MANAGING BOTRYTIS BLIGHT IN GREENHOUSE ORNAMENTALS THROUGH HOST RESISTANCE AND BIORATIONAL PRODUCTS

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ABSTRACT

MANAGING BOTRYTIS BLIGHT IN GREENHOUSE ORNAMENTALS THROUGH HOST RESISTANCE AND BIORATIONAL PRODUCTS

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Botrytis cinerea, an airborne necrotrophic fungus is one of the most important and destructive pathogens of greenhouse-grown ornamental crops. The pathogen incites leaf, stem, and flower blight reducing plant quality and marketability. The overall goal was to provide growers with disease management options by combining host resistance and biorational products. Geranium and petunia cultivars were screened for resistance and biorational products were evaluated for efficacy against *B. cinerea*. 'Ringo 2000 Violet' and 'Maverick Scarlet Picotee' geranium was significantly more susceptible than 'Pinto Premium Orange' and 'Horizon Coral Spice'. Most of the biorational products applied to 'Pinto Premium Orange' effectively controlled *B. cinerea.* In contrast, only few products provided effective control in 'Ringo 2000 Violet'. In 'Pinto Premium Orange', disease assessment indicated that *Bacillus amyloliquefaciens*, *Pseudomonas chlororaphis*, *Aureobasidium pullulans*, and extract of *Swinglea glutinosa* provided a level of efficacy similar to the fungicide standard fenhexamid. In 'Ringo 2000 Violet', *S. glutinosa* resulted in protection similar to the fenhexamid across both trials. 'Tidal Wave Cherry' petunia had significantly higher disease severity and AUDPC values than 'Sophistica Blackberry' for each trial. According to AUDPC value, 'Shock Wave Red' had significantly less disease than 'Tidal Wave Cherry' and was similar to 'Sophistica Blacberry'. Efficacy of biorational products on 'Shock Wave Red' petunia showed that *A. pullulans* and *Gliocladium catenulatum* effectively limited disease similar to fenhexamid. Host resistance could reduce fungicide inputs and be used in combination with biorational controls for effective Botrytis blight control.

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INTRODUCTION

Ornamental production is an important agricultural enterprise in the U.S.; plants may be grown in the field/outdoor shaded areas or in greenhouses (Daughtrey and Benson, 2005). The value of floricultural crops in the U.S. for the 6,386 growers who produce a revenue of $10,000 or more, was nearly $4.63 billion in 2018. The total production area included 859 and 39.3 million m^2 for covered and greenhouse space, respectively (USDA, National Agricultural Statistics, 2019). Michigan ranks third in the U.S., behind California and Florida, in the production of floriculture crops, accounting for 10% of the total wholesale value of $467 million in 2018. In the same year, there were 569 floriculture crop producers in the state with $10,000+ in sales with a total of 4.45 million m^2 of greenhouse space (USDA, National Agricultural Statistics, 2019). Michigan leads the nation in production of flats of seeded geraniums, petunias, begonias and impatiens; hanging baskets of geraniums from either seed and vegetative cuttings, petunias, begonias, impatiens and pansies/violas; and potted geranium, petunias, peony and Easter lilies (USDA, National Agricultural Statistics, 2019). The wholesales value for production of flats of geraniums (vegetative cuttings) in the U.S. in 2018 was $7.6 million with total sales of $1.15 million in Michigan just behind California with sales of $3.84 million. The total wholesale value of hanging baskets and geranium pots (vegetative cuttings) were $30.56 million and $81.64 million respectively in 2018, Michigan being the highest producer in nation with sales value of $7.47 million for hanging baskets and $12.45 million for geranium pots. Similarly, petunias have a total wholesale value of $141.7 million when sold in 2018 as flat, pots and as hanging baskets in the U.S; the sales value in Michigan was $31.3 million. Michigan ranks first for the production of geranium (17%) and petunia (22%) with the highest total wholesale values throughout the nation (USDA, National Agricultural Statistics, 2019).

Disease management is a concern of ornamental crop growers as marketing depends on the aesthetics of the plant (Daughtrey and Benson, 2005). Greenhouse-grown ornamentals are susceptible to Botrytis blight or grey mold disease (Hausbeck and Moorman, 1996) incited by *Botrytis cinerea* (telemorph: *Botryotinia fuckeliana*), an airborne necrotropic fungi belonging to Sclerotiniaceae family on Helotiales order under Ascomycota division (Whetzel, 1945). Considered to be one of the most destructive pathogens both pre-and post-harvest (Dean et al., 2012), it causes damping-off, stem canker, blossom and leaf blight, and bud, stem, crown and blossom end fruit rot (Williamson et. al., 2007, Jiang et. al., 2018). Other diseases include damping-off of young seedlings, leaf spot and root rot of corms, rhizomes, tubers, seeds (Hausbeck and Moorman, 1996). Globally, more than 200 crop species are affected by *B. cinerea* including ornamental plants, vegetables and fruits (Moyano et al., 2004; Williamson et al., 2007; Hahn, 2014). The average cost to protect crops from this pathogen (cultural measures, fungicides, biocontrol) is approximately $51.98 /ha with a global expense of approximately $1.3 billion annually (Steiger, 2007; Dean et al., 2012). Average protection costs against *B. cinerea* vary between $19.5/ha for pumpkin in China to more than $169/ha for citrus in Japan (Steiger, 2007). The cost of limiting *B. cinerea* in grape represents 50% the total market value. However, the pathogen also causes noble rot in grape bunches used to produce valuable sweet wines (Dean et al., 2012).The cost of controlling this pathogen is 5% of total botrytis market for ornamentals, bulb vegetables and leafy vegetables, 7% in cucurbits and 9% in solanaceous crops (Steiger, 2007).

B. cinerea is problematic all over the world ranging from tropical and subtropical to temperate cold regions and can remain active at the temperature of 0^0c which makes it an important pathogen even during storage and shipping (Elad et al., 2007). It infects crops

growing in both the greenhouse and field, causing crop damage when conditions favor disease (Elmhirst et al., 2011). Production of vegetables and ornamentals in the greenhouses favors grey mold as warm temperatures, high relative humidity, free moisture, and a lack of air exchange provide favorable environmental conditions for the pathogen (Elad and Shtienberg, 1995; Paulitz and Belanger, 2001).

SIGN AND SYMPTOMS

Grey mold symptoms and signs include water-soaked tissue, necrotic spots, soft rot, and powdery grey conidial masses on the surface of infected tissue (Williamson et al., 2007). In some cases, tiny, round, black resting spores called sclerotia may form on infected tissue. *B. cinerea* reproduces on dead, decaying host tissue and organic matter and sporulates producing grey conidia (Punja and Utkhede, 2003). Infection may be initiated on dead flowers and then spread to other tissue. Infection may occur via conidia that germinate and infect susceptible tissue or from mycelium growing from infected to healthy tissue (Moorman and Lease, 1992).

EPIDEMIOLOGY

B. cinerea is an ubiquitous fungus that infects the leaves, flowers, and fruits of ornamentals, small fruit crops and vegetables (Elad and Shtienberg, 1995). It may survive in the short term as mycelium, conidia or chlamydospores or for longer periods as sclerotia (Holz et al., 2007; Williamson et al., 2007). The pathogen produces large amount of conidia in the asexual cycle which serve as primary inoculum. Sclerotia are the primary structures for pathogen survival which germinate primarily by producing conidiophores. Germination of sclerotia is favored by low temperature with the optimum temperature of 5^0C. Sclerotia may germinate and produce apothecia in the field to initiate the sexual cycle but the apothecial stage is rarely found

for most of the *Botrytis* species including *B. cinerea* (Coley-Smith, 1980; Hahn, 2014).

Conidia are the primary inoculum with optimum germination occurring at 20^0C. The optimum temperature for infection is between 15 to 25^0C. (Jarvis, 1989). Temperature influences the germination of conidia and lesion development and can occur between 4 and 25^0C; germination is inhibited at 30^0C (Salinas et al., 1989). The optimum temperature for conidial germination is 22 to 25^0C with RH > 90%. Relative humidity of 100% for 5 hrs is sufficient for disease infection at room temperature (Salinas et al., 1989). The wet and humid conditions in the greenhouse from misting during propagation promotes conidial germination and expansion and coalescence of lesions which reduces plant quality (Hausbeck and Pennypacker, 1991b).

Conidia are oval or globose one-celled hyaline structures produced by conidiophores and borne in clusters on short sterigmata (Pande et al., 2002). They are short lived and influenced by temperature, light, moisture and microbial activity (Holz et al., 2007). In some *Botrytis* species, the septate and brown mycelium can survive for relatively longer periods in bulbs and other vegetative parts and can overwinter as mycelium in the bark and buds of infected grape vines. (Coley-Smith, 1980). *Botrytis cinerea* is a problem in the storage and shipping of geranium cuttings as conidia are deposited on the plant's surface and may infect and cause disease during the environmental conditions associated with shipping (Hausbeck and Pennypacker, 1991b).

Conidia are dispersed from infected plants when there is a rapid decline in relative humidity which often occurs mid-morning. When there is rapid fluctuation in the relative humidity, the conidia are released through a hygroscopic mechanism (Jarvis, 1989). Maximum conidial dispersal occurs when the relative humidity fluctuates rapidly between 85 and 65%; vigorous hygroscopic movement of the conidiophore occurs with a 5% change within this range (Jarvis, 1960). The peak atmospherical conidial concentration among geraniums in a greenhouse

was associated with grower activity including watering, fertilization, pesticide application and harvesting cuttings (Hausbeck and Pennypacker, 1991a, 1991b). Conidial dispersal within the greenhouse is influenced by the magnitude of previous dispersals; a high concentration occurring on one day may be followed by a reduced concentration the following day (Hausbeck and Moorman, 1996). *B. cinerea* conidia can be carried by the insect *Drosophila melanogaster* on its cuticle and in its digestive canal. Conidia germinate in the insect's gut and develop into mycelium which may differentiate into microsclerotia and survive throughout the insect's life (Louis et al., 1996)

The geranium foliage infected with *B. cinerea* was greater than that of petunia and impatiens when inoculated and incubated under similar environmental conditions (Pritchard, 1995). Sporulation incidence when geranium leaves were inoculated was high for one-wk-old leaves, declined when the leaves were 4 wks old and increased when leaves were 4- to 10-wks old (Sirjusingh et al., 1996). *B. cinerea* sporulated more rapidly in one and 10-wk old leaves at 25^0C when leaves were wet for 8 – 24 hr (Sirjusingh and Sutton, 1996). Sporulation of the pathogen on inoculated geranium flowers increased when the wetness duration increased from 8 to 24 h at 15^0C and from 4 to 6 h at 30^0C. Sporulation was more efficient and prominent when conidia were inoculated directly to leaves compared to petals of geranium (Sirjusingh and Sutton, 1996).

GROWTH IN CULTURE MEDIA

The maximum germination of *B. cinerea* conidia occurred at 20^0C after 24 hrs of incubation on potato dextrose agar (PDA) media. *B. cinerea* mycelial growth on PDA media at 98-100% RH for 24 hr increased up to 20^0C, but decreased rapidly above 25^0 and died at 35^0C

(Ahmed et al., 2014, Van Den Berg and Lentz, 1968). *Botrytis convulata* grows on PDA when incubated for 5 to 7 days at 24^0C in darkness and then exposed to continuous white fluorescent light for 6 days or until colonies are covered with conidiophores (Maas, 1969). Prune extract lactose yeast extract agar (PLY) is used to culture *B. allii*; growth increases from 5^0C to 20^0C but is slowed above 30^0C with no growth at 35^0C (Alderman and Lacy, 1981). The optimum temperature for mycelial growth of *B. cinerea* on potato sucrose agar (PSA) medium was 24-28^0C with sporulation observed at 24^0C after 3 days of inoculation and reached a maximum 4 and 6 days after inoculation (Shiraishi et al., 1970b). Conidia germinate in the range of temperature 5-32^0C but at 10^0C germination is delayed with only 60% conidia germination within 48 hrs with an optimum temperature of 20-30^0C. (Shiraishi et al., 1970a). A selective medium for growth and sporulation of *B. cinerea* known as *Botrytis* selective medium (BSM) has been prepared by Kritzman and Netzer (1978) that contains fungicides and tannic acid resulting in brown pigmentation after oxidization indicating the growth of *Botrytis*.

DISEASE ASSESSMENT

Disease may be assessed based on the number of necrotic leaves and sporulation of *B. cinerea*. Scoring is based on the total diseased leaf area on each plant with visual scale rating of 0 to 10 where 0 = no lesions, 1= lesions with 1-10% leaf area covered, 2= 11-20%, 3= 21-30%, 4= 31-40%, 5= 41-50%, 6= 51-60%, 7=61-70%, 8= 71-80%, 9= 81-90%, and 10= 91-100% of the leaf area affected by the pathogen (Elmhirst et al., 2011). Köhl et al. (1998) assessed plants in the greenhouse based on the area (%) covered with conidiophores of *B. cinerea* ranging from >0 to 1 at an interval of 0.1. The disease severity indicates the number of leaves covered with *B. cinerea* sporulation (spore producing leaf area) and is estimated using the formula: Severity=

$\frac{\sum_{i=1}^{n} \sum_{j=1}^{m_i} P_{ij}}{n}$, where i= number of plants (1 to n), m_i= no. of sporulated leaves, P_{ij} = proportion of jth leaf on plant i which have sporulation (Köhl et al., 1998).

DISEASE MANAGEMENT

Effective management of *B. cinerea* in the greenhouse requires an integrated approach including manipulation of the environment, biocontrol agents, and fungicides (Jarvis, 1989). Hausbeck and Pennypacker (1991a) suggested that applying fungicides or modifying the greenhouse environment should be timed immediately after the harvest of cuttings from geranium stock plants. Sanitation, use of a photo-selective greenhouse covering, heating systems, and timely application of fungicides are important tools to manage *B. cinerea* in greenhouses (Hausbeck and Moorman, 1996).

CULTURAL CONTROL

Botrytis cinerea commonly occurs in the greenhouse where the relative humidity may be high. Reducing the relative humidity in the greenhouse can be achieved by venting and heating (Hausbeck and Moorman, 1996). Removing diseased, dead plant tissue, providing proper air circulation, increasing plant spacing, and avoiding plant wounds is necessary (Hausbeck et al., 1996). *Botrytis cinerea* can be managed using several management strategies but keeping the atmosphere dry is important (Gerlagh et al., 2001). Reducing moisture in the greenhouse can be achieved by installing a heating system under the bench, using plastic mulch on top of the pots in a stock plant scenario, and reducing plant density (Hausbeck and Moorman, 1996). Combining plastic mulch and an under the bench heating system reduced pathogen sporulation on stock plant leaves more effectively than the single treatments. Forced heated air was more effective in

reducing disease incidence than the plastic mulch (Hausbeck et al., 1996).

BIOLOGICAL CONTROL

Biological control is used predominantly as a preventive disease control measure and is generally not used post infection (Jacometti et al., 2010). Generally, biocontrol refers to the use of microbial organisms and natural product extracts that suppresses plant pathogens and limits disease (Pal and Gardener, 2006). Biocontrol agents for *B. cinerea* includes bacteria within the genera of *Bacillus* and *Pseudomonas*, filamentous fungi within the genera of *Ulocladium*, *Gliocladium* and *Trichoderma* and also within the genera of *Pichia* and *Candida* of yeast (Jacometti et al., 2010; Paulitz and Belanger, 2001). *Bacillus* species including *B. subtilis, B. amyloliquefaciens* and *B. mycoides* have various modes of action including competition, parasitism, antibiosis, and induction of systemic acquired resistance (Choudhary and Johri, 2009; Pal and Gardener, 2006; Paulitz and Belanger, 2001). The mode of action of *Trichoderma harzianum* includes mycoparasitism, competition for nutrients or space and inactivation of enzymes produced by pathogens (Vidhyasekaran, 2004). Similarly, *Gliocladium catenulatum* offers antagonistic activity through antibiosis and *Ulocladium oudemansi*, *Aureobasidium pullulans* competes with *B. cinerea* for nutrition (Castoria et al., 2001; Jacometti et al., 2010; Pal and Gardener, 2006)

Applying *Gliocladium catenulatum* (Prestop^{R} WP) on geranium limited Botrytis blight in the greenhouse and significantly reduced disease incidence and severity (Elmhirst et al., 2011). This biological control agent also limited Botrytis stem canker on greenhouse tomatoes (Utkhede and Mathur, 2006). *Trichoderma harzianum* (RootShield) and *Rhodosporidium diobavatum* S33 strain sprayed as curative treatment on the wounded surface of greenhouse tomato stems reduced

lesion expansion caused by *B. cinerea* increasing yield (Utkhede and Mathur, 2002). Elad (1994) reported the application of *T. harzianum* (0.5-1.0 g/l) in the vineyards significantly reduced grape gray mold disease incidence up to 78%. The *T. hamatum* 382 (T382) isolate suppressed Botrytis blight severity in begonia and geranium when applied as an amended form in the potting mix (Horst et al., 2005; Olson and Benson, 2007). Binucleate *Rhozoctonia* (BNR) applied in the potting mix before transplanting also induced systemic resistance and resulted in a reduction of disease symptoms on the geranium foliage (Olson and Benson, 2007).

Ingram and Meister (2006) found that *Bacillus subtilis* (Serenade ASO) and extract of *Reynoutria sachalinensis* (Milsana) significantly reduced grey mold disease severity of greenhouse tomatoes. Application of *Ulocladium atrum* on geranium stock plants decreased *B. cinerea* conidial production with reduced severity on necrotic leaves (Gerlagh et al., 2001). *Bacillus velezensis* (strains 5YN8 and DSN012) suppressed growth and conidial formation of *B. cinerea* on pepper through the secretion of secondary metabolites and release of volatile organic compounds (Jiang et al., 2018). Mycelial growth of *B. cinerea* was inhibited by *Azotobacter chroococcum*. Sporulation and severity on strawberry was reduced when *Chlorella vulgaris* was sprayed (El-ghanam et. al., 2015).

Aureobasidium pullulans, Gliocladium catenulatum and *Chaetomium globosum* reduced the Botrytis disease incidence and sporulation by 75% on stems of tomato and cucumber. Both *A. pullulans* and *G. roseum* completely prevented the disease on cucumbers grown in the greenhouse (Dik et. al.,1999). In cyclamen, applications of *U. atrum* and *G. roseum* decreased disease incidence with a reduced number of petioles becoming infected in the greenhouse (Köhl et al., 1998). Under highly conducive environmental conditions, *T. hamatum* 382 and binucleate *Rhozoctonia* (BNR) did not effectively control the *B. cinerea* on geranium. However, under a

less conducive environment they reduced the disease severity as effectively as chemical fungicides (Olson and Benson, 2007).

