positively affected rooting of geranium, impatiens and poinsettia (Dole and Faust, personal communication). Some of these treatments may be able to overcome environmental stresses during shipping and allow production schedules to be maintained. However, there may be a limit to how well these solutions work depending on the species, how long cuttings have been in stressed conditions or the type of stress cuttings are experience.

Analysis of carbohydrates

Cultivars differed in total soluble carbohydrates (TS) (fructose + glucose + sucrose) (Table 3). WS had an average of 1.4 $mg \cdot g^{-1}$ DW TS more than PR, but PR had more TS at day 0.

As storage duration increased, TS levels decreased (Table 1), but PR lost TS faster than WS. Reducible sugars (RS), fructose and glucose, consistently made up over 50% of the TS, of which fructose was the main constituent. There was a significant interaction between cultivar and storage duration ($P = 0.0085$) for sucrose (Table 3). PR initially had higher concentrations of sucrose than WS, which decreased to be no different from sucrose level in WS by day two. After 2 d, PR concentrations of sucrose continued to decrease as storage duration increased. Sucrose is the main transportable carbohydrate in plants and correlated to rooting in *Pelargonium* (Rapaka, et al., 2005). Fructose and glucose decreased as storage duration increased, but levels were not significantly lower than unstored cuttings until stored for eight days. WS had significantly higher concentrations of fructose and glucose than PR.

Rooting performance and correlations

When the unrooted cuttings were placed under mist and propagated, WS had a higher root rating compared to PR ($P = 0.0032$) (Table 3). Across cultivars, cuttings stored for 2 d had the highest root rating, but it was not significantly higher than unstored cuttings or cuttings stored for 4 days. A storage duration of 6 or 8 d caused a significant reduction in root rating, so poinsettia could be stored for 4 days and still be readily propagated (Table 1). These findings match with results obtained by Einfield (2011), except a storage duration of 10 d did not affect rooting which could be caused by the difference in seasons when experiments were implemented.

Overall, PR had lower RR and carbohydrate levels than WS, and reduced levels of carbohydrates negatively impact rooting (Faust et al, 2016). One reason why root ratings showed a reduction after 2 days could be explained by the loss in carbohydrates needed for root formation. Glucose showed the strongest correlation with root rating (0.4824, $P \leq 0.01$) followed

by RS (0.4222, $P \leq 0.05$) and TS (0.4056, $P \leq 0.05$). Einfield (2011) also found that higher concentrations of RS increased rooting ($R^2 = 0.44$, $P < 0.01$). Besides nitrogen, rooting was also correlated to, sucrose concentrations for poinsettias tested by Zerche and Druege (2009). Rapaka et al. (2005) found the similar results with sucrose being connected to rooting in *Pelargonium*. Sucrose is a main transportable sugar, hence the need for carbohydrates for adventitious root formation. When multivariate correlation was run with PR and WS separate (data not shown), sucrose had the second strongest correlation for PR, but was not significant. Cultivars may differ in their ability to store carbohydrates as reducible sugars or in their ability to use reducible sugars or transport sucrose. Cultivars of apples (*Malus* sp.) and chrysanthemum (*Chrysanthemum* × *grandiflora*) were shown to have a wide variation in concentrations of individual sugars (Suni, 2000; Rajapakse et al., 1996). Carbohydrates could be converting to different forms throughout the storage times which was not studied in this experiment.

According to Zerche and Druege (2009), "adventitious root formation in poinsettia is basically not subject to carbohydrate availability", and that nitrogen is the main factor. However, Einfield (2011) and the current study both show some correlation between rooting and carbohydrates It could be that nitrogen allows carbohydrates to be effectively used for rooting. There is no doubt that nitrogen along with many other constituents within plants affect and work together to form adventitious roots. There is a large amount of research supporting interplay between hormones, nutrients and genetics in the role of adventitious root formation (da Costa et al., 2013). Determining which factor is most important for poinsettia rooting has yet to be determined. It will also be important to understand how to induce that factor's concentration during stock plant growth and maintain it during shipping. Cuttings can have the optimum

constituents needed for root formation when harvested and packaged, but if cuttings become stressed during shipping, those constituents could become depleted or unavailable.

Three major processes need to be implemented into the vegetative cutting industry. One, real time tracking of environmental conditions of shipments should be implemented to allow suppliers to determine shipments' condition before reaching the customer. A new shipment could then be sent before the spoiled shipment reaches a grower or rooting station. This will reduce lag time between receiving a damaged shipment and sending new cuttings, which will help keep planting schedules on time. Second, growers need a simple method of assessing the status of cutting shipments, which would allow them to determine if they should take the time and labor to stick the cuttings or request new cuttings and push back the finish date. Three, if a shipment has been stressed, growers need solutions and processes that will rehydrate and replace vital constituents, such as carbohydrates, so cuttings will root and grow at rates similar to a healthy cutting.