CHEMICAL FUNGICIDES

Complete host resistance to *B. cinerea* has not been identified for greenhouse ornamentals so fungicides are important (Yourman and Jeffers, 1999). The fungicides used to control of *B. cinerea* include: (i) the benzimidazole fungicides (carbendazim, benomyl, thiophanate methyl) with anti-microbial properties; (ii) phenylpyrrole fungicide (fludioxonil) and dicarboximide fungicide (iprodione) affecting fungal content of polyols, probably involved in osmoregulation; (iii) anilinopyrimidine fungicides (cyprodinil and pyrimethanil), a methionine biosynthesis inhibitor whose toxicity ability is reversed by amino acids; (iv) strobilurins (Quinone outside inhibitors or QoIs) fungicides (pyraclostrobin) being inhibitor of mitochondrial electron transport complex III and binding Qo site of cytochrome b; (v) phenylpyridinamine fungicide (fluazinam) and Succcinate dehydrogenase (SDHI) fungicide (boscalid) a toxicants affecting fungal respiration; and (vi) hydroxyanilide fungicide (fenhexamid) a sterol biosynthesis inhibitor (Leroux, 2007; Bardas et al., 2010; Hahn, 2014). In addition to these site-specific fungicides representing different mode of action, multisite inhibitors (dithiocarbamates, captan, chlorothalonil) have been used widely for a long period of time (Hahn, 2014). Two fungicide groups; benzimidazole and dicarboximides were initially highly effective against *B. cinerea* and were used intensively over decades (Elad and Shtienberg, 1995). According to a study regarding the efficacy of six different classes of fungicides by Kim et al. (2016), the phenylpyrrole fungicide (fludioxonil) was most effective in inhibiting mycelial growth, germination and conidiation (EC50 < 0.1µg/ml). Boscalid, tebuconazole, iprodione and fenpyrazamine have an EC50 in the range of 0.3 to 0.9 µg/ml. Pyrimethanil has an EC50 of 50 1µg/ml and is less

effective in inhibiting mycelial growth compared to fludioxonil. (Kim et al., 2016). Mixing azoxystrobin with carbendazim or iprodione or applying azoxystrobin in alternation with carbendazim and iprodione were not effective in limiting fungicide resistance in a *B. cinerea* population indicating multiple-resistance to different families of fungicides (Jiang et al., 2009). Using chemical fungicides with the same mode of action for a longer period to control *Botrytis* may result in pathogen resistance (Gerlagh et al., 2001).

FUNGICIDE RESISTANCE

Resistance to fungicides has developed among *B. cinerea* isolates (Kim et al., 2016). Resistance to benzimidazole (thiophanate-methyl) and dicarboximide (vinclozolin) was frequently detected in populations of *B. cinerea* in greenhouse-grown ornamentals (Yourman and Jeffers, 1999). Isolates resistant to the benzimidazole fungicide (benomyl) was detected in all greenhouse and double resistance to both benzimidazole and dicarboximide was detected in six greenhouses (Moorman and Lease, 1992). Yourman and Jeffers (1999) found that *B. cinerea* isolates resistant to dicarboximide were also resistant to benzimidazoles even though there had not been exposure to benzimidazole previously nor had the products been used for a long period. Negative-cross resistance has been reported between benzimidazoles (e.g. carbendazim) and phenylcarbamates (e.g. diethofencarb) (Leroux, 2007). Fungicide resistance may result from excessive use of fungicides with same mode of action, stability of fungicide-resistant isolates, or movement of resistant isolates via plant material while shipping from propagation to production greenhouses during various stages of plant growth. (Moorman and Lease, 1992; Yourman and Jeffers, 1999).

Cross resistance to dicarboximide and benzimidazole fungicides was found among isolates accounting for 65.8% of the total (Moyano et al., 2004). Resistance to three fungicide classes including dicarboximides (procymidone), benzimidazole (carbendazim) and N-phenylcarbamates (diethofencarb) was found in 14% of the isolates. Resistance to these three fungicide classes and anilinopyrimidines (pyrimethanil) was found in 3% of the isolates collected from commercial greenhouses of vegetable crops (cucumber, bean, tomato, squash, eggplant and pepper) in Spain (Moyano et al., 2004). Isolates collected from orchards treated with a pyraclostrobin and boscalid mixture were resistant to both with an EC50 value greater than 50mg/l for boscalid and 16 to >50 mg/l for pyraclostrobin; none were resistant to fludioxonil or fenhexamid (Bardas et al., 2010; Markoglou et al., 2006). Cross resistance studies showed that the mutation for pyraclostrobin resistance can reduce the sensitivity of mutant strains to other QoIs including azoxystrobin, fluoxastrobin, trifloxystrobin and picoxystrobin (Markoglou et al., 2006).

CULTIVAR RESISTANCE

Screening different cultivars of ornamental crops in the greenhouse has shown some partial resistance against *B. cinerea*. Uchneat et al. (1999a) found different levels of resistance when evaluating forty-five genotypes of *Pelargonium* against *B. cinerea* infection; two genotypes were consistently more resistant as measured by foliar lesion diameter. Uchneat et al. (1999b) studied floral infection to *B. cinerea* using sixty-two genotypes of *Pelargonium* species and found varying level of resistance with diploid genotypes having greater resistance than tetraploid. Also, *P. peltatum* cultivars were found more resistant than *P. x hortorum* with regards to floral infection and no correlation was observed between floral and foliar resistance (Uchneat et al., 1999b). Tian et al., (2019) screened 15 tree peonies for resistance to *B. cinerea* and found

different resistance levels with early flowering cultivars more resistant than late flowering cultivars. Krahl and Randle (1999) evaluated forty-eight petunia cultivars for resistance to *B. cinerea* and found a range of variation among cultivars over two seasons in the greenhouse; only one cultivar was consistently resistant. Also, inconsistencies among cultivars regarding resistance were observed when different methods of inoculation were used for screening. Similarly, fluctuations in ranking lisianthus cultivars were observed by Wegulo and Vilchez (2007) when comparing different inoculation methods for resistance against *B. cinerea.* Selected ornamental crops were favored by *B. cinerea* infection as measured by the proportion infection: geranium plants were more susceptible than petunia and impatiens under similar environmental conditions (Prichard et.al, 1999). Lisianthus plants were more susceptible than rose and gerbera (Vrind, 2005).

Plant resistance to *B. cinerea* depends on the rate of senescence, structural defense and defenses accelerated by the production of different hormones (Elad and Evensen, 1995). In biotrophs hypersensitive response is considered a major component for host resistance whereas the necrotrophic pathogens such as *B. cinerea* trigger a hypersensitive response for its pathogenicity. Hypersensitive response enhanced generation of reactive oxygen species which facilitates its colonization and increases pathogenicity by using the host defense mechanism (Govrin and Levine, 2000). Disease control for *B. cinerea* is difficult due to the ability of the pathogen to attack any plant growth stage. Senescent plant parts are easily invaded by the pathogen so the changes related with senescence can play a role in host resistance (Elad and Evensen, 1995). Defense mechanisms against *B.* cinerea may be mediated by jasmonic acid, salicylic acid, abscisic acid and ethylene signaling pathways; and are linked among a complex network (AbuQamar et al., 2017). Likewise, nitric oxide was found to have an important role in

host resistance of geranium against *B. cinerea* with early nitric oxides bursts and production of secondary nitric oxide stimulating noncell-death-associated defense (Floryszak-wieczorek et. al, 2007).

Host resistance is important due to the development of fungicide resistance by key pathogens (Elad and Evensen, 1995). Transgenic geranium plants with antimicrobial protein *Ace*-AMP1 have increased resistance to *B. cinerea* based on sporulation density. There was a significant negative correlation between disease incidence and protein level signaling the inhibitory effect of protein on disease development (Bi et al., 1999). Similarly, plants expressing mannitol dehydrogenase (MTD) protein also exhibited defense against *B. cinerea* although mannitol may act as a pathogenicity factor. Williamson et al. (2013) assessed effects of MTD expression on zonal geranium and showed that plants with overexpression of MTD have higher resistance to *B. cinerea.*

So, Botrytis blight is one of the most important disease of greenhouse ornamentals causing higher economic loss to the growers. Chemical fungicides have been used intensively to control this disease and the pathogen has developed resistance against the different classes of fungicides. Growers were interested on the alternative management options that helps to better control of disease and avoid the fungicide resistance. Exploring the host resistance of the greenhouse ornamentals and making choice of the resistance cultivar could help the growers to better design their management strategies. Also, the use of biorational products could be next alternatives for the control of Botrytis blight. The better understanding of the host resistance of cultivars and coupling with the biorational products could provide the growers with the good option for the management of Botrytis blight on the greenhouse ornamentals.

LITERATURE CITED

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1. AbuQamar, S., Moustafa, K., and Tran, L. S. P. 2017. Mechanisms and strategies of plant defense against *Botrytis cinerea*. Crit. Rev. Biotechnol. 37:262–274.

2. Ahmed, A., Zaman, S., Mazid, M., Rahman, M., Sarkar, M., Arbia, L., Ud-deen, M., and Kabir, G. 2016. Studies of *Botrytis cinerea* causing botrytis gray mold disease in chickpea (*Cicer arietinum* L.). J. Biosci. 22:69-76.

3. Alderman, S. C., and Lacy, M. L. 1984. Influence of temperature and water potential on growth of *Botrytis allii*. Can. J. Bot. 62:1567-1570.

4. Bardas, G. A., Veloukas, T., Koutita, O., and Karaoglanidis, G. S. 2010. Multiple resistance of *Botrytis cinerea* from kiwifruit to SDHIs, QoIs and fungicides of other chemical groups. Pest Manag. Sci. 66:967–973.

5. Bi, Y. M., Cammue, B. P. A., Goodwin, P. H., KrishnaRaj, S., and Saxena, P. K. 1999. Resistance to *Botrytis cinerea* in scented geranium transformed with a gene encoding the antimicrobial protein Ace-AMP1. Plant Cell Rep. 18: 835–840.

6. Castoria, R., De Curtis, F., Lima, G., Caputo, L., Pacifico, S., and De Cicco, V. 2001. *Aureobasidium pullulans* (LS-30) an antagonist of postharvest pathogens of fruits: Study on its modes of action. Postharvest Biol. and Technol. 22:7–17.

7. Choudhary, D. K., and Johri, B. N. 2009. Interactions of *Bacillus spp*. and plants - With special reference to induced systemic resistance (ISR). Microbiol. Res. 164:493–513.

8. Coley-Smith, J. 1980. Sclerotia and other structures in survival. Pages 85–114 In: The Biology of Botrytis. J. R. Coley-Smith, K. Verhoeff, and W. R. Jarvis, eds. Academic Press, London, UK.

9. Daughtrey, M. L., and Benson, D. M. 2005. Principles of plant health management for ornamental plants. Annu. Rev. Phytopathol. 43:141–169.

10. Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P.D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., and Foster, G.D. 2012. The top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 134:414–430.

11. Dik, A. J., Koning, G., and Köhl, J. 1999. Evaluation of microbial antagonists for biological control of *Botrytis cinerea* stem infection in cucumber and tomato. Eur. J. Plant Pathol. 105:115–122.

12. Elad, Y., and Shtienberg, D. 1995. *Botrytis cinerea* in greenhouse vegetables: chemical, cultural, physiological, and biological controls and their integration. Integr. Pest Manag. Rev. 1:15–29.

13. Elad, Ygal, and Evensen, K. 1995. Physiological aspcects of resistance to *Botrytis cinerea*. Phytopathology. 85:637-643.

14. Elad, Yigal. 1994. Biological control of grape grey mould by *Trichoderma harzianum*. Crop Prot. 13:35–38.

15. Elad, Yigal, Williamson, B., Tudzynski, P., and Delen, N. 2007. *Botrytis* Spp and disease they cause in agricultural system- An introduction. Pages 1-8. In: *Botrytis*: Biology, Pathology and Control. Y. Elad, B. Williamson, P. Tudzynski and N. Delen. (eds.) Kluwer Academic Publishers. Dordrecht, The Netherlands.

16. El-ghanam A. A, Farfour S. A., and Ragab, S. S. 2015. Bio-suppression of strawberry fruit rot disease caused by *Botrytis cinerea*. J. Plant Pathol. Microbiol. S3:005.

17. Elmhirst, J. F., Haselhan, C., and Punja, Z. K. 2011. Evaluation of biological control agents for control of botrytis blight of geranium and powdery mildew of rose. Can. J. Plant Pathol. 33:499–505.

18. Floryszak-wieczorek, J., Arasimowicz, M., Milczarek, G., Jelen, H., and Jackowiak, H. 2007. Only an early nitric oxide burst and the following wave of secondary nitric oxide generation enhanced effective defense responses of *Pelargonium* to a necrotrophic pathogen, New Phytol. 175:718–730.

19. Gerlagh, M., Amsing, J. J., Molhoek, W. M. L., Bosker-Van Zessen, A. I., Lombaers-Van Der Plas, C. H., and Köhl, J. 2001. The effect of treatment with *Ulocladium atrum* on *Botrytis cinerea*-attack of geranium (*Pelargonium zonale*) stock plants and cuttings. Eur. J. Plant Pathol. 107:377–386.

20. Govrin, E. M., and Levine, A. 2000. The hypersensitive response facilitates plant infection by the necotrophic pathogen *Botrytis cinerea*. Curr. Biol. 10:751–757.

21. Hahn, M. 2014. The rising threat of fungicide resistance in plant pathogenic fungi: *Botrytis* as a case study. J. Chem. Biol. 7:133–141.

22. Hausbeck, M. K., and Moorman, G. W. 1996. Managing *Botrytis* in greenhouse-grown flower Crops. Plant Dis. 80:1212-1219.

23. Hausbeck, M. K., and Pennypacker, S. P. 1991a. Influence of grower activity and disease incidence on concentrations of airborne conidia of *Brotytis cinerea* among geranium stock plants. Plant Dis. 75:798–803.

24. Hausbeck, M. K., and Pennypacker, S. P. 1991b. Influence of grower activity on concentrations of airborne conidia of *Botrytis cinerea* among geranium cuttings. Plant Dis. 75:1236–1243.

25. Hausbeck, M., Pennypacker, S., and Stevenson, R. 1996. The effect of plastic mulch and forced heated air on *Botrytis cinerea* on geranium stock plants in a research greenhouse. Plant Dis. 80:170-173.

26. Holz, G., Coertze, S., and Williamson, B. 2007. The ecology of *Botrytis* on plant surfaces. Pages 9-27 In: *Botrytis*: Biology, Pathology and Control. Y. Elad, B. Williamson, P. Tudzynski and N. Delen. (eds.) Kluwer Academic Publishers. Dordrecht, The Netherlands.

27. Horst, L. E., Locke, J., Krause, C. R., McMahon, R. W., Madden, L. V., and Hoitink, H. A. J. 2005. Suppression of *Botrytis* blight of begonia by *Trichoderma hamatum* 382 in peat and compost-amended potting mixes. Plant Dis. 89:1195–1200.

28. Ingram, D. M., and Meister, C. W. 2006. Managing *Botrytis* gray mold in greenhouse tomatoes using traditional and bio-fungicides. Plant Health Prog. 7:1–7.

29. Jacometti, M. A., Wratten, S. D., and Walter, M. 2010. Review: Alternatives to synthetic fungicides for *Botrytis cinerea* management in vineyards. Aust. J. Grape Wine R. 16:154–172.

30. Jarvis, W. R. 1960. An apparatus for studying hygroscopic responses in fungal conidiophores. Trans. Brit. Mycol. Soc. 43:525-528.

31. Jarvis, W. R. 1989. Managing diseases in greenhouse crops. Plant Disease. 73(3):190-194.

32. Jiang, C., Liao, M., Wang, H., Zheng, M., and Xu, J. 2018. *Bacillus velezensis*, a potential and efficient biocontrol agent in control of pepper gray mold caused by *Botrytis cinerea*. Biol. Control. 126:147–157.

33. Jiang, J., Ding, L., Michailides, T. J., Li, H., and Ma, Z. 2009. Molecular characterization of field azoxystrobin-resistant isolates of *Botrytis cinerea*. Pestic. Biochem. Phys. 93:72–76.

34. Kim, J.-O., Shin, J.-H., Gumilang, A., Chung, K., Choi, K. Y., and Kim, K. S. 2016. Effectiveness of different classes of fungicides on *Botrytis cinerea* causing gray mold on fruit and vegetables. Plant Pathol. J. 32:570–574.

35. Köhl, J., Gerlagh, M., De Haas, B. H., and Krijger, M. C. 1998. Biological control of *Botrytis cinerea* in cyclamen with *Ulocladium atrum* and *Gliocladium roseum* under commercial growing conditions. Phytopathology. 88:568–575.

36. Krahl, K. H., and Randle, W. M. 1999. Resistance of petunia phenotypes to *Botrytis cinerea*. HortScience. 34:690–692.

37. Kritzman, G., and Netzer, D. 1978. A selective medium for isolation and identification of *Botrytis spp.* from soil and onion seed. Phytoparasitica. 6:3–7.

38. Leroux, P. 2007. Chemical control of botrytis and its resistance to chemical fungicides. Pages 195-222. In: *Botrytis*: Biology, Pathology and Control. Y. Elad, B. Williamson, P. Tudzynski and N. Delen. (eds). Kluwer Academic Publishers. Dordrecht, The Netherlands.

39. Louis, C., Girard, M., Kuhl, G., and Lopez-Ferber, M. 1996. Persistence of *Botrytis cinerea* in its vector *Drosophila melanogaster*. Phytopathology. 86:934-939.

40. Maas, J.L. (1969) Effect of time and temperature of storage on viability of *Botrytis convoluta* conidia and sclerotia. Plant Disease Reporter. 53:141-144.

41. Markoglou, A. N., Malandrakis, A. A., Vitoratos, A. G., and Ziogas, B. N. 2006. Characterization of laboratory mutants of *Botrytis cinerea* resistant to QoI fungicides. Eur. J. Plant Pathol. 115:149–162.

42. Moorman, G. W., and Lease, R. J. 1992. Benzimidazole and dicarboximide resistant *Botrytis cinerea* from Pennsylvania greenhouses. Plant Dis. 76:477–480.

43. Moyano, C., Gómez, V., and Melgarejo, P. 2004. Resistance to pyrimethanil and other fungicides in *Botrytis cinerea* populations collected on vegetable crops in Spain. J. Phytopathol. 152:484–490.

44. Olson, H. A., and Benson, D. M. 2007. Induced systemic resistance and the role of binucleate *Rhizoctonia* and *Trichoderma hamatum* 382 in biocontrol of Botrytis blight in geranium. Biol. Control. 42:233-241.

45. Pal, K. K., and Gardener, B. M. 2006. Biological control of plant pathogens. The Plant Health Instructor. 2:1117-1142.

46. Pande, S., Singh, G., Narayana Rao, J., Bakr, M. A., Chaurasia, P. C. P., Joshi, S., Johansen, C., Singh, S. D., Kumar, J., Rahman, M.M., and Gowda, C. L. L. 2001. Integrated management of *Botrytis* gray mold of chickpea. Information Bulletin no. 61. ICRISAT, Patancheru, Andhra Pradesh, India.

47. Paulitz, T. C., and Belanger, R. R. 2001. Biological control in greenhouse system. Annu. Rev. Phytopathol. 39:103–133.

48. Pritchard, P. M. 1995. Influence of DIF on the susceptibility of floral crops to *Botrytis cinerea*. MS thesis. Michigan State University. East Lansing.

49. Punja, Z. K., and Utkhede, R. S. 2003. Using fungi and yeasts to manage vegetable crop diseases. Trends Biotechnol. 21:400–407.

50. Salinas, J., Glandorf, D. C. M., Picavet, F. D., and Verhoeff, K. 1989. Effects of temperature, relative humidity and age of conidia on the incidence of spotting on gerbera flowers caused by *Botrytis cinerea*. Neth. J. Plant Pathol. 95:51–64.

51. Shiraishi, M., Fukutomi, M. and Akai, S. 1970a. Effect of temperature on the conidium germination and appressorium formation of *Botrytis cinerea* Pers. Ann. Phytopathol. Soc. Japan. 36:234-236.

52. Shiraishi, M., Fukutomi, M., and Akai, S. 1970b. On the mycelial growth and sporulation of *Botrytis cinerea* Pers. and the conidium germination and appressorium formation as affected by the conidial age. Ann. Phytopathol. Soc. Japan. 36:230-233.

53. Sirjusingh, C., and Sutton, J. C. 1996. Effects of wetness duration and temperature on infection of geranium by *Botrytis cinerea*. Plant Dis. 80:160–165.

54. Sirjusingh, C., Sutton, J. C., and Tsujita, M. J. 1996. Effects of inoculum concentration and host age on infection of geranium by *Botrytis cinerea*. Plant Dis. 80:154-159.

55. Steiger, D. 2007. Global ecoonomic importance of *Botrytis* protection. Page 7. In: 14th International Botrytis Symposium. African Sun MeDIA Pty (Ltd.).

56. Tian, Y., Che, Z., Sun, D., Yang, Y., Lin, X., and Liu, S. 2019. Resistance identification of tree peony cultivars of different flowering time to gray mold pathogen *Botrytis cinerea*. HortScience. 54:328–330.

57. Uchneat, M. S., Spicer, K., and Craig, R. 1999a. Differential response to floral infection by *Botrytis cinerea* within the genus *Pelargonium*. HortScience. 34:718-720.

58. Uchneat, M. S., Zhigilei, A., and Craig, R. 1999b. Differential response to foliar infection with *Botrytis cinerea* within the genus *Pelargonium*. J. Am. Soc. Hortic. Sci. 124:76–80.

59. USDA-National Agricultural Statistics Service. 2019. Floriculture crops 2018 summary. Retrieved from: https://www.nass.usda.gov/Publications/Todays_Reports/reports/floran19.pdf

60. Utkhede, R. S., and Mathur, S. 2002. Biological control of stem canker of greenhouse tomatoes caused by *Botrytis cinerea*. Can. J. Microbiol. 48:550–554.

61. Utkhede, R. S., and Mathur, S. 2006. Preventive and curative biological treatments for control of *Botrytis cinerea* stem canker of greenhouse tomatoes. Biol. Control. 51:363–373.

62. Van Den Berg, L., and Lentz, C. P. 1968. The effect of relative humidity and temperature on survival and growth of *Botrytis cinerea* and *Sclerotinia sclerotiorum*. Can. J. Bot. 46:1477–1481.

63. Vidhyasekaran, P. 2004. Biological control – Microbial pesticides. Pages 239–270. In: Concise encyclopedia of plant pathology. Food Products Press. Binghamton, USA.

64. Vrind, T. A. 2005. The *Botrytis* problem in figures. Acta Hortic. 669: 99–102.

65. Wegulo, S. N., and Vilchez, M. 2007. Evaluation of lisianthus cultivars for resistance to *Botrytis cinerea*. Plant Dis. 91:997–1001.

66. Whetzel, H. H. 1945. A synopsis of the genera and species of the sclerotiniaceae, a family of stromatic inoperculate discomycetes. Mycologia. 37:648-714.

67. Williamson, B., Tudzynski, B., Tudzynski, P., and Van Kan, J. A. L. 2007. *Botrytis cinerea*: The cause of grey mould disease. Mol. Plant Pathol. 8:561–580.

68. Williamson, J. D., Desai, A., Krasnyanski, S. F., Ding, F., Guo, W., Nguyen, T., Olson, H. A., Dole, J. M., and Allen, G. C. 2013. Overexpression of mannitol dehydrogenase in zonal geranium confers increased resistance to the mannitol secreting fungal pathogen *Botrytis cinerea*. Plant Cell Tiss. Org. 115:367–375.

69. Yourman, L. F., and Jeffers, S. N. 1999. Resistance to benzimidazole and dicarboximide fungicides in greenhouse isolates of *Botrytis cinerea*. Plant Dis. 83:569–575.

CHAPTER 1.

EVALUATION OF GERANIUM CULTIVARS AND BIORATIONAL PRODUCTS TO CONTROL BOTRYTIS BLIGHT IN GREENHOUSE

ABSTRACT

Botrytis blight caused by the fungus *Botrytis cinerea* is one of the most important disease of greenhouse-grown ornamental crops. On geranium, it causes leaf, stem, and flower blight and decreases its marketability. Our objectives were to evaluate (i) susceptibility of geranium cultivars to *B. cinerea* and (ii) efficacy of different biorational products for control of Botrytis blight on geranium. Disease assessment included the number of blighted leaves, foliar lesions, and leaves with *B. cinerea* sporulation. Area under disease progress curve (AUDPC) was calculated to determine overall disease progress. Among the ten geranium cultivars evaluated, 'Pinto Premium Orange', 'Horizon Coral Spice' and 'Ivy Tornado White' were significantly more resistant than 'Ringo 2000 Violet', and 'Maverick Scarlet Picotee' for all measured parameters and AUDPC data. When ten treatments were compared in the efficacy trial of biorational products, *Aureobasidium pullulans* (Botector) and *Gliocladium catenulatum* (Prestop) effectively controled the disease according to AUDPC for blighted leaves and leaves with sporulating *Botrytis* in both 'Ringo 2000 Violet' and 'Pinto Premium Orange'. *Pseudomonas chlororaphis* (Zio), *Bacillus amyloliquefaciens* (Serifel), *B. subtilis* (Serenade Opti) and *B. mycoides* (LifeGard) were also effective in the moderately resistant 'Pinto Premium Orange' geranium based on AUDPC values for all measured parameters. AUDPC for leaves with sporulating *B. cinerea* showed that all biorational products included in the study effectively controlled *B. cinerea* except *Streptomyces lydicus* (Actinovate) in 'Pinto Premium Orange' geranium. The moderately resistant geranium cultivars could be used in combination with biorational controls for effective Botrytis blight control for a more sustainable management approach.

INTRODUCTION

The floricultural industry is an important contributor to U.S. agriculture producing $4.63 billion in revenue in 2018 (USDA-NASS, 2019). Geraniums are a popular flowering annual with a yearly revenue of $119.8 million which includes seeded flats ($7.6 million), hanging baskets ($30.5 million) and pots of cutting-propagated geraniums ($81.6 million) (USDA-NASS, 2019). Geranium is susceptible to the plant pathogen *Botrytis cinerea* (teleomorph: *Botryotinia fuckeliana*), an airborne necrotrophic fungus, considered to be one of the most common and destructive pathogens (Dean et al., 2012; Chandel and Kumar, 2018). Initial symptoms include water-soaked tissue, brown spotting, and soft rot resulting in blossom blight, leaf blight, bud rot, stem and crown rot, stem canker, and damping-off (Hausbeck and Moorman, 1996; Jiang et al., 2018; Williamson et al., 2007). The grey conidial masses that form on infected tissue are diagnostic and are commonly called grey mold (Punja and Utkhede, 2003; Williamson et al., 2007). The optimum temperature for conidial germination is 22 to 25^{0}C although germination of conidia and lesion development may occur between 4 and 25^{0}C (Salinas et al., 1989). The optimum temperature for infection is 15 to 25^{0}C. (Jarvis, 1989) Wet and humid greenhouse conditions especially during propagation promote conidial germination and coalescence of lesions which reduces plant quality (Hausbeck and Pennypacker, 1991). Infection may be intitated on senescing flowers or leaves near the moist soil surface (Hausbeck and Harlan, 2020).

Host resistance against *B. cinerea* in greenhouse ornamentals has not been identified (Yourman and Jeffers, 1999) although quantitative resistance to *B. cinerea* was observed among different genotypes of geranium by Uchneat et al. (1999b). Currently, successful limitation of *B. cinerea* requires an integrated approach including sanitation, environmental manipulation such as heating and venting, and timely application of effective fungicides (Jarvis, 1989; Hausbeck and

Moorman, 1996). The greenhouse environment including warm temperatures, high relative humidity, periods of leaf wetness, and limited air exchange favor Botrytis blight (Elad and Shtienberg, 1995; Paulitz and Belanger, 2001). Reliance on chemical fungicides with the same mode of action may result in pathogen resistance (Gerlagh et al., 2001). Resistance to fungicides has developed among *B. cinerea* isolates (Hahn, 2014; Kim et al., 2016). Resistance to benzimidazole (thiophanate-methyl) and dicarboximide (vinclozolin) were frequently detected in populations of *B. cinerea* in greenhouse-grown ornamentals (Yourman and Jeffers, 1999). Multiple fungicide resistance among *B. cinerea* isolates to various chemical classes with different modes of action has been reported on cut roses (Muñoz et al., 2019) and petunia (Samarakoon et al., 2017). Fungicide resistant *B. cinerea* isolates may be disseminated via plant material that is shipped from propagation to production greenhouses (Moorman and Lease, 1992; Yourman and Jeffers, 1999).

Biorational products offer an alternative to chemical fungicides. Suppression of *B. cinerea* has been reported from products containing species of *Bacillus, Pseudomonas, Ulocladium, Gliocladium, Trichoderma, Pichia* or *Candida* (Jacometti et al., 2010; Paulitz and Belanger, 2001). There are various mechanisms by which living organisms may suppress *B. cinerea* including induction of systemic acquired resistance (Choudhary and Johri, 2009), competition for nutrients, mycoparasitism and antibiosis (Pal and Gardener, 2006; Paulitz and Belanger, 2001; Vidhyasekaran, 2004). Foliar sprays of *Gliocladium catenulatum* reduced Botrytis blight incidence and severity on geranium (Elmhirst et al., 2011). Application of *Ulocladium atrum* to geranium stock plants decreased the percentage of necrotic leaves and pathogen sporulation (Gerlagh et al., 2001). *Trichoderma hamatum* (isolate 382) suppressed Botrytis blight severity in begonia and geranium when incorporated into the potting medium

(Horst et al., 2005; Olson and Benson, 2007). Binucleate *Rhizoctonia* (BNR) isolates BNR621 and P9023 added to the potting mix prior to transplanting induced systemic resistance and reduced foliar disease on geranium (Olson and Benson, 2007).

Host resistance combined with birational products would provide a sustainable disease approach. Our objectives were to: 1) Identify resistance to *B. cinerea* among selected geranium cultivars and 2) Evaluate the efficacy of biorational products in limiting Botrytis blight.

MATERIALS AND METHODS

Inoculation and incubation. A *B. cinerea* isolate was obtained from symptomatic geraniums growing in the Plant Science Greenhouses at Michigan State University (MSU), East Lansing, MI. Hyphae from symptomatic foliage was teased out of the tissue using a needle and placed onto potato dextrose agar media (PDA) (39 g PDA, 1000 ml H_20) in 10-cm diameter petri plates and grown for 14 days at 20-25^{0}C under continuous fluorescent light to prompt sporulation. Single spore cultures from this isolate were obtained by transferring conidia onto water agar media (16g agar, 1000 ml H_20) and placing them under the fluorescent light for approximately one week. A single hypha was selected from the water-agar media using a stereo light microscope and transferred to another culture plate containing PDA to establish a pure colony which was then stored in silica gel in the refrigerator at -4^{0}C.

Iron baskets (n=100) were bleached (Sodium hypochlorite (0.65%), Clorox germicidal bleach, The Clorox company, Oakland, CA) and placed in translucent plastic bags (21 x 5.5 x 38 cm^3) containing enough water to cover the bottom of basket for increased relative humidity (RH). Plants were selected and placed inside the iron basket and arranged in completely randomized design on a bench in the Plant Science Greenhouses at MSU that was shaded (80%).

To inoculate the geraniums, a conidial suspension was prepared by flooding 11-day-old *B. cinerea* cultures grown on PDA with sterilized distilled water and scraping with a spatula to dislodge the conidia. The conidial suspension was strained through cheesecloth and standardized to 1 x 10^6 conidia/ml solution using a hemocytometer. On 26 Oct, the *B. cinerea* conidial suspension was sprayed on each plant with a hand sprayer until run off. Each plant was enclosed in a translucent plastic bag containing water at the bottom that was sealed to provide high RH for incubation. The plants remained in the bags for the entire duration of the experiment. A Watchdog A-series data logger (Spectrum technologies Inc., Aurora, IL) was placed in one bag to record temperature and RH at hourly intervals.

Cultivar evaluation. Ten geranium cultivars (*Pelargonium x hortorum* and *P. peltatum*) representing different colors were chosen (Ball Horticultural Company, West Chicago, IL) (Table 1). Seed was sown in 128-cell plug trays containing soilless root medium (Suremix Perlite, Michigan Growers Products Inc, Galesburg, MI) on 16 Aug 2018 and placed on a bench in the Plant Science Greenhouses at MSU. Seedlings were transplanted on 1 Oct 2018 into square pots (10*10 cm^2) filled with soilless media and fertilized daily with 200 ppm water-soluble 20-20-20 water soluble NPK fertilizer (ICL Specialty fertilizers, Dublin, OH). Transplanted geraniums were drenched with the fungicide Subdue Maxx (0.08 ml/l, mefenoxam 22%, Syngenta Crop Protection, LLC, Greensboro, NC) to prevent root rot incited by *Pythium* spp. Average greenhouse air temperature during the growing period (16 Aug to Oct 26) was 23.6^{0}C and the maximum/minimum temperatures were 33.5^{0}C/13.5^{0}C. Ten plants from each cultivar served as single plant replicates. Disease was assessed 7 days after the inoculation on 2 Nov 2018 with subsequent assessments 13 (8 Nov) and 20 (15 Nov) days post inoculation. Average temperature was 18.4^{0}C, ranging from 12.2^{0}C – 21.9^{0}C with RH of 89 to 100% inside

the plastic bags during incubation. The experiment was repeated following the same procedure with geranium plants grown on the greenhouse (7 Mar to 17 May 2019). Plants were inoculated with *Botrytis* conidial suspension (17 May) and incubated inside the plastic bag. Disease was assessed 7, 13 and 20-days post inoculation on 24, 30 May and 6 Jun, respectively. Maximum/minimum temperature inside plastic bag during the incubation were 32.7^0C/21.3^0C with the average of 25^0C and RH of 91-100%.

Evaluation of biorational products. 'Pinto Premium Orange' and 'Ringo 2000 Violet' geraniums determined to be moderately resistant and highly susceptible to Botrytis blight, respectively, were included. Seeds were planted into 128-cell plug trays and grown in the Plant Science Greenhouses of MSU for 45 days after which they were transplanted into square pots (10*10 cm^2) filled with soilless root medium and fertilized daily with 200 ppm 20-20-20 water soluble NPK fertilizer (ICL Specialty Fertilizers, Dublin, OH). Treatments included ten biorational products, a fungicide standard, and an untreated inoculated control (Table 2). Five and six, single-plant replications of 'Pinto Premium Orange' (n=60) and 'Ringo 2000 Violet' (n=72), respectively, were included. Treatments were applied three times with a hand compressed air sprayer at weekly intervals on 19, 26 Sep and 3 Oct 2019, except *Gliocladium catenulatum* (Prestop) which was applied once due to the labeled application interval of 21 days. Plants were inoculated 1 day after the first treatment was applied on 20 Sep. Disease assessment was conducted on 26 Sep, and 3 and 10 Oct. Average temperature of 22.9^0C was recorded during the incubation inside plastic bag with max./min. temperature of 30.8^0C/18.9^0C. The experiment was repeated following the same procedure from 17 Oct to 7 Nov 2019. Treatments were applied on 17, 24 and 31 Oct at weekly interval. Plants were inoculated once with *Botrytis* conidial suspension (18 Oct), a day after the first application and incubated inside the plastic bag. Disease

assessment was done on 6, 13 and 20-days post inoculation on 24, 31 Oct and 7 Nov, respectively. Maximum/minimum temperature inside the plastic bags during the incubation were 21.4^0C/16.4^0C with the average of 21^0C and RH of 92-100%.

Table 1. Geranium species and cultivars evaluated for susceptibility to *Botrytis cinerea*.

Cultivars	**Species**
Bullseye Red	*Pelargonium x hortorum*
Horizon Coral Spice	*P.* x *hortorum*
Ivy Tornado White	*P. peltatum*
Maverick Scarlet Picotee	*P.* x *hortorum*
Multibloom Lavender	*P.* x *hortorum*
Nano Deep Rose	*P.* x *hortorum*
Pinto Pink	*P.* x *hortorum*
Pinto Premium Orange	*P.* x *hortorum*
Quantum Salmon	*P.* x *hortorum*
Ringo 2000 Violet	*P.* x *hortorum*

Table 2. Biorational products and a standard fungicide evaluated for efficacy against *Botrytis cinerea* on geranium.