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Table 1. Analysis of the storage duration effect for weight loss (mg·g^{-1} FW), shoot rating loss, root rating, fructose, glucose, reducible sugars (RS), total sugars (TS) (mg·g^{-1} DW). Shoot ratings were based on a scale of 1 to 5: 1, dead; 5, fully turgid. Root ratings were based on a scale of 1 – 5:1, no roots; 5, 10+ roots 2.5 cm or longer. For each storage duration, means were an average of 72 cuttings for weight loss, shoot rating loss, and 36 for root rating, fructose, glucose, sucrose, RS, and TS.

	Storage duration (d)					
Response	0	2	4	6	8	Significance
Weight loss (mg·g^{-1} FW)	0.00 d[z]	71.96 c	91.82c	123.37 b	151.69 b	<0.0001
Shoot rating loss	0.00 d	0.80 c	1.30 b	1.5 ab	1.7 a	<0.0001
Root rating	2.10 ab	2.3 a	1.70 ab	1.6 b	1.6 b	0.0043
Fructose (mg·g^{-1} DW)	6.60 a	6.41 ab	6.03 ab	6.24 ab	5.43 b	0.0273
Glucose (mg·g^{-1} DW)	3.09 a	2.86 a	2.60 a	2.39 ab	1.79 b	<0.0001
RS[y] (mg·g^{-1} DW)	9.70 a	9.27 a	8.64 ab	8.62 ab	7.22 b	0.0020
TS[x] (mg·g^{-1} DW)	13.57 a	12.21 ab	11.01 bc	10.77 bc	9.04 c	<0.0001

[z]Mean separation in rows followed by the same letter are not significantly different according to Tukey's Studentized range test procedure at $\alpha = 0.05$.

[y]RS: reducing sugars (glucose + fructose)

[x]TS: total soluble sugars (RS + sucrose)

Table 2. Pearson Product-Moment Correlation matrix showing the strength of the linear relationship between the measured responses. Correlation used six replications made from averages across replications, storage duration, cultivar and 3 replicates of the experiment (n = 6).

	Weight loss	Shoot rating loss	Root rating	Fructose	Glucose	Sucrose	RS[z]	TS[y]
Weight loss	-	0.8528***	NS	NS	NS	-0.5835***	NS	-0.4144*
Shoot rating Loss	0.8528***	-	NS	NS	-0.3917*	-0.6768***	NS	-0.4813**
Root rating	NS	NS	-	NS	0.4824**	NS	0.4222*	0.4056*
Fructose	NS	NS	NS	-	0.8743***	0.5273**	0.9775***	0.9075***
Glucose	NS	-0.3917*	0.4824**	0.8743***	-	0.6724***	0.9570***	0.9473***
Sucrose	-0.5835**	-0.6768***	NS	0.5273**	0.6724***	-	0.6073***	0.8177***
RS[z]	NS	NS	0.4222*	0.9775***	0.9570***	0.6073***	-	0.9539***
TS[y]	-0.4144*	-0.4813**	0.4056*	0.9075***	0.9473***	0.8177***	0.9539***	-

[z]RS: reducing sugars (glucose + fructose)

[y]TS: total soluble sugars (RS + sucrose)

NS, non-significant or significant at *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Table 3. Analysis of weight loss (mg·g^{-1} FW), shoot rating loss, root rating, fructose, glucose, reducible sugars (RS), total sugars (TS) (mg·g^{-1} DW). Shoot ratings were based on a scale of 1 to 5: 1, dead; 5, fully turgid. Root ratings were based on a scale of 1 – 5: 1, no roots; 5, 10+ roots 2.5 cm or longer for the effect of cultivar. For each cultivar, means were an average of 180 cuttings for weight loss and shoot rating loss, and 90 for root rating, fructose, glucose, sucrose, RS, and TS.

Responses	'Prestige Red'	'White Star'	Significance
Weight loss (mg·g^{-1} FW)	91.03	84.33	NS
Shoot rating loss	1.0	1.1	NS
Root rating	1.6 b[z]	2.1 a	0.0032
Fructose (mg·g^{-1} DW)	5.55 b	6.75 a	<0.0001
Glucose (mg·g^{-1} DW)	2.31 b	2.78 a	0.0071
RS[y] (mg·g^{-1} DW)	9.52 b	7.86 a	<0.0001
TS[x] (mg·g^{-1} DW)	12.02 b	10.62 a	0.0052

[z]Means within a row followed by the same letter are not significantly different according to Tukey's Studentized range test procedure at $\alpha = 0.05$.