Products	**Active Ingredients**	**Company**	**Rate/ 100 gal**	**Application Interval (days)**
Actinovate® SP	*Streptomyces lydicus* WYEC108 (0.037%)	Novozymes BioAg Inc.	12 oz	7
Botector®	*Aureobasidium pullulans* strain DSM 14940 (40%), DSM 14941 (40%)	Bio-ferm	10 oz	7
BotryStop™	*Ulocladium oudemansii* strain U3	BioWorks, Inc.	4 lb	7
EcoSwing™	Extract of *Swinglea glutinosa* (82%)	Gowan Company	2 pt	7
LifeGard™ WG	*Bacillus mycoides* (40%)	Certis USA	4.5 oz	7
Prestop® WP	*Gliocladium catenulatum* strain J1446 (32%)	Danstar Ferment AG	70 oz	21
PureCrop1	Soybean oil (10%), Corn oil (5%)	PureCrop1	200 oz	7
Serenade Opti® WP	*Bacillus subtilis* QST713 (26.2%)	Bayer CropScience Inc.	20 oz	7
Serifel®	*Bacillus amyloliquefaciens* strain MBI600 (11%)	BASF Corporation	16 oz	7
Zio™	*Pseudomonas chlororaphis* strain AFS009	SePRO Corporation	100 oz	7
Decree® 50 WDG	Fenhexamid (50%)	SePro Corporation	1 lb	7

Disease assessment and statistical analysis. The number of blighted leaves, foliar lesions and leaves with sporulating *B. cinerea* were counted. Area under the disease progression curve (AUDPC) was calculated to express the cumulative disease on the geranium plants by using the formula AUDPC = $\sum_{i=1}^{n-1}$ $[(y_i + y_{i+1})/2] \times (t_{i+1} - t_i)$ where y_i is the assessment of disease at i^{th} observation, t_i is the time (days) at the i^{th} observation and n is the total number of observations (Simko and Piepho, 2012). AUDPC was calculated for blighted leaves, foliar lesions and leaves with sporulating *B. cinerea*.

Data were analyzed with a one-way ANOVA using PROC GLM procedure of SAS statistical analysis software (SAS Institute Inc., Cary, NC, 2013) for the total number of blighted leaves, foliar lesions and leaves with the sporulating pathogen. The AUDPC of all the assessed parameters was calculated based on three ratings. The assumption of the normality was satisfied in all of the trials which was checked using residual plots. The homogeneity of the variance through Levene's test showed equal variance for the cultivar resistance trials and most of the biorational trials. Analysis of data for the biorational Trial 2 with 'Pinto Premium Orange' showed unequal variance so the re-analysis was conducted using Satterthwaite test for unequal variance for the adjustment in degree of freedom. LS Means were determined using PROC GLIMMIX procedure in SAS and statistical differences between treatments within the trials were compared by using Fisher's Least Significant Difference (LSD) t- test at the significance level of 0.05 (P = 0.05).

RESULTS

Cultivar evaluation. 'Horizon Coral Spice', 'Pinto Premium Orange' and 'Ivy Tornado White' had low numbers of blighted leaves, foliar lesions and leaves with sporulating *B. cinerea* for all assessed parameters in both trials and were significantly more resistant than 'Ringo 2000 Violet' and 'Maverick Scarlett Picotee' ($P<0.0001$) (Table 2). 'Quantum Salmon' was also less susceptible than 'Ringo 2000 Violet' and 'Maverick Scarlett Picotee' for all parameters (Trials 1 and 2) except for leaves with pathogen sporulation (Trial 2). 'Horizon Coral Spice', 'Pinto Premium' and 'Ivy Tornado White' were similar for blighted leaves and leaves with sporulating *B. cinerea* in both trials, but there were significantly more lesions for 'Horizon Coral Spice' than 'Ivy Tornado White' in Trial 1. 'Multibloom Lavender' and 'Nano Deep Rose' were similar to 'Ringo 2000 Violet' and 'Maverick Scarlett Picotee' in Trial 1. However, in Trial 2, 'Multibloom Lavender' and 'Nano Deep Rose' were significantly less susceptible ($P<0.0001$) than 'Ringo 2000 Violet' and 'Maverick Scarlett Picotee' for all parameters.

According to the AUDPC data for blighted leaves, foliar lesions and leaves with sporulating *B. cinerea* ($P=0.0032$), 'Horizon Coral Spice' and 'Pinto Premium Orange' (Trials 1 and 2) and 'Ivy Tornado White' (Trial 1) were more resistant than 'Ringo 2000 Violet' (Table 3). In Trial 1, 'Maverick Scarlet Picotee' and 'Multibloom Lavender' had similar AUDPC values for all parameters and were similar to 'Ringo 2000 Violet'. In Trials 1 and 2, 'BullsEye Red' and 'Pinto Pink' were susceptible to Botrytis blight and were similar to 'Ringo 2000 Violet' for all parameters according to AUDPC data.

Table 3. Mean number of blighted leaves, foliar lesions and leaves with sporulating *Botrytis cinerea* on geranium cultivars 20 days following inoculation.

Cultivars	Blighted leaves (no.)		Foliar lesions (no.)		Leaves with *B. cinerea* sporulation (no.)	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Ringo 2000 Violet	26.9 a	19.4 a	28.2 a	20.0 a	20.8 a	17.3 a[x]
Maverick Scarlet Picotee	25.2 ab	18.1 a	27.1 ab	20.3 a	18.7 a-c	15.4 ab
Multibloom Lavender	24.2 a-c	11.2 c	26.2 ab	12.0 c	19.6 ab	10.0 c
Nano Deep Rose	23.0 a-d	9.1 c	23.1 a-c	9.1 c	16.6 a-d	8.4 c
BullsEye Red	21.3 b-d	18.5 a	23.0 a-c	18.6 a	15.5 b-d	17.1 a
Pinto Pink	19.9 b-e	17.1 ab	22.3 bc	17.4 ab	14.1 c-e	16.2 ab
Quantum Salmon	19.1 c-e	12.4 c	19.9 cd	12.4 c	13.7 de	11.9 bc
Horizon Coral Spice	18.0 d-f	11.4 c	19.7 cd	12.3 c	12.8 d-f	9.9 c
Pinto Premium Orange	15.2 ef	11.4 c	16.4 de	11.8 c	10.5 ef	10.2 c
Ivy Tornado White	13.1 f	12.5 bc	13.6 e	12.9 bc	8.9 f	10.6 c

[x]Column means with a letter in common are not statistically different (Fisher's Protected LSD; P=0.05).

Table 4. Area under disease progress curve (AUDPC) values for blighted leaves, foliar lesions and leaves with sporulating *Botrytis cinerea* on geranium cultivars when inoculated.

Cultivars	AUDPC for blighted leaves		AUDPC for foliar lesions		AUDPC for leaves with *B. cinerea* sporulation[x]	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Ringo 2000 Violet	186.20 a-c	141.40 ab	201.9 a-c	106.00 ab	124.65 a-c	106.00 ab[y]
Maverick Scarlet Picotee	220.15 a	134.55 a-c	242.75 a	102.10 a-d	131.45 ab	102.10 a-d
Multibloom Lavender	194.60 ab	87.40 de	209.50 ab	70.05 de	141.30 a	70.05 de
Nano Deep Rose	181.70 a-c	54.40 e	184.65 b-e	44.55 e	122.35 a-c	44.55 e
BullsEye Red	166.55 b-d	149.15 a	190.25 b-d	125.25 a	95.15 cd	125.25 a
Pinto Pink	158.50 b-d	148.85 a	181.90 b-e	127.75 a	93.25 cd	127.75 a
Quantum Salmon	149.35 cd	115.45 a-d	158.30 c-e	103.90 a-c	99.25 b-d	103.90 a-c
Horizon Coral Spice	135.05 d	91.80 d	151.25 de	70.00 de	86.05 d	70.00 de
Pinto Premium Orange	132.25 d	102.45 cd	147.45 de	73.55 c-e	84.50 d	73.55 c-e
Ivy Tornado White	129.60 d	111.50 b-d	136.65 e	85.15 b-d	82.65 d	85.15 b-d

[x]Disease assessment were done on 7, 13 and 20-days post inoculation on 2, 8 and 15 Nov 2018 (Trial 1) and 24, 30 May and 6 Jun 2019 (Trial 2).
[y]Column means with a letter in common are not statistically different (Fisher's Protected LSD; P=0.05).

Figure 1. Highly susceptible (A,B) and moderately resistant (C,D) geranium cultivars observed 20 days after inoculation with *Botrytis cinerea* A: Ringo 2000 Violet, B: Maverick Scarlet Picottee, C: Pinto Premiun Orange, D: Horizon Coral Spice

Biorational evaluation on 'Ringo 2000 Violet'. Disease pressure was higher in Trial 1 than Trial 2. In Trial 2, Botrytis blight on the control plants was not advanced and was similar to the fungicide fenhexamid (Decree) for blighted leaves and foliar lesions (Table 4). However, according to the AUDPC data, plants treated with fenhexamid were less diseased than the control in both trials for all parameters. In Trial 1, most of the biorational products provided control similar to both the fungicide standard and the control according to the last observation and the AUDPC data. While applications of extract of *Swinglea glutinosa* (Ecoswing) resulted in protection similar to fenhexamid based on the last disease assessment (Trials 1 and 2) and the AUDPC values (Trial 2), AUDPC results from Trial 1 indicated that this biorational product was less effective than fenhexamid. *Pseudomonas chlororaphis* (Zio) (Trials 1 and 2) and *Streptomyces lydicus* (Actinovate) (Trial 2) were less effective than fenhexamid.

Table 5. Mean number of blighted leaves, foliar lesions and leaves with sporulating *Botrytis cinerea* on 'Ringo 2000 Violet' geranium treated with biorational products and a fungicide standard 20 days following inoculation.

Treatment (Trade name/active ingredient)	Blighted leaves (no.)		Foliar lesions (no.)		Leaves with *B. cinerea* sporulation	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Fenhexamid (Decree)	25.2 c	4.50 d	25.5 d	4.7 d	22.5 b	2.3 d[x]
Aureobasidium pullulans (Botector)	25.8 bc	13.0 bc	26.3 cd	13.0 bc	22.2 b	11.7 a-c
Bacillus amyloliquefaciens (Serifel)	28.0 a-c	18.0 ab	28.0 b-d	18.5 ab	26.2 ab	15.3 a
Gliocladium catenulatum (Prestop)	28.8 a-c	18.3 ab	29.8 a-d	18.3 ab	25.5 ab	15.5 a
Bacillus mycoides (LifeGard)	28.8 a-c	13.2 bc	29.3 a-d	13.0 bc	26.3 ab	11.8 a-c
Extract. *Swinglea glutinosa* (Ecoswing)	29.7 a-c	9.2 cd	30.3 a-d	9.2 cd	27.8 ab	6.7 cd
Bacillus subtilis (Serenade Opti)	29.8 a-c	15.5 a-c	30.3 a-d	15.5 a-c	28.2 ab	12.5 ab
Soybean and corn oil (PureCrop1)	31.3 a-c	15.5 a-c	32.3 a-d	15.5 a-c	29.5 ab	13.3 ab
Ulocladium oudemansii (BotryStop)	32.5 a-c	19.7 a	33.7 a-c	19.7 a	28.0 ab	16.2 a
Streptomyces lydicus (Actinovate)	33.5 ab	14.8 a-c	34.5 ab	14.8 a-c	30.5 a	12.7 ab
Pseudomonas chlororaphis (Zio)	35.2 a	12.2 bc	35.5 ab	12.2 bc	31.3 a	10.5 a-c
Untreated inoculated control	35.0 a	10.7 cd	36.2 a	10.7 cd	32.2 a	9.2 bc

[x]Column means with a letter in common are not statistically different (Fisher's Protected LSD; P=0.05).

Table 6. Area under disease progress curve (AUDPC) data for blighted leaves, foliar lesions and leaves with sporulating *Botrytis cinerea* on 'Ringo 2000 Violet' geranium treated with biorational products.

Treatment (Trade name/active ingredient)	AUDPC for blighted leaves		AUDPC for foliar lesions		AUDPC for leaves with *B. cinerea* sporulation[x]	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Fenhexamid (Decree)	191.92 c	35.58 d	207.67 d	39.67 d	125.42 c	13.42 c[y]
Aureobasidium pullulans (Botector)	215.83 bc	93.92 bc	224.00 cd	99.75 bc	157.50 bc	75.83 ab
Bacillus amyloliquefaciens (Serifel)	214.08 bc	141.17 ab	224.58 cd	147.58 ab	172.67 a-c	117.83 a
Gliocladium catenulatum (Prestop)	215.25 bc	135.33 ab	229.25 cd	138.83 ab	135.33 c	104.42 a
Bacillus mycoides (LifeGard)	242.67 a-c	113.75 a-c	255.50 a-d	113.75 a-c	184.92 ab	95.08 a
Extract. *Swinglea glutinosa* (Ecoswing)	258.42 ab	74.08 cd	282.33 a-c	75.25 cd	196.58 ab	50.75 bc
Bacillus subtilis (Serenade Opti)	240.92 a-c	120.75 a-c	250.25 b-d	127.17 a-c	172.08 a-c	96.25 a
Soybean and corn oil (PureCrop1)	274.17 ab	119.00 a-c	289.92 a-c	123.08 a-c	195.42 ab	93.91 a
Ulocladium oudemansii (BotryStop)	234.50 a-c	154.00 a	244.42 cd	159.83 a	152.83 bc	113.17 a
Streptomyces lydicus (Actinovate)	254.92 a-c	117.25 a-c	269.50 a-d	119.58 a-c	196.58 ab	89.25 ab
Pseudomonas chlororaphis (Zio)	292.83 a	105.00 a-c	323.17 a	105.58 bc	219.92 a	82.25 ab

Table 6 (cont'd)

Untreated inoculated control	289.33 a	95.08 bc	318.50 ab	99.17 bc	210.00 a	76.42 ab

[x]Disease assessment were done on 6, 13 and 20-days post inoculation on 26 Sept, 3 and 10 Oct 2019 (Trial 1) and 24 and 31 Oct; 7 Nov 2019 (Trial 2).
[y]Column means with a letter in common are not statistically different (Fisher's Protected LSD; P=0.05).

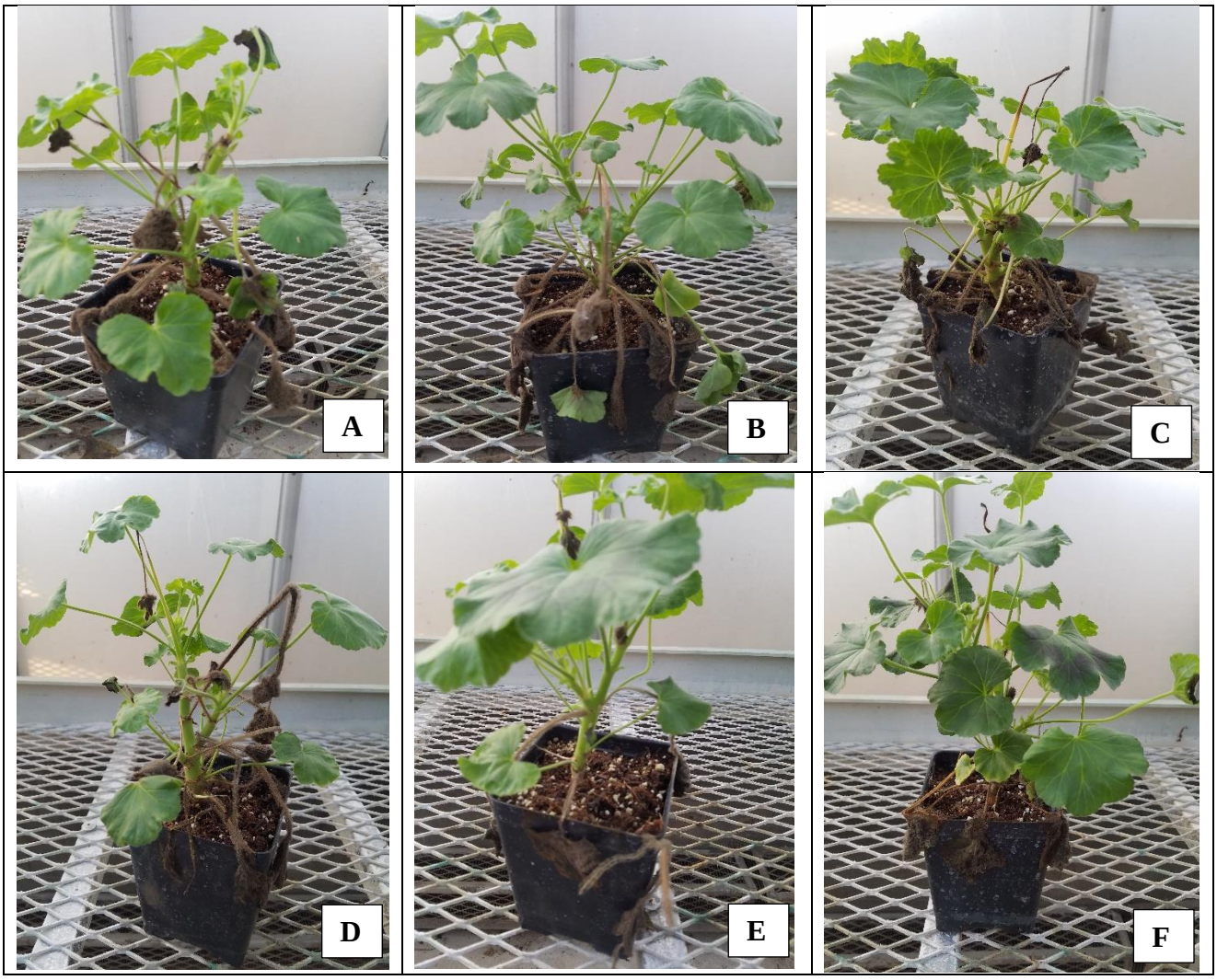

Figure 2. Botrytis blight on ‘Ringo 2000 Violet’ geranium when inoculated with *Botrytis cinerea* and treated with biorational products A: Untreated inoculated control, B: *Streptomyces lydicus* (Actinovate), C: *Pseudomonas chlororaphis* (Zio), D: Extract of *Swinglea glutinosa* (Ecoswing), E: *Aureobasidium pullulans* (Botector), F: Fenhexamid (Decree)

Biorational evaluation on 'Premium Orange'. In both trials, the disease assessment at the last observation and the AUDPC data for the fungicide fenhexamid (Decree) showed effective control with an exception of foliar lesions in Trial 2. Data associated with the last disease assessment for both trials indicated that *Bacillus amyloliquefaciens* (Serifel), *Pseudomonas chlororaphis* (Zio), *Aureobasidium pullulans* (Botector), and extract of *Swinglea glutinosa* (Ecoswing) were similar to the fungicide standard for the number of blighted leaves and lesions. The AUDPC data for these parameters indicated that *A. pullulans* (Trials 1 and 2), *Bacillus subtilis* (Serenade Opti) (Trial 2) and *B. amyloliquefaciens* (Trial 1) provided a level of efficacy similar to the fungicide fenhexamid.

Table 7. Mean number of blighted leaves, foliar lesions and leaves with sporulating *Botrytis cinerea* on 'Pinto Premium Orange' geranium treated with biorational products and a fungicide standard 20 days following inoculation.

Treatment (Trade name/active ingredient)	Blighted leaves (no.)		Foliar lesions (no.)		Leaves with sporulation	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Fenhexamid (Decree)	13.2 d	8.0 cd	14.6 d	10.2 bc	9.0 c	4.2 d[x]
Bacillus amyloliquefaciens (Serifel)	16.0 cd	13.4 a-c	16.2 cd	15.0 ab	13.8 bc	11.8 a-c
Pseudomonas chlororaphis (Zio)	16.6 cd	13.6 ab	19.2 b-d	15.2 ab	14.2 bc	12.0 a-c
Bacillus subtilis (Serenade Opti)	18.8 b-d	7.8 d	19.6 b-d	7.8 c	18.0 b	6.8 cd
Aureobasidium pullulans (Botector)	19.2 b-d	13.4 a-c	21.8 b-d	14.4 ab	16.2 bc	10.4 a-c
Extract. *Swinglea glutinosa* (Ecoswing)	20.0 b-d	12.8 a-d	21.0 b-d	13.0 a-c	16.8 bc	10.8 a-c
Gliocladium catenulatum (Prestop)	20.8 b-d	16.4 a	23.0 bc	16.4 a	16.0 bc	14.6 a
Bacillus mycoides (LifeGard)	22.4 bc	13.4 a-c	24.0 bc	13.4 a-c	21.2 ab	12.2 a-c
Streptomyces lydicus (Actinovate)	22.4 bc	15.2 ab	24.2 ab	15.6 ab	19.2 b	14.6 a
Soybean and corn oil (PureCrop1)	23.8 a-c	10.4 b-d	25.8 ab	11.4 a-c	19.6 b	8.8 b-d
Ulocladium oudemansii (BotryStop)	25.4 ab	12.8 a-d	26.2 ab	12.8 a-c	21.4 ab	11.8 a-c
Untreated inoculated control	31.2 a	14.2 ab	32.0 a	14.2 ab	29.0 a	13.6 ab

[x]Column means with a letter in common are not statistically different (Fisher's Protected LSD; P=0.05).

Table 8. Area under disease progress curve (AUDPC) data for blighted leaves, foliar lesions and leaves with sporulating *B. cinerea* on 'Pinto Premium Orange' geranium treated with biorational products.

Treatment (Trade name/active ingredient)	AUDPC for blighted leaves		AUDPC for foliar lesions		AUDPC for leaves with sporulation[x]	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Fenhexamid (Decree)	95.2 d	67.2 b	113.4 d	75.6 cd	44.8 d	27.3 c[y]
Bacillus amyloliquefaciens (Serifel)	129.5 cd	130.2 a	135.1 cd	139.3 ab	86.8 cd	112.0 ab
Pseudomonas chlororaphis (Zio)	175.7 bc	161.7 a	236.6 ab	190.4 a	110.6 bc	134.4 a
Bacillus subtilis (Serenade Opti)	169.4 bc	71.4 b	182.0 b-d	72.8 d	124.6 bc	57.4 b
Aureobasidium pullulans (Botector)	158.9 b-d	113.4 ab	178.5 b-d	119.7 b-d	105.7 bc	88.9 ab
Extract. *Swinglea glutinosa* (Ecoswing)	189.7 a-c	123.2 ab	214.2 ab	134.4 a-c	123.2 bc	92.4 ab
Gliocladium catenulatum (Prestop)	179.9 bc	126.7 a	211.4 ab	136.5 ab	90.3 b-d	105.7 ab
Bacillus mycoides (LifeGard)	177.8 bc	136.5 a	195.3 bc	142.8 ab	132.3 bc	110.6 ab
Streptomyces lydicus (Actinovate)	204.4 ab	167.3 a	231.0 ab	181.3 a	137.2 ab	149.8 a
Soybean and corn oil (PureCrop1)	195.3 a-c	103.6 ab	221.9 ab	112.7 b-d	126.7 bc	84.0 ab
Ulocladium oudemansii (BotryStop)	203.0 ab	139.3 a	231.7 ab	160.3 ab	114.8 bc	113.4 a

Table 8 (cont'd)

Untreated inoculated control	247.1 a	133.7 a	280.0 a	149.8 ab	184.8 a	107.1 ab

[x]Disease assessment were done on 6, 13 and 20-days post inoculation on 26 Sept, 3 and 10 Oct 2019 (Trial 1) and 24 and 31 Oct; 7 Nov 2019 (Trial 2).
[y]Means with the same letter are not significantly different (α=0.05) based on Fisher's LSD t-test.

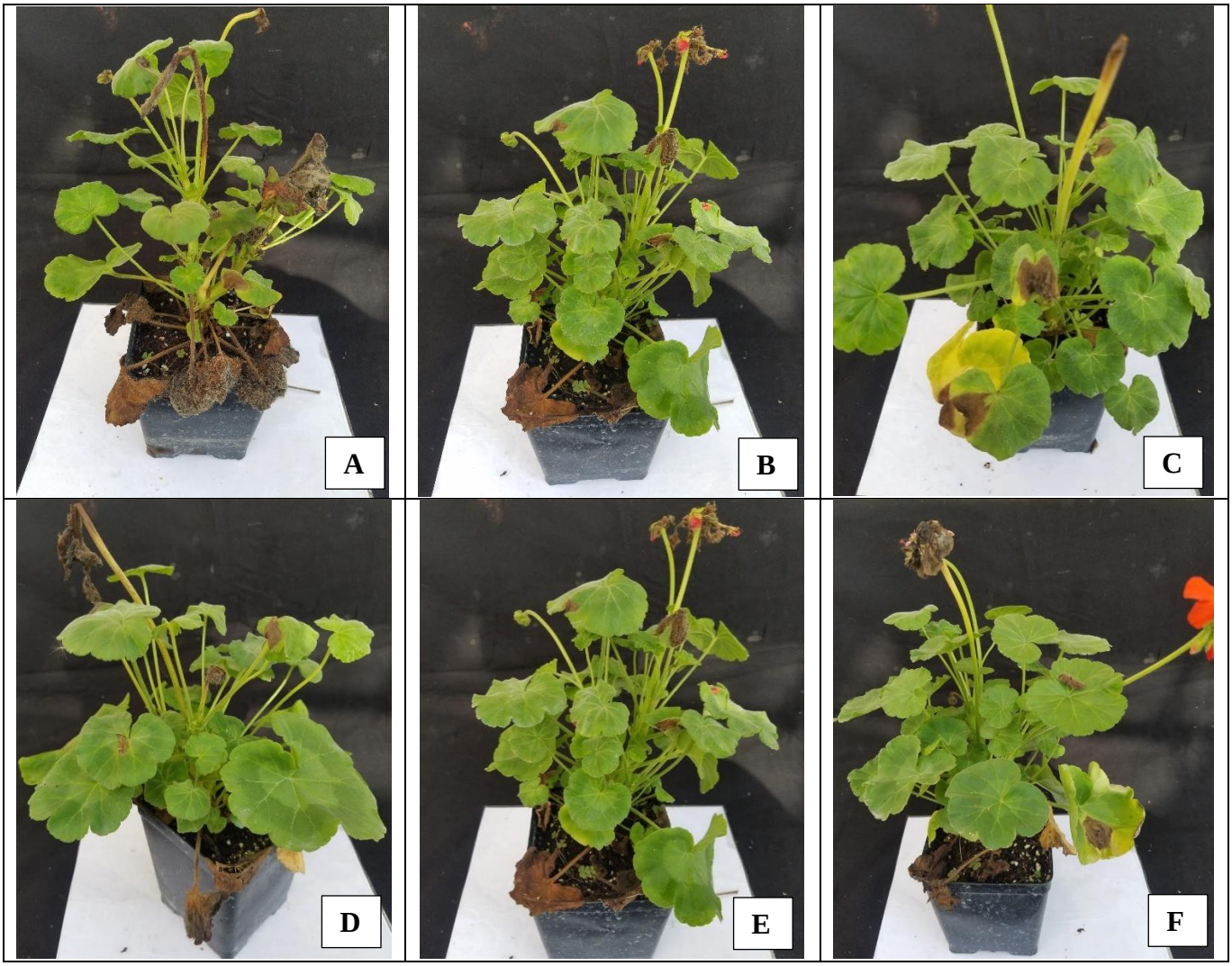

Figure 3. Botrytis blight on ‘Pinto Premium Orange’ geranium when inoculated with *Botrytis cinerea* and treated with biorational products. A: Untreated control, B: *Pseudomonas chlororaphis* (Zio), C: *Bacillus subtilis* (Serenade Opti), D: *Aureobasidium pullulans* (Botector), E: *Gliocladium catenulatum* (Prestop), F: Fenhexamid (Decree)

DISCUSSION

Botrytis blight requires intensive management efforts to reduce crop loss of floriculture crops (Grinstein et al., 1997). While all geranium cultivars included in our trial were susceptible to *B. cinerea*, significant differences were observed. Disease incidence was less for 'Horizon Coral Spice', 'Pinto Premium Orange' and 'Ivy Tornado White' than 'Ringo 2000 Violet' and 'Maverick Scarlet Picotee'. In a previous study, an ivy geranium accession 86-23-1 (diploid, *P. peltatum*) and zonal geranium 'Fox' (tetraploid, *P. x hortorum*) had consistently high levels of resistance compared to 'Ben Franklin'(diploid, *P. x hortorum*) (Uchneat, et al., 1999b). In our study, we found that the ivy geranium (*P. peltatum*) was less susceptible to *B. cinerea* than zonal geraniums (*P. x hortorum*) cultivars. Among the *P. x hortorum* cultivars included in this trial, there were significant differences in resistance to *B. cinerea*.

Differences in *B. cinerea* susceptibility among cultivars has been observed for geranium (Uchneat, et al., 1999a, 1999b), petunia (Krahl and Randle, 1999), lisianthus (Wegulo and Vilchez, 2007), and cut roses (Hammer and Evensen, 1994; Muñoz et al., 2019). Host resistance to *B. cinerea* has been attributed to genetics, rate of senescence, structural defense, secondary metabolites and defenses accelerated by hormone production (Elad and Evensen, 1995). Senescent plant tissues is readily invaded by the pathogen so changes related to senescence can play a role in host resistance (Elad and Evensen, 1995). Defense may be mediated through jasmonic acid, salicylic acid, abscisic acid and ethylene signaling pathways which are linked in a complex network (AbuQamar et al., 2017). Nitric oxide has an important role in resistance of geranium to *B. cinerea* with early nitric oxides bursts and production of secondary nitric oxide stimulating noncell-death-associated defense (Floryszak-wieczorek et. al, 2007).

Inconsistencies among the geranium cultivars were observed in this research when the experiment was repeated using the similar procedures. Inconsistencies among cultivars in their susceptibility to *B. cinerea* was noted in studies including petunia (Krahl and Randle 1999) and lisianthus (Wegulo and Vilchez, 2007). In our research, 'Multibloom Lavender' and 'Nano Deep Rose' were highly susceptible in Trial 1 but not in Trial 2. Similarly, 'Bullseye Red' and 'Pinto Pink' appeared to be more susceptible in Trial 2 than in Trial 1. The difference in disease pressure between the two trials may be due to variation in the environment. During the incubation period there were differences in temperature between the trials. In Trial 1, average temperature was 18.4^0C with minimum/maximum temperatures of $12.2/21.9^0$C. In Trial 2, the average temperature was 25^0C with minimum/maximum temperatures of $21.3/32.7^0$C. RH was greater than 90% in both trials. The optimum temperature for *B. cinerea* conidial germination is 22 to 25^0C with RH > 90% (Salinas et al., 1989) with infection occurring between 15 to 25^0C (Jarvis, 1989). Conidial germination decreases at temperature $>25^0$C and is inhibited at 30^0C (Salinas et al., 1989). Thus, the reduced disease pressure in Trial 2 may have been the result of the temperature exceeding the optimum requirements for disease development.

Although the flowers remained on the plant during our assessment, we did not evaluate flower blighting as the timing of flowering was inconsistent among the cultivars. While Uchneat et al. (1999a) determined that there is no correlation between floral and foliar resistance. Flower petals are highly susceptible to infection and may serve as the initial source of inoculum for foliar infection ((Williamson et al., 2007).

Several biorational products evaluated in our study effectively limited Botrytis blight in geranium. In Trial 1, *Aureobasidium pullulans* (Botector), *Bacillus amyloliquefaciens* (Serifel) and *Gliocladium catenulatum* (Prestop) were effective when tested on a highly susceptible and

moderately resistant geranium cultivar according to the AUDPC values for blighted leaves, foliar lesions and leaves with sporulating *B. cinerea*. *G. catenulatum* has antagonistic activity through antibiosis and mycoparasitism of *B. cinerea* conidia and germ tube which limits the disease whereas *A. pullulans* competes with the pathogen for nutrients (Castoria et al., 2001; Jacometti et al., 2010; Pal and Gardener, 2006; Vidhyasekaran, 2004). Elmhirst et al. (2011) also reported that on greenhouse geraniums, *G. catenulatum* effectively reduced disease incidence and severity. This product also effectively limited *B. cinerea* on greenhouse tomatoes and cucumber through an antagonistic mode of action (Dik et al., 1999; Utkhede and Mathur, 2006). Applications of *Gliocladium roseum* also decreased disease incidence in greenhouse cyclamen (Köhl et al., 1998). In the present study, *A. pullulans* effectively limited *B. cinerea* in both geranium cultivars. In a previous study, *A. pullulans* was moderately to highly effective for *B. cinerea* control on greenhouse tomato and cucumber and reduced the number of diseased fruits and stem lesions (Dik and Elad, 1999). Efficacy of *A. pullulans* for *B. cinerea* control has also been reported on strawberry (Lima et al., 1997; Sylla et al., 2015) and grape crops (Fedele et al., 2020; Pertot et al., 2017).

Pseudomonas chlororaphis (Zio) and the *Bacillus* products including *B. subtilis* (Serenade Opti) and *B. mycoides* (LifeGard) effectively controlled Botrytis blight on the moderately resistant 'Pinto Premium Orange' as indicated by AUDPC date for the number of blighted leaves, foliar lesions and number of leaves with pathogen sporulation. *Bacillus* species including *B. subtilis, B. amyloliquefaciens* and *B. mycoides* limit *B. cinerea* through induction of systemic acquired resistance (Choudhary and Johri, 2009), mycoparasitism, and antibiosis (Pal and Gardener, 2006; Paulitz and Belanger, 2001). According to AUDPC data for leaves with

sporulating *B. cinerea*, all tested products effectively limited disease except *Streptomyces lydicus* (Actinovate) for 'Pinto Premium Orange' geraniums in Trial 1.

Biorational products did not provide consistent results between trials. This has been reported by others as the suppression of *B. cinerea* is highly affected by the environment which influences the survival of the biocontrol agents on the phyllosphere and their ability to control the pathogen (Guetsky et al., 2001; Shtienberg and Elad, 1997). Combining two or more biocontrol products with different mechanism may reduce the variability of the biocontrol products and effectively control Botrytis blight (Guetsky et al., 2001; Guetsky et al., 2002; Pertot et al., 2017).

In summary, results from the present study indicate that 'Pinto Premium Orange' and 'Horizon Coral Spice' were moderately resistant to *B. cinerea* whereas 'Ringo 2000 Violet' and 'Maverick Scarlet Picotee' were highly susceptible. None of the cultivars included in this study were immune to *B. cinerea* as all became infected. Cultivars with a moderate resistance could be combined with biorational products including *Aureobasidium pullulans* (Botector), *Gliocladium catenulatum* (Prestop) and *Bacillus amyloliquefaciens* (Serifel) to achieve a sustainable disease management strategy. Cultivars that are highly susceptible to *B. cinerea* resistance may need to be protected using conventional fungicides along with other control strategies.

LITERATURE CITED

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1. AbuQamar, S., Moustafa, K., and Tran, L. S. P. 2017. Mechanisms and strategies of plant defense against *Botrytis cinerea*. Crit. Rev. Biotechnol. 37:262–274.

2. Castoria, R., De Curtis, F., Lima, G., Caputo, L., Pacifico, S., and De Cicco, V. 2001. *Aureobasidium pullulans* (LS-30) an antagonist of postharvest pathogens of fruits: Study on its modes of action. Postharvest Biol. Technol. 22:7–17.

3. Chandel, S., and Kumar, V. 2018. Diseases of geranium and their management. 223-242.

4. Choudhary, D. K., and Johri, B. N. 2009. Interactions of *Bacillus spp*. and plants - With special reference to induced systemic resistance (ISR). Microbiol. Res. 164:493–513.

5. Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P.D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., and Foster, G.D. 2012. The Top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 134:414–430.

6. Dik, A. J., and Elad, Y. 1999. Comparison of antagonists of *Botrytis cinerea* in greenhouse-grown cucumber and tomato under different climatic conditions. Eur. J. Plant Pathol. 105:123–137.

7. Dik, A. J., Koning, G., and Köhl, J. 1999. Evaluation of microbial antagonists for biological control of *Botrytis cinerea* stem infection in cucumber and tomato. Eur. J. Plant Pathol. 105:115–122.

8. Elad, Y., and Evensen, K. 1995. Physiological aspects of resistance to *Botrytis cinerea*. Phytopathology. 85:637–343.

9. Elad, Y., and Shtienberg, D. 1995. *Botrytis cinerea* in greenhouse vegetables: chemical, cultural, physiological, and biological controls and their integration. Integr. Pest Manag. Rev. 1:15–29.

10. Elmhirst, J. F., Haselhan, C., and Punja, Z. K. 2011. Evaluation of biological control agents for control of botrytis blight of geranium and powdery mildew of rose. Can. J. Plant Pathol. 334:499–505.

11. Fedele, G., González-Domínguez, E., Ammour, M. S., Languasco, L., and Rossi, V. 2020. Reduction of *Botrytis cinerea* colonization of and sporulation on bunch trash. Plant Dis. 104:808–816.