[y]RS: reducing sugars (glucose + fructose),

[x]TS: total soluble sugars (RS + sucrose)

Table 4. Analysis of the interaction effect cultivar and storage duration had on sucrose concentrations ($mg \cdot g^{-1}$ DW) of unrooted cuttings ($P = 0.0085$). Means were averaged across 18 replications.

	Storage duration[z] (d)				
Cultivar	0	2	4	6	8
Prestige Red	4.48 a[y]	3.25 b	2.27 bc	2.18 bc	1.63 c
White Star	3.27 b	2.62 bc	2.48 bc	2.11 c	2.01 c

[z]Duration of storage in number of days.

[y]Means followed by the same letter are not significantly different according to Tukey's Studentized range test procedure at $\alpha = 0.05$.

Figures

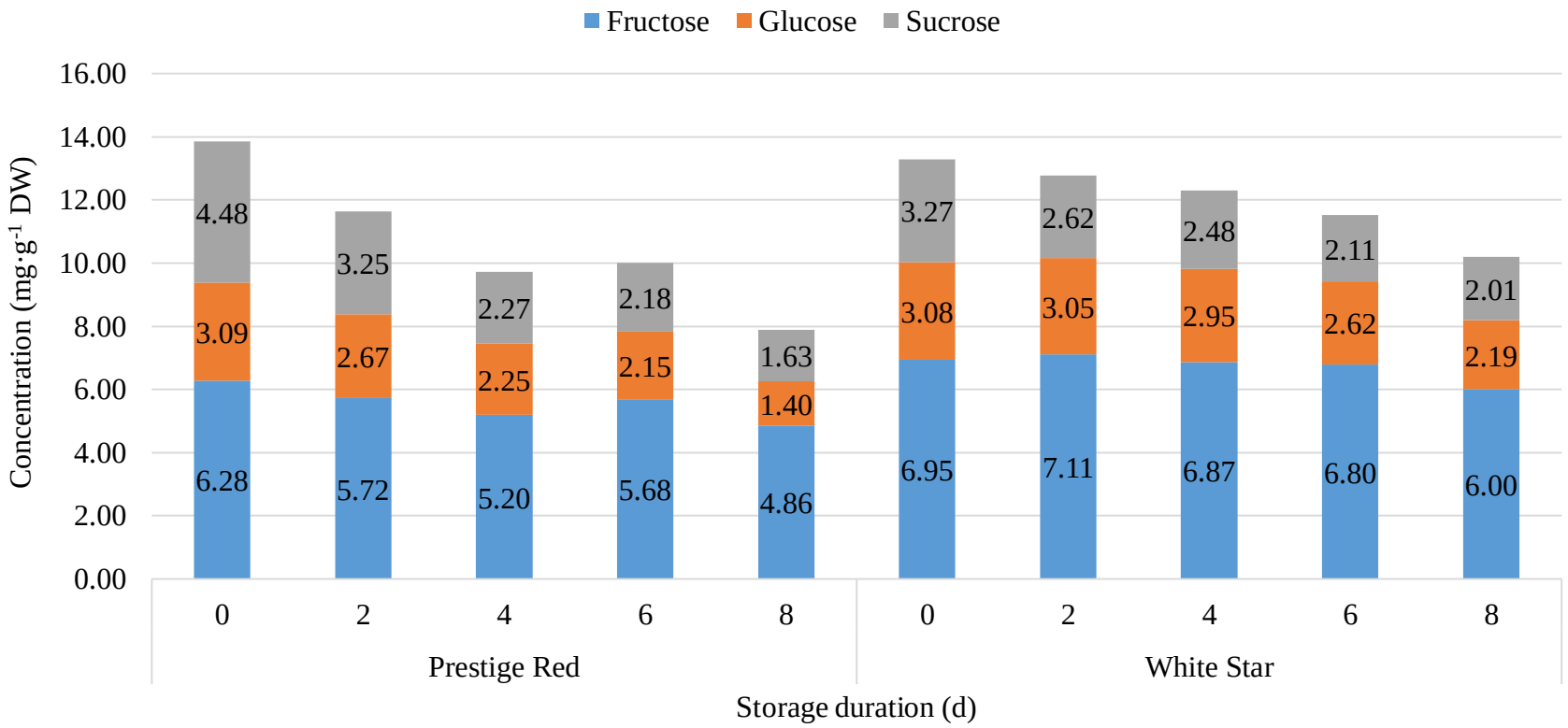

Fig. 1. Soluble carbohydrate concentrations of 'Prestige Red' and 'White Star' (WS) as storage duration increases. Interaction between cultivar and storage time was only significant for sucrose concentrations (P = 0.0085). All other responses were significant to cultivar and storage duration main effects, in which 'White Star' had significantly higher concentrations across storage time for fructose, glucose, fructose + glucose (reducible sugars), and total soluble carbohydrates. Carbohydrate means were averages of 18 replications for each storage duration and cultivar.