12. Floryszak-wieczorek, J., Arasimowicz, M., Milczarek, G., Jelen, H., and Jackowiak, H. 2007. Only an early nitric oxide burst and the following wave of secondary nitric oxide generation enhanced effective defence responses of *Pelargonium* to a necrotrophic pathogen. New Phytol. 175:718–730.

13. Gerlagh, M., Amsing, J. J., Molhoek, W. M. L., Bosker-Van Zessen, A. I., Lombaers-Van Der Plas, C. H., and Köhl, J. 2001. The effect of treatment with *Ulocladium atrum* on *Botrytis cinerea*-attack of geranium (*Pelargonium zonale*) stock plants and cuttings. Eur. J. Plant Pathol. 107:377–386.

14. Grinstein, A., Riven, Y., and Elad, Y. 1997. Improved chemical control of botrytis blight in roses. Phytoparasitica. 25:87–92.

15. Guetsky, R., Shtienberg, D., Elad, Y., and Dinoor, A. 2001. Combining biocontrol agents to reduce the variability of biological control. Phytopathology. 91:621–627.

16. Guetsky, Ruth, Shtienberg, D., Elad, Y., Fischer, E., and Dinoor, A. 2002. Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. Phytopathology. 92:976–985.

17. Hahn, M. 2014. The rising threat of fungicide resistance in plant pathogenic fungi: *Botrytis* as a case study. J. Chem. Biol. 7:133–141.

18. Hammer, P. E., and Evensen, K. B. 1994. Differences between rose cultivars in susceptibility to infection by *Botrytis cinerea*. Phytopathology. 84:1305-1312.

19. Hausbeck, M.K., and Harlan, B. 2020. Recommendations for *Botrytis* fungicides for 2020. MSU Extension, Floriculture and Greenhouse Crop Production.

20. Hausbeck, M. K., and Moorman, G. W. 1996. Managing *Botrytis* in greenhouse-grown flower Crops. Plant Dis. 80:1212-1219.

21. Hausbeck, M. K., and Pennypacker, S. P. 1991. influence of grower activity on concentrations of airborne conidia of *Botrytis cinerea* among geranium cuttings. Plant Dis. 75:1236–1243.

22. Horst, L. E., Locke, J., Krause, C. R., McMahon, R. W., Madden, L. V., and Hoitink, H. A. J. 2005. Suppression of Botrytis blight of begonia by *Trichoderma hamatum* 382 in peat and compost-amended potting mixes. Plant Dis. 89:1195–1200.

23. Jacometti, M. A., Wratten, S. D., and Walter, M. 2010. Review: Alternatives to synthetic fungicides for *Botrytis cinerea* management in vineyards. Aust. J. Grape Wine R. 16:154–172.

24. Jarvis, W. R. 1989. Managing Diseases in Greenhouse Crops. Plant Dis. 73:190-194.

25. Jiang, C., Liao, M., Wang, H., Zheng, M., and Xu, J. 2018. *Bacillus velezensis*, a potential and efficient biocontrol agent in control of pepper gray mold caused by *Botrytis cinerea*. Biol. Control. 126:147–157.

26. Kim, J.-O., Shin, J.-H., Gumilang, A., Chung, K., Choi, K. Y., and Kim, K. S. 2016. Effectiveness of different classes of fungicides on *Botrytis cinerea* causing gray mold on fruit and vegetables. Plant Pathol. J. 32:570–574.

27. Köhl, J., Gerlagh, M., De Haas, B. H., and Krijger, M. C. 1998. Biological control of *Botrytis cinerea* in cyclamen with *Ulocladium atrum* and *Gliocladium roseum* under commercial growing conditions. Phytopathology. 88:568–575.

28. Krahl, K. H., and Randle, W. M. 1999. Resistance of petunia phenotypes to *Botrytis cinerea*. HortScience. 34:690–692.

29. Lima, G., Ippolito, A., Nigro, F., and Salerno, M. 1997. Effectiveness of *Aureobasidium pullulans* and *Candida oleophila* against postharvest strawberry rots. Postharvest Biol. Technol. 10:169–178.

30. Moorman, G. W., and Lease, R. J. 1992. Benzimidazole- and dicarboximide- resistant *Botrytis cinerea* from Pennsylvania greenhouses. Plant Dis. 76:477–480.

31. Muñoz, M., Faust, J. E., and Schnabel, G. 2019. Characterization of *Botrytis cinerea* from commercial cut flower roses. Plant Dis. 103:1577–1583.

32. Olson, H. A., and Benson, D. M. 2007. Induced systemic resistance and the role of binucleate *Rhizoctonia* and *Trichoderma hamatum* 382 in biocontrol of Botrytis blight in geranium. Biol. Control. 42(2):233-241.

33. Pal, K. K., and Gardener, B. M. 2006. Biological control of plant pathogens. The Plant Health Instructor. 2:1117-1142.

34. Paulitz, T. C., and Belanger, R. R. 2001. Biological control in greenhouse system. Annu. Rev. Phytopathol. 39:103–133.

35. Pertot, I., Giovannini, O., Benanchi, M., Caffi, T., Rossi, V., and Mugnai, L. 2017. Combining biocontrol agents with different mechanisms of action in a strategy to control *Botrytis cinerea* on grapevine. Crop Prot. 97:85–93.

36. Punja, Z. K., and Utkhede, R. S. 2003. Using fungi and yeasts to manage vegetable crop diseases. Trends Biotechnol. 21:400–407.

37. Salinas, J., Glandorf, D. C. M., Picavet, F. D., and Verhoeff, K. 1989. Effects of temperature, relative humidity and age of conidia on the incidence of spotting on gerbera flowers caused by *Botrytis cinerea*. Neth. J. Plant Pathol. 95:51–64.

38. Samarakoon, U. C., Schnabel, G., Faust, J. E., Bennett, K., Jent, J., Hu, M. J., Basnagala, S., and Williamson, M. 2017. First report of resistance to multiple chemical classes of fungicides in *Botrytis cinerea*, the causal agent of gray mold from greenhouse-grown petunia in Florida. Plant Dis. 101:1052.

39. Shtienberg, D., and Elad, Y. 1997. Incorporation of weather forecasting in integrated, biological-chemical management of *Botrytis cinerea*. Phytopathology. 87:332–340.

40. Simko, I., and Piepho, H-P. 2012. The area under the disease progress stairs: Calculation, advantage, and application. Phytopathology 102:381-389.

41. Sylla, J., Alsanius, B. W., Krüger, E., and Wohanka, W. 2015. Control of *Botrytis cinerea* in strawberries by biological control agents applied as single or combined treatments. Eur. J. Plant Pathol. 143:461–471.

42. Uchneat, M. S., Spicer, K., and Craig, R. 1999a. Differential response to floral infection by *Botrytis cinerea* within the genus *Pelargonium*. HortScience. 34:718-720.

43. Uchneat, M. S., Zhigilei, A., and Craig, R. 1999b. Differential Response to Foliar Infection with *Botrytis cinerea* within the Genus *Pelargonium*. J. Am. Soc. Hortic. Sci. 124:76–80.

44. USDA-National Agricultural Statistics Service. 2019. Floriculture crops 2018 summary. Retrieved from: USDA-National Agricultural Statistics Service. 2019. Floriculture crops 2018 summary. Retrieved from: https://www.nass.usda.gov/Publications/Todays_Reports/reports/floran19.pdf

45. Utkhede, R. S., and Mathur, S. 2006. Preventive and curative biological treatments for control of *Botrytis cinerea* stem canker of greenhouse tomatoes. Biol. Control. 51:363–373.

46. Vidhyasekaran, P. 2004. Biological control – Microbial pesticides. In: Concise encyclopedia of plant pathology. Food Products Press. Binghamton. USA:239–270.

47. Wegulo, S. N., and Vilchez, M. 2007. Evaluation of Lisianthus Cultivars for Resistance to *Botrytis cinerea*. Plant Dis. 91:997–1001.

48. Williamson, B., Tudzynski, B., Tudzynski, P., and Van Kan, J. A. L. 2007. *Botrytis cinerea*: The cause of grey mould disease. Mol. Plant Pathol. 8:561–580.

49. Yourman, L. F., and Jeffers, S. N. 1999. Resistance to Benzimidazole and Dicarboximide Fungicides in Greenhouse Isolates of *Botrytis cinerea*. Plant Dis. 83:569–575.

CHAPTER 2.

MANAGEMENT OF *BOTRYTIS CINEREA* IN PETUNIA USING CULTIVAR RESISTANCE AND BIORATIONAL PRODUCTS

ABSTRACT

Botrytis cinerea, causes blight on the leaves, stems, and flowers of petunia (*Petunia x hybrida*), a popular annual bedding plant. Our objectives were to evaluate: (i) petunia cultivars for susceptibility to Botrytis blight and (ii) biorationals that limit Botrytis blight. Thirteen traditional and spreading type (wave) petunia cultivars were selected. Ten biorational products were evaluated for control of Botrytis blight and compared to the standard fungicide fenhexamid and an untreated control. The area under the disease progress curve (AUDPC) was calculated. 'Tidal Wave Cherry' had significantly higher disease severity and AUDPC values than 'Sophistica Blackberry' in the trials. According to AUDPC data, 'Shock Wave Red' had significantly less disease than 'Tidal Wave Cherry' and was similar to 'Sophistica Blackberry'. 'Shock Wave Coconut' was also susceptible to *B. cinerea* and had a disease severity rating and AUDPC data similar to 'Tidal Wave Cherry' in both trials. When evaluated on 'Shock Wave Red' petunia, *Aureobasidium pullulans* (Botector) and *Gliocladium catenulatum* (Prestop) provided *B. cinerea* control similar to fungicide standard fenhexamid (Decree) in both trials. Applications of *Pseudomonas chlororaphis* (Zio) resulted disease severity ratings and AUDPC values similar to the fungicide standard for both trials with the exception of AUDPC data in Trial 1. According to final disease severity assessment, treatment with soybean and corn oil (PureCrop1) and *Ulocladium oudemansii* (BotryStop) and *Bacillus mycoides* (LifeGard) also provided control similar to the fungicide standard but was not significantly different from untreated control. Results from this study illustrate that certain biorational products can limit *B. cinerea* when used in conjunction with a cultivar that has disease resistance.

INTRODUCTION

Petunia (*Petunia x hybrida*) is one of the most popular annual bedding plants and is availble in a range of flower colors and growth habits. In 2018, the total U.S. sales of petunia sold in pots, flats or hanging baskets was $141.7 million (USDA-NAS, 2019). The “wave” petunia has become popular due to its vigorous nature and trailing growth which are ideal for hanging baskets. Botrytis blight is one of the most important disease of greenhouse ornamentals and is incited by the airborne necrotrophic fungus *Botrytis cinerea* (teleomorph: *Botryotinia fuckeliana*). Considered the second most destructive pathogen in the world (Williamson et al., 2007; Dean et al., 2012), *B. cinerea* affects more than 200 crop species causing blossom and leaf blight, stem canker, damping off, bud, crown and fruit rot (Hausbeck and Moorman, 1996; Moyano et al., 2004; Williamson et al., 2007; Hahn, 2014; Jiang et al., 2018). *B. cinerea* produces grey masses of conidia on the surface of infected plant tissue which is diagnostic (Punja and Utkhede, 2003; Williamson et al., 2007).

Production of ornamentals in the greenhouses favors grey mold as warm temperatures, high relative humidity, free moisture, and a lack of air exchange provide favorable environmental conditions for the pathogen (Elad and Shtienberg, 1995; Paulitz and Belanger, 2001). *B. cinera* may enter the greenhouse through young seedlings and cuttings which later forms a source of inoculum in the production greenhouse (Dik and Wubben, 2004). Dispersal of conidia in the greenhouse occurs through air current or water splash (Jarvis, 1989) and the peak atmospherical conidial concentration was often associated with grower activity including watering, fertilization, pesticide application and harvesting cuttings (Hausbeck and Pennypacker, 1991).

Flower infection is the major concern of producers as the lesions render them unsuitable for marketing. Latent infections may occur during production and become active during storage or transportation (Dik and Wubben, 2004). For instance, asymptomatic petunia plants may harbor latent infections which develop during the cool moist conditions during shipping. When retailers receive the plants, they are severely diseased with wilted and necrotic flowers (Samarakoon et al., 2016).

Resistance to *B. cinerea* was observed by Krahl and Randle (1999) on select petunia phenotypes but resistant cultivars are not commercially available. Cultural control of Botrytis blight includes sanitation, heating and venting, minimizing the duration of leaf wetness, increased plant spacing and air circulation (Jarvis, 1989; Hausbeck and Moorman, 1996; Elad, 2016). Removing dead and decaying plant parts excludes the source of the inoculum and delays the onset of the disease (Elad and Shtienberg, 1995). Heating and venting the greenhouse reduces the relative humidity and duration of dew periods (Elad, 2016; Elad and Shtienberg, 1995; Hausbeck et al., 1996).

Application of biorationals or fungicides is often needed as an additional control measure for Botrytis blight. Multisite and site-specific fungicides (Hahn, 2014) have been relied upon to limit the disease in the greenhouse but fungicide resistance to single or multiple chemical classes has been noted in several cropping systems including strawberry (Fernández-Ortuño et al., 2015; Hu et al., 2016), grape (Bertetti et al., 2020; Saito et al., 2019), greenhouse cucumber and tomato (Moyano et al., 2004), cut roses (Muñoz et al., 2019), and petunia (Samarakoon et al., 2017). *B. cinerea* from greenhouse grown cut roses was resistant to four different classes of fungicides (Muñoz et al., 2019). Samarakoon et al. (2017) found that the pathogen from diseased petunias was resistant to six different fungicides classes. Use of biorationals could decrease reliance on

fungicides and delay *B. cinerea* resistance. Yeasts (*Pichia spp*., *Candida spp.*) (Jacometti et al., 2010), bacteria (*Bacillus* spp., *Pseudomonas* spp.) and filamentous fungi (*Ulocladium* spp., *Gliocladium* spp., *Trichoderma* spp.) have effectively controlled *B. cinerea* on different crops (Jacometti et al., 2010; Paulitz and Belanger, 2001). Specifically, biorationals have proven effective in greehouse tomato, cucumber (Dik et al., 1999), pepper (Jiang et al., 2018) and ornamentals including begonia (Horst et al., 2005) and geranium (Olson and Benson, 2007). Biorationals offer various modes of action including competition for space and nutrients, parasitism, antibiosis, and induction of systemic acquired resistance (Choudhary and Johri, 2009; Pal and Gardener, 2006; Paulitz and Belanger, 2001).

Our objective was to evaluate selected petunia cultivars for susceptibility to Botrytis blight and the ability of biorational products to limit disease. Integrating host resistance with effective biorational products could offer growers sustainable control options.

MATERIAL AND METHODS

Cultivar screening: The following petunias (*Petunia x hybrida*) included the following: 'Shock Wave Coconut', 'Tidal Wave Cherry', 'Easy Wave Blue', 'Tidal Wave Silver', 'Shock Wave Red', 'Easy Wave Red Improved', 'Wave Purple Classic', 'Debonair Lime Green' and 'Sophistica Blackberry' (Ball Horticultural Company, IL, USA). Seeds were sown in 128-cell plug trays containing soilless root medium (Suremix Perlite, Michigan Growers Products Inc, Galesburg, MI) on 20 Dec 2018 and incubated in the Plant Science Greenhouse at Michigan State University (MSU), East Lansing, MI. Seedlings were transplanted 42 days after seeding (9 Oct 2019) into square pots (10*10 cm^2) filled with soilless root medium and fertilized daily with 200 ppm water-soluble 20:20:20 NPK fertilizer (ICL Specialty fertilizers, Dublin, OH).

A *B. cinerea* isolate from geranium was cultured on potato dextrose agar (PDA) media and grown under florescent light under laboratory conditions to induce sporulation. Iron baskets were sanitized (10% solution, Clorox germicidal bleach, The Clorox company, Oakland, CA) and placed inside translucent plastic bags (21 cm x 5.5 cm x 38 cm) containing water at the bottom to achieve high relative humidity (RH). Eight plants, single-plant replication per treatment, from each cultivar were selected and placed inside the basket and arranged in completely randomized design on the bench in 80% shaded greenhouse at MSU. The conidial suspension was prepared by dislodging 11-day-old *B. cinerea* cultures flooded with distilled water and strained through cheesecloth. The conidial concentration was standardized to $1x10^6$ conidia/ml solution with a hemocytometer. Plants were inoculated by spraying the *B. cinerea* conidial suspension on 12 Mar 2019 on the plant surface uniformly with a hand sprayer until run off. Inoculated plants were incubated by closing the translucent plastic bags with a rubber band to provide high RH. A Watchdog data logger (Spectrum technologies Inc., Aurora, IL) was installed in one basket to monitor daily temperature and RH inside the bag. The experiment was conducted for 21 days (12 Mar to 2 Apr 2019) with disease assessed three times at 7-day intervals (19, 26 Mar and 2 Apr). Average temperature of 20.7^0C was recorded during the incubation with max./min. temperature of 22.5^0C/20.3^0C. The experiment was repeated twice (9 to 30 Apr and 13 Sept to 4 Oct 2019) using the procedure as previously described with four additional cultivars including 'Wave Lavender', 'Easy Mix Flag Wave', 'Success Burgundy' and 'Ramblin Red' for a total of 13 cultivars. Plants were inoculated with conidial suspension of *Botrytis* on 9 Apr and 12 Sept for Trial 2 and 3, respectively. Disease assessment was done 7, 14 and 21-days post inoculation on 16, 23 and 30 Apr (Trial 2) and 20, 27 Sept and 4 Oct 2019 (Trial 3). Average temperature during the incubation period were 22.8^0C and 23.7^0C with

max./min. temperature of 33.4^{0}C/17.2^{0}C and 30.8^{0}C/22.1^{0}C for Trial 2 and 3 respectively.

Efficacy of biorational products: Wave petunia 'Shock Wave Red', identified as one of the less susceptible petunia cultivars to *B. cinerea* in our studies was selected. Seed was sown in the 128-cell plug trays in the Plant Science Greenhouse at MSU, East Lansing, MI on 28 Aug 2019 and seedlings were transplanted six weeks later (9 Oct 2019) into square pots (10*10 cm^2) filled with soilless root medium (Suremix Perlite, Michigan Growers Products Inc, Galesburg, MI). The transplanted plants were fertilized daily with 200 ppm water-soluble 20-20-20 NPK fertilizer (ICL Specialty fertilizers, Dublin, OH). Ten biorational products and the standard fungicide Decree (fenhexamid) were each applied at 7-day intervals using a hand compressed air sprayer (Table 1). Three application (14, 21 and 28 Nov) were made for each product with the exception of *Gliocladium catenulatum* (PreStop) which was applied one time as the label specifies a 21-day application interval. Five replications each comprising of a single plant, for a total of 60 plants were arranged in a completely randomized design on a shaded (80%) bench at the Plant Science Greenhouses at MSU, East Lansing, MI. The experiment was conducted from 14 Nov to 5 Dec 2019. Conidial suspension of *B. cinerea* conidia (10^6 conidia/ml) was applied one day following treatment (15 Nov) by spraying the conidial suspension to the plants until runoff using a hand sprayer. Disease was assessed 6, 13 and 20-days post inoculation on 21, 28 Nov and 5 Dec. A watchdog data logger was used to monitor the environmental conditions as described previously. Max./min. temperature inside plastic bag were 24.1^{0}C /15.8^{0}C with average of 19.5^{0}C during the period of experiment. The experiment was repeated from 14 Jan to 4 Feb 2020 using the same procedure as described above. Plants were inoculated with *B. cinerea* (15 Jan) and treatments were applied for three times (14, 21 and 28 Jan). Disease was assessed at 7-day interval on 21, 28 Jan and 4 Feb 2020.

Table 9. Biorational products and a standard fungicide evaluated for efficacy against *Botrytis cinerea* on petunia.

Product	Active Ingredient	Registrant	Rate/ 100 gal
Actinovate® SP	*Streptomyces lydicus* WYEC108 (0.037%)	Novozymes BioAg Inc.	12 oz
Botector®	*Aureobasidium pullulans* strain DSM 14940 (40%), DSM 14941 (40%)	Bio-ferm	10 oz
BotryStop™	*Ulocladium oudemansii* strain U3	BioWorks, Inc.	4 lb
EcoSwing™	Extract of *Swinglea glutinosa* (82%)	Gowan Company	2 pt
LifeGard™ WG	*Bacillus mycoides* (40%)	Certis USA	4.5 oz
Prestop® WP	*Gliocladium catenulatum* strain J1446 (32%)	Danstar Ferment AG	70 oz
PureCrop1	Soybean oil (10%), Corn oil (5%)	PureCrop1	200 oz
Serenade Opti® WP	*Bacillus subtilis* QST713 (26.2%)	Bayer CropScience Inc.	20 oz
Serifel®	*Bacillus amyloliquefaciens* strain MBI600 (11%)	BASF Corporation	16 oz
Zio™	*Pseudomonas chlororaphis* strain AFS009	SePRO Corporation	100 oz
Decree® 50 WDG	Fenhexamid (50%)	SePro Corporation	1 lb

Disease assessment: The total diseased area (%) of each plant's diseased foliage was assessed visually using a scale of 0 to 10 (0 = no disease, 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90% and defoliation, 10 = >91% and plant death (Elmhirst et al., 2011). Assessments were conducted 7, 14 and 21-days post inoculation. The area under disease progression curve (AUDPC) was calculated to express the cumulative disease severity using the formula AUDPC = $\sum_{i=1}^{n-1}$ $[(y_i + y_{i+1})/2] \times (t_{i+1} - t_i)$ where y_i is the assessment of disease at ith observation, t_i is the time (days) at the i^{th} observation and n is the total number of observations (Simko and Piepho, 2012).

Statistical Analysis: Data were analyzed with a one-way ANOVA using PROC GLIMMIX procedure on SAS Statistical Analyzing Software (SAS Institute Inc., Cary, NC, 2013) for disease severity and determined least square means among the treatments. Normal distribution of the data was met when checked through residual plots and homogeneity analysis using Levene's test which established equal variance among replicates. Area under disease progress curve (AUDPC) for disease severity was calculated from the three ratings. Statistical differences among treatments in all trials were determined by using Fisher's Least Significant Difference t- test (P=0.05).

RESULTS

Cultivar screening: According to the final disease assessment and AUDPC data, ‘Sophistica Blackberry’ had significantly less disease than ‘Tidal Wave Cherry’ in each trial. ‘Tidal Wave Cherry’ also had significantly more disease according to ratings and AUDPC data than ‘Easy Wave Red Improved’, ‘Wave Purple Classic’, ‘Tidal Wave Silver’, ‘Shock Wave Red’ and ‘Easy Wave Blue’ in Trials 1 and 3; in Trial 2, the disease levels and AUDPC data in ‘Tidal Wave Cherry’ was similar to these same cultivars. In Trials 2 and 3, ‘Success Burgundy’ had significantly more disease according to the final disease assessment. According to AUDPC data, ‘Shock Wave Red’ had significantly less disease than ‘Tidal Wave Cherry’ and was similar to ‘Sophistica Blackberry’. ‘Shock Wave Coconut’ was also susceptible to *B. cinerea* and had a disease severity rating and AUDPC data similar to ‘Tidal Wave Cherry’ in Trials 1 and 2.

Table 10. Disease severity on petunia cultivars in the greenhouse observed 21 days following inoculation with *Botrytis cinerea*.

Cultivars	Disease Severity[z]		
	Trial 1	Trial 2	Trial 3
Tidal Wave Cherry	6.88 a	4.75 bc	6.67 a[y]
Success Burgundy	- [x]	8.00 a	6.67 a
Debonair Lime Green	4.63 bc	7.00 a	6.33 ab
Wave lavender	-	5.25 b	5.67 a-c
Easy Wave Red Improved	4.00 cd	4.75 bc	5.17 bc
Sophistica Blackberry	4.13 cd	3.00 d	5.00 c
Wave Purple Classic	3.25 d	5.50 b	4.83 c
Shock Wave Coconut	7.38 a	5.25 b	4.67 cd
Tidal Wave Silver	4.88 bc	4.38 bc	4.67 cd
Easy Mix Flag	-	3.63 cd	4.50 c-e
Ramblin Red	-	5.25 b	4.50 c-e
Shock Wave Red	4.63 bc	4.88 b	3.50 de
Easy Wave Blue	5.25 b	4.88 b	3.33 e

[z]Disease rating scale 0 to 10 (0=no disease, 1=1-10%, 2=11-20%, 3=21-30%, 4=31-40%, 5=41-50%, 6=51-60%, 7=61-70%, 8=71-80%, 9=81-90% and defoliation, 10= >91% blighting and plant death.
[y]Column means with a letter in common are not statistically different (Fisher's Protected LSD; P=0.05).
[x]Petunia cultivars not included in Trial 1.

Table 11. Area under disease progress curve (AUDPC) for disease severity on petunia cultivars in the greenhouse when inoculated with *Botrytis cinerea*.

Cultivars	AUDPC for disease severity[z]		
	Trial 1	Trial 2	Trial 3
Tidal Wave Cherry	76.56 a	56.44 c-e	71.75 a[y]
Debonair Lime Green	49.00 cd	67.37 bc	70.00 a
Success Burgundy	-[x]	81.81 a	57.17 b
Wave Lavender	-	66.06 b-d	51.33 bc
Shock Wave Coconut	80.94 a	60.81 b-e	50.75 bc
Easy Wave Red Improved	40.25 d	50.75 ef	50.17 bc
Tidal Wave Silver	55.13 bc	54.69 de	47.83 bc
Sophistica Blackberry	44.63 cd	33.25 g	45.50 cd
Easy Mix Flag	-	37.19 g	43.75 cd
Wave Purple Classic	37.63 d	71.31 ab	42.58 cd
Ramblin Red	-	58.19 c-e	40.83 c-e
Shock Wave Red	49.00 cd	41.12 fg	35.0 de
Easy Wave Blue	63.88 b	58.62 c-e	31.50 e

[z]Disease assessment were done on 7, 14 and 21-days post inoculation on 19, 26 Mar and 2 Apr (Trial 1), 16, 23 and 30 Apr (Trial 2) and 20, 27 Sept and 4 Oct 2019 (Trial 3).
[y]Column means with a letter in common are not statistically different (Fisher's Protected LSD; P=0.05).
[x]Petunia cultivars not included in Trial 1.

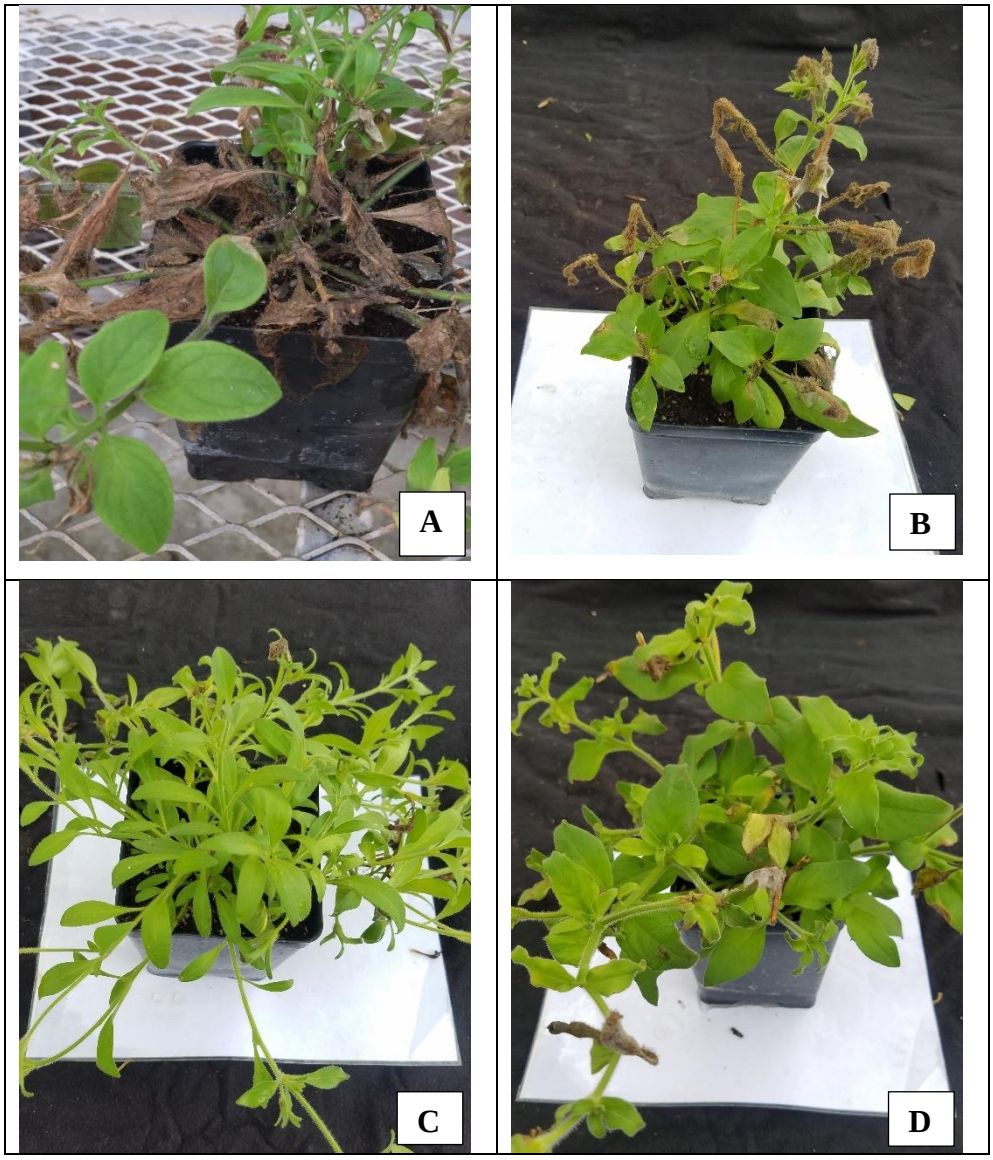

Figure 4. Highly susceptible (A, B) and least susceptible (C, D) petunia cultivars observed 21 days following the inoculation with *Botrytis cinerea*. A: 'Tidal Wave Cherry', B: 'Success Burgundy', C: 'Shock Wave Red', D: 'Sophistica Blackberry'

Efficacy of biorational products: The final disease severity rating for the untreated control was 4.0 to 5.4 for Trials 1 and 2, respectively. The fungicide standard Decree (fenhexamid) resulted in significantly less disease than the untreated control according to disease severity in Trial 1 and AUDPC data in Trial 2. According to disease severity ratings and AUDPC data, applications of *Aureobasidium pullulans* (Botector) and *Gliocladium catenulatum* (Prestop) limited disease and was similar to the fungicide standard fenhexamid (Decree) for both trials. Treatments of *Pseudomonas chlororaphis* (Zio) provided disease severity ratings and AUDPC data similar to the fungicide standard fenhexamid for both trials with the exception of AUDPC data in Trial 1. Many of the biorational products provided control similar to the fungicide standard fenhexamid in both trials according to the final disease severity. According to disease severity assessments, soybean and corn oil (PureCrop1), *Ulocladium oudemansii* (BotryStop), and *Bacillus mycoides* (LifeGard) provided control similar to the fungicide Decree standard in both trials; these products were also similar to the untreated control.

Table 12. Disease severity and area under disease progress curve (AUDPC) of 'Shock Wave Red' petunia when inoculated with *Botrytis cinerea* and treated with biorational products and a fungicide standard.

Treatments	Disease severity[z]		AUDPC[y]	
	Trial 1	Trial 2	Trial 1	Trial 2
Fenhexamid (Decree)	2.8 cd	4.4 a-c	24.5 de	41.3 d[x]
Aureobasidium pullulans (Botector)	3.2 b-d	3.8 c	32.2 cd	43.4 cd
Pseudomonas chlororaphis (Zio)	3.2 b-d	4.0 bc	35.0 a-c	46.2 b-d
Gliocladium catenulatum (Prestop)	2.2 d	4.4 a-c	21.0 e	46.9 b-d
Bacillus mycoides (LifeGard)	3.8 a-c	4.6 a-c	35.0 a-c	47.6 b-d
Ulocladium oudemansii (BotryStop)	3.2 b-d	4.8 a-c	35.0 a-c	52.5 a-c
Bacillus amyloliquefaciens (Serifel)	4.8 a	5.4 a	44.1 a	52.5 a-c
Soybean and corn oil (PureCrop1)	3.8 a-c	4.8 a-c	34.3 bc	53.9 ab
Extract of *Swinglea glutinosa* (Ecoswing)	4.4 a	4.8 a-c	34.3 bc	54.6 ab
Bacillus subtilis (Serenade Opti)	4.6 a	5.0 a	42.0 ab	56.0 ab
Streptomyces lydicus (Actinovate)	4.2 ab	5.4 a	41.3 a-c	58.1 a
Untreated inoculated control	4.0 ab	5.4 a	32.9 b-d	58.8 a

[z]Disease rating scale 0 to 10 (0 = no blighting, 1 = 1-10% blighting, 2 = 11-20% blighting, 3 = 21-30% blighting, 4 = 31-40% blighting, 5 = 41-50% blighting, 6 = 51-60% blighting, 7 = 61-70% blighting, 8 = 71-80% blighting, 9 = 81-90% blighting and defoliation, 10 = >91% blighting and plant death.
[y]Disease was assessed 6, 13 and 20-days post inoculation on 21, 28 Nov and 5 Dec 2019 (Trial 1) and 21, 28 Jan and 4 Feb 2020 (Trial 2).
[x]Column means with a letter in common are not statistically different (Fisher's Protected LSD; P=0.05).

Figure 5. Botrytis blight on 'Shock Wave Red' petunia when inoculated with *Botrytis cinerea* and treated with biorational products A: Untreated control, B: *Bacillus subtilis* (Serenade Opti), C: *Streptomyces lydicus* (Actinovate), D: *Ulocladium oudemansii* (BotryStop), E: *Pseudomonas chlororaphis* (Zio), F: *Aureobasidium pullulans* (Botector), G: *Gliocladium catenulatum* (Prestop), H: Fenhexamid (Decree)

DISCUSSION

Botrytis blight causes a loss of millions of dollars each year (Steiger, 2007; Dean et al., 2012). In our study, all cultivars evaluted were susceptible but significant differences were observed. In Trial 1, 'Tidal Wave Cherry' and 'Shock Wave Coconut' petunias were highly susceptible according to final disease assessment and AUDPC data. For Trials 2 and 3, 'Success Burgundy' and 'Debonair Lime Green' had high disease severity and were similar to 'Tidal Wave Cherry' (Trial 3). 'Tidal Wave Cherry' was highly susceptible across all trials; 'Sophistica Blackberry' was significantly less susceptible. According to AUDPC values, 'Shock Wave Red' had less disease across all trials and was similar to 'Sophistica Blackberry'.

Some petunia cultivars have been found to be resistant to *B. cinerea* (Krahl and Randle, 1999; Weddle, 1976) but are no longer available. Floryszak-wieczorek et al. (2007) reported that in resistant geranium cultivars, there was an early nitric oxide (NO) burst with subsequent secondary waves of NO, whereas in the susceptible cultivar, there was an overproduction of NO as the disease progressed but an early burst and secondary wave of NO was lacking. An early and high concentration of NO generates a strong signal for effective defense in resistant cultivars. The accumulation of secondary metabolites may induce the host response to the pathogen as no qualitative resistance to *B. cinerea* has been found.

Inconsistent results were observed among the trials. 'Shock Wave Red' was highly susceptible in Trial 1 but was among the least susceptible cultivars in Trial 3. 'Easy Wave Red Improved' and 'Wave Purple Classic' that were least susceptible in Trial 1 were moderately susceptible in Trials 2 and 3 according to disease severity assessments. When Krahl and Randle (1999) evaluated 48 petunia cultivars for *B. cinerea* resistance, they observed variation among cultivars over two seasons but 'Pink Sensation Improved' was consistently resistant.

Inconsistency has been reported for *B. cinerea* on lisianthus (Wegulo and Vilchez, 2007) and geranium cultivars (Uchneat et al.,1999) against *B. cinerea*.

Biorationals could be used as an alternative or in conjunction with traditional fungicides to limit *B.cinerea*. Commercially available biorationals were tested for their ability to control Botrytis blight on 'Shock Wave Red' petunia, a cultivar that was more resistant than others included in our trial. Many of the biorationals provided a similar level of control as the fungicide standard. Higher disease severity was observed in the Trial 2 compared to Trial 1. Among the tested products, *Aureobasidium pullulans* (Botector) and *Gliocladium catenulatum* (Prestop) provided effective control similar to the fungicide standard in both trials with reduced disease severity and AUDPC values. *A. pullulans* is a yeast that inhibits mycelial growth and conidial germination of *B. cinerea* through the production of diffusible and volatile inhibitory antifungal compounds (Yalage et al., 2020) and secretion of hydrolytic enzymes including chitinase, β-1,3-glucanase, and protease (Zhang et al., 2010; Chen et al., 2018). It also acts as an indirect antagonist by suppressing *B. cinerea* by competing for space and nutrition (Castoria, et al., 2001; Zhang et al., 2010). Our findings that *A. pullulans* effectively limited *B. cinerea* is supported by previous studies where *A. pullulans* was moderate to highly effective for *B. cinerea* control on greenhouse tomato and cucumber and reduced the number of diseased fruits and stem lesions (Dik and Elad, 1999). The efficacy of *A. pullulans* against *B. cinerea* has been reported for apples (Zhang et al., 2010), strawberry (Lima et al., 1997; Sylla et al., 2015), and grapes (Fedele et al., 2020; Pertot et al., 2017). When applied in combination with *T. harzianum* T39, *A. pullulans* significantly reduced stem lesions on tomato compared to *A. pullulans* alone (Dik et al., 1999).

Suppression of *Botrytis* by *G. catenulatum* has been described through the antagonistic mechanism of antibiosis and mycoparasitism (Pal and Gardener, 2006; Jacometti et al., 2010). *G. catenulatum* (Prestop) similar to our study have successfully control the *B. cinerea* on greenhouse tomatoes (Utkhede and Mathur, 2006) and geranium (Elmhirst et al., 2011). Other species of *Gliocladium* (*G. roseum*) effectively controlled Botrytis blight by suppressing spore production, reducing disease incidence on strawberry, raspberry and greenhouse flowers (begonia, cyclamen and geranium) and vegetables (cucumber, pepper and tomato) (Sutton et al., 1997). In our study, *Pseudomonas chlororaphis* (Zio) resulted in disease severity ratings and AUDPC values similar to the fungicide standard fenhexamid for both trials: an exception was observed with AUDPC data for Trial 1. *Pseudomonas* spp. inhibits the conidial germination of *B. cinerea* by secreting volatile metabolites with fungistatic effects (Swadling and Jeffries, 1998; Redouan, et al., 2018). *Pseudomonas fluorescens* effectively supressed *B. cinerea* sporulation and significantly reduced disease on greenhouse petunia (Gould et al., 1996) and tomato (Yildiz et al., 2007). In vitro evaluation of *Pseudomonas* spp. showed *B. cinerea* mycelial growth inhibition of 65% with 100% radial growth inhibition through the production of votalite antifungal compounds (Redouan et al., 2018). According to South et al. (2020) *P. protegens* AP54, *P. chlororaphis* 14B11 and *P. fluorescens* 89F1 effectively controlled *B. cinerea* in petunia based on a disease severity index and AUDPC values.

None of the products with *Bacillus* species; *B. subtilis* (Serenade Opti), *B. amyloliquefaciens* (Serifel) and *B. mycoides* (LifeGard), tested in this study reduced disease severity compared to the untreated inoculated control, although *B. mycoides* (LifeGard) was similar to the standard fungicide fenhexamid (Decree). *Bacillus* species secrete antimicrobial compounds, antibiotics and lipopeptide- like compounds and acts directly as an antagonist

against *B. cinerea* hyphae (Salvatierra-Martinez et al., 2018). In contrast to our result, *B.cinerea* has been effectively suppressed in many other crops by *B. subtilis* (Abbey et al., 2020; Elmhirst et al., 2011; Pertot et al., 2017) and *B. amyloliquefaciens* (Nakkeeran et al., 2020; Salvatierra-Martinez et al., 2018; Zhou et al., 2020).

Ruiz-Moyano et al. (2020) conducted in-vivo assays of strawberry and cherries and determined that *Hanseniaspora* spp. isolates provided increased efficacy and reduced *B. cinerea* mycelial growth and development: *H. uvarum* 793 was selected as potential biorational. Combining biorational products with different mechanism could reduce performance variability and improve efficacy (Guetsky et al., 2001; Guetsky et al., 2002). Biorational products can be combined with fungicides to reduce the number of fungicide applications thereby reducing the risk of pathogen resistance and providing effective control of *B. cinerea* (Rotolo et al., 2018).

In conclusion, ‘Sophistica Blackberry’ and ‘Shock Wave Red’ were less susceptible than others in our study. Growers interested in using biorationals may want to consider selecting cultivars that are less suceptible to *B. cinerea*. *Gliocladium catenulatum* (Prestop), *Aureobasidium pullulans* (Botector) and *Pseudomonas chlororaphis* (Zio) effectively limited disease in our trials when tested on a petunia cultivar determined to have a level of resistance to Botrytis blight.

LITERATURE CITED

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1. Abbey, J. A., Percival, D., Asiedu, S. K., Prithiviraj, B., and Schilder, A. 2020. Management of Botrytis blossom blight in wild blueberries by biological control agents under field conditions. Crop Prot. 131:105078.

2. Bertetti, D., Monchiero, M., Garibaldi, A., and Gullino, M. L. 2020. Monitoring activities on fungicide resistance in *Botrytis cinerea* carried out in vineyards in North-West Italy in 2018. J. Plant Dis. Protect. 127:123–127.

3. Castoria, R., De Curtis, F., Lima, G., Caputo, L., Pacifico, S., and De Cicco, V. 2001. *Aureobasidium pullulans* (LS-30) an antagonist of postharvest pathogens of fruits: Study on its modes of action. Postharvest Biol. Technol. 22:7–17.

4. Chen, P. H., Chen, R. Y., and Chou, J. Y. 2018. Screening and evaluation of yeast antagonists for biological control of *Botrytis cinerea* on strawberry fruits. Mycobiology. 46:33–46.

5. Choudhary, D. K., and Johri, B. N. (2009). Interactions of *Bacillus* spp. and plants - With special reference to induced systemic resistance (ISR). Microbiol. Res. 164:493–513.

6. Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P.D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., and Foster, G.D. 2012. The Top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13:414–430.

7. Dik, A. J., and Elad, Y. 1999. Comparison of antagonists of *Botrytis cinerea* in greenhouse-grown cucumber and tomato under different climatic conditions. Eur. J. Plant Pathol. 105:123–137.

8. Dik, A. J., Koning, G., and Köhl, J. 1999. Evaluation of microbial antagonists for biological control of *Botrytis cinerea* stem infection in cucumber and tomato. Eur. J. Plant Pathol. 105:115–122.

9. Dik A. J., and Wubben, J. P. 2004 Epidemiology of *Botrytis cinerea* diseases in greenhouses. In: Botrytis: biology, pathology, and control. Elad Y, Williamson B, Tudzynski P, Delen N (eds). Kluwer Academic Publishers, Dordrecht, pp 319–333.

10. Elad, Y. 2016. Cultural and integrated control of *Botrytis* spp. In: *Botrytis* - The Fungus, the Pathogen, and its Management in Agricultural Systems. Fillinger, S. and Y. Elad (Eds.):149–164.

11. Elad, Y., and Shtienberg, D. 1995. *Botrytis cinerea* in greenhouse vegetables: chemical, cultural, physiological, and biological controls and their integration. Integr. Pest Manag. Rev. 1:15–29.

12. Elmhirst, J. F., Haselhan, C., and Punja, Z. K. 2011. Evaluation of biological control agents for control of botrytis blight of geranium and powdery mildew of rose. Can. J. Plant Pathol. 33:499–505.

13. Fedele, G., González-Domínguez, E., Ammour, M. S., Languasco, L., and Rossi, V. 2020. Reduction of *Botrytis cinerea* colonization of and sporulation on bunch trash. Plant Dis. 104:808–816.

14. Fernández-Ortuño, D., Grabke, A., Li, X., and Schnabel, G. 2015. Independent Emergence of Resistance to Seven Chemical Classes of Fungicides in *Botrytis cinerea*. Phytopathology. 105:424–432.

15. Floryszak-wieczorek, J., Arasimowicz, M., Milczarek, G., Jelen, H., and Jackowiak, H. 2007. Only an early nitric oxide burst and the following wave of secondary nitric oxide generation enhanced effective defense responses of *Pelargonium* to a necrotrophic pathogen. New Phytol. 175:718–730.

16. Gould, A. B., Kobayashi, D. Y., and Bergen, M. S. 1996. Identification of bacteria for biological control of *Botrytis cinerea* on petunia using a petal disk assay. Plant Dis. 80:1029-1033.

17. Guetsky, R., Shtienberg, D., Elad, Y., and Dinoor, A. 2001. Combining biocontrol agents to reduce the variability of biological control. Phytopathology. 91:621–627.

18. Guetsky, Ruth, Shtienberg, D., Elad, Y., Fischer, E., and Dinoor, A. 2002. Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. Phytopathology. 92:976–985.

19. Hahn, M. 2014. The rising threat of fungicide resistance in plant pathogenic fungi: *Botrytis* as a case study. J. Chem. Biol. 7:133–141.

20. Hausbeck, M. K., and Moorman, G. W. 1996. Managing *Botrytis* in greenhouse-grown flower Crops. Plant Dis. 80:1212-1219.

21. Hausbeck, M. K., and Pennypacker, S. P. 1991. Influence of grower activity and disease incidence on concentrations of airborne conidia of *Brotytis cinerea* among geranium stock plants. Plant Dis. 75:798–803.

22. Hausbeck, M., Pennypacker, S., and Stevenson, R. 1996. The effect of plastic mulch and forced heated air on *Botrytis cinerea* on geranium stock plants in a research greenhouse. Plant Dis. 80:170-173.

23. Horst, L. E., Locke, J., Krause, C. R., McMahon, R. W., Madden, L. V., and Hoitink, H. A. J. 2005. Suppression of Botrytis blight of begonia by *Trichoderma hamatum* 382 in peat and compost-amended potting mixes. Plant Dis. 89:1195–1200.

24. Hu, M.-J., Cox, K. D., and Schnabel, G. 2016. Resistance to Increasing Chemical Classes of Fungicides by Virtue of "Selection by Association" in *Botrytis cinerea*. Phytopathology. 106:1513–1520.

25. Jacometti, M. A., Wratten, S. D., and Walter, M. 2010. Review: Alternatives to synthetic fungicides for *Botrytis cinerea* management in vineyards. Australian Journal of Grape and Wine Research. 16(1):154–172.

26. Jarvis, W. R. 1989. Managing diseases in greenhouse crops. Plant Dis. 73:190-194.

27. Jiang, C., Liao, M., Wang, H., Zheng, M., and Xu, J. 2018. *Bacillus velezensis*, a potential and efficient biocontrol agent in control of pepper gray mold caused by *Botrytis cinerea*. Biol. Control. 126:147–157.

28. Krahl, K. H., and Randle, W. M. 1999. Resistance of petunia phenotypes to *Botrytis cinerea*. HortScience. 34:690–692.

29. Lima, G., Ippolito, A., Nigro, F., and Salerno, M. 1997. Effectiveness of *Aureobasidium pullulans* and *Candida oleophila* against postharvest strawberry rots. Postharvest Biol. Technol. 10:169–178.

30. Moyano, C., Gómez, V., and Melgarejo, P. 2004. Resistance to pyrimethanil and other fungicides in *Botrytis cinerea* populations collected on vegetable crops in Spain. J. Phytopathol. 152:484–490.

31. Muñoz, M., Faust, J. E., and Schnabel, G. 2019. Characterization of *Botrytis cinerea* from commercial cut flower roses. Plant Dis. 103:1577–1583.

32. Nakkeeran, S., Priyanka, R., Rajamanickam, S., and Sivakumar, U. 2020. *Bacillus amyloliquefaciens* alters the diversity of volatile and non-volatile metabolites and induces the expression of defense genes for the management of Botrytis leaf blight of Lilium under protected conditions. J. Plant Pathol.

33. Olson, H. A., and Benson, D. M. 2007. Induced systemic resistance and the role of binucleate *Rhizoctonia* and *Trichoderma hamatum* 382 in biocontrol of Botrytis blight in geranium. Biol. Control. 42:233-241.

34. Pal, K. K., and Gardener, B. M. 2006. Biological control of plant pathogens. The Plant Health Instructor. 2:1117-1142.

35. Paulitz, T. C., and Belanger, R. R. 2001. Biological control in greenhouse system. Annu. Rev. Phytopathol. 39:103–133.

36. Pertot, I., Giovannini, O., Benanchi, M., Caffi, T., Rossi, V., and Mugnai, L. 2017. Combining biocontrol agents with different mechanisms of action in a strategy to control *Botrytis cinerea* on grapevine. Crop Prot. 97:85–93.

37. Punja, Z. K., and Utkhede, R. S. 2003. Using fungi and yeasts to manage vegetable crop diseases. Trends Biotechnol. 21:400–407.

38. Redouan, Q., Rachid, B., Abedrahim, A., Hassan, M. EL, and Bouchra, C. 2018. Effectiveness of beneficial bacteria *Pseudomonas spp.* to control grey and green mold. In Proceedings of the IX International Agricultural Symposium "Agrosym 2018" PP:933–939.

39. Rotolo, C., De Miccolis Angelini, R. M., Dongiovanni, C., Pollastro, S., Fumarola, G., Di Carolo, M., Perrelli, D., Natale, P., and Faretra, F. 2018. Use of biocontrol agents and botanicals in integrated management of *Botrytis cinerea* in table grape vineyards. Pest Manag. Sci. 74:715–725.

40. Ruiz-Moyano, S., Hernández, A., Galvan, A. I., Córdoba, M. G., Casquete, R., Serradilla, M. J., and Martín, A. 2020. Selection and application of antifungal VOCs-producing yeasts as biocontrol agents of grey mould in fruits. Food Microbiol. 92:103556.

41. Saito, S., Michailides, T. J., and Xiao, C. L. 2019. Fungicide-resistant phenotypes in *Botrytis cinerea* populations and their impact on control of gray mold on stored table grapes in California. Eur. J. Plant Pathol. 154:203–213.

42. Salvatierra-Martinez, R., Arancibia, W., Araya, M., Aguilera, S., Olalde, V., Bravo, J., and Stoll, A. 2018. Colonization ability as an indicator of enhanced biocontrol capacity—An example using two *Bacillus amyloliquefaciens* strains and *Botrytis cinerea* infection of tomatoes. J. Phytopathol. 166:601–612.

43. Samarakoon, U., Bennett, K., Jent, J., Chiu, C., and Sch, G. 2016. Alternative compounds to control gray mold. In: Petunia flower meltdown. *Grower Talks*.

44. Samarakoon, U. C., Schnabel, G., Faust, J. E., Bennett, K., Jent, J., Hu, M. J., Basnagala, S., and Williamson, M. 2017. First report of resistance to multiple chemical classes of fungicides in *Botrytis cinerea*, the causal agent of gray mold from greenhouse-grown petunia in Florida. Plant Dis. 101:1052.

45. Simko, I., and Piepho, H. P. 2012. The area under the disease progress stairs: Calculation, advantage, and application. Phytopathology. 102:381–389.

46. South, K. A., Peduto Hand, F., and Jones, M. L. 2020. Beneficial bacteria identified for the control of *Botrytis cinerea* in petunia greenhouse production. Plant Dis. 104:1801–1810.

47. Steiger, D. 2007. Global ecoonomic importance of *Botrytis* protection. In: 14th International Botrytis Symposium. African Sun MeDIA Pty (Ltd.). Page 7.

48. Sutton, J. C., Li, D., Peng, G., Yu, H., Zhang, P., and Valdebenito-Sanhueza, R. M. 1997. *Gliocladium roseum*: A versatile adversary of *Botrytis cinerea* in crops antagonistic. Plant Dis. 81:316–328.

49. Swadling, I. R., and Jeffries, P. 1998. Antagonistic properties of two bacterial biocontrol agents of grey mould disease. Biocontrol Sci. Technol. 8:439–448.

50. Sylla, J., Alsanius, B. W., Krüger, E., and Wohanka, W. 2015. Control of *Botrytis cinerea* in strawberries by biological control agents applied as single or combined treatments. Eur. J. Plant Pathol. 143:461–471.

51. Uchneat, M. S., Zhigilei, A., and Craig, R. 1999. differential response to foliar infection with *Botrytis cinerea* within the genus *Pelargonium*. J. Am. Soc. Hortic. Sci. 124:76–80.

52. USDA-National Agricultural Statistics Service. 2019. Floriculture crops 2018 summary. Retrieved from: https://www.nass.usda.gov/Publications/Todays_Reports/reports/floran19.pdf

53. Utkhede, R. S., and Mathur, S. 2006. Preventive and curative biological treatments for control of *Botrytis cinerea* stem canker of greenhouse tomatoes. Biol. Control. 51:363–373.

54. Weddle, C.L. 1976. Petunias. In: Bedding plants: A manual on the culture of bedding plants as a greenhouse crop. Mastalerz, J.W. (ed.). Penn. Flower Growers, University Park:252–270

55. Wegulo, S. N., and Vilchez, M. 2007. Evaluation of lisianthus cultivars for resistance to *Botrytis cinerea*. Plant Dis. 91:997–1001.

56. Williamson, B., Tudzynski, B., Tudzynski, P., and Van Kan, J. A. L. 2007. *Botrytis cinerea*: The cause of grey mould disease. Mol. Plant Pathol. 8:561–580.

57. Yalage Don, S. M., Schmidtke, L. M., Gambetta, J. M., and Steel, C. C. 2020. *Aureobasidium pullulans* volatilome identified by a novel, quantitative approach employing SPME-GC-MS, suppressed *Botrytis cinerea* and *Alternaria alternata* in vitro. Sci. Rep. 10:1–13.

58. Yildiz, F., Yildiz, M., Delen, N., Coşkuntuna, A., Kinay, P., and Türküsay, H. 2007. The effects of biological and chemical treatment on gray mold disease in tomatoes grown under greenhouse conditions. Turk. J. Agric. For. 31:319–325.

59. Zhang, D., Spadaro, D., Garibaldi, A., and Gullino, M. L. 2010. Efficacy of the antagonist *Aureobasidium pullulans* PL5 against postharvest pathogens of peach, apple and plum and its modes of action. Biol. Control. 54:172–180.

60. Zhou, Q., Fu, M., Xu, M., Chen, X., Qiu, J., Wang, F., Yan, R., Wang, J., Zhao, S., Xin, X., and Chen, L. 2020. Application of antagonist *Bacillus amyloliquefaciens* NCPSJ7 against *Botrytis cinerea* in postharvest Red Globe grapes. Food Sci. Nutr. 8:1499–1508.