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Summary. ***Botrytis cinerea* is one of the problematic and notorious postharvest pathogens of bigleaf hydrangea (*Hydrangea macrophylla*) cut flowers. It causes flower blight, leaf blight, and stem rot, reducing the ornamental value (such as longevity, color, and texture) of flowers, ultimately making them unsalable. The objective of this study was to identify effective conventional fungicides and biorational products for botrytis blight management on bigleaf hydrangea cut flowers that can be easily and readily adopted by growers of ornamentals. Preventive preharvest whole-plant spray and postharvest dip treatment applications were used in this study. For the whole-plant spray applications, bigleaf hydrangea plants were sprayed with treatment solution 3 days before harvesting flowers. For the dip applications, cut flowers were dipped in treatment solutions after harvest. For both application types, flowers were inoculated with *B. cinerea* spores once treatment solutions dried. Flowers were stored in cold storage for 3 days and then displayed in conditions similar to retail stores. Botrytis blight disease severity, marketability of flower (postharvest vase life), phytotoxicity, and application residue were assessed in the study. Treatments showed variable efficacy in managing postharvest *B. cinerea* infection in bigleaf hydrangea cut flowers. Preventive preharvest whole-plant spray and postharvest dip applications of isofetamid and fluxapyroxad + pyraclostrobin significantly reduced the postharvest botrytis blight disease severity and area under disease progress curve (AUDPC) compared with the positive control (nontreated, inoculated with *B. cinerea*). When applied as a postharvest dip, the fungicide fludioxonil and biofungicide *Aureobasidium pullulans* strains DSM 14940 and DSM 14941 effectively lowered the disease severity and disease progress (AUDPC). These effective treatments also maintained a significantly longer postharvest vase life of bigleaf hydrangea cut flowers compared with the nontreated, inoculated control. The longer vase life may be attributed to lowered botrytis blight disease severity and the resultant proper physiological functioning of flowers.**

Hydrangeas (*Hydrangea* sp.) are popular deciduous flowering shrubs that are widely used as cut flowers, potted plants, and landscape plants (Adkins et al., 2003; Arafa et al., 2017; Kazaz et al., 2020; Pagter and Williams, 2011). Hydrangeas are the second top-selling ornamental shrub in the United States and are produced in more than 1500 nurseries nationwide (Fulcher et al., 2016). The market for hydrangea cut flowers is increasing, with hydrangea sales growing 64% between 2007 and 2014 to over $120 million per year (U.S. Department of Agriculture, 2014). The most popular species of hydrangea in the cut flower market is bigleaf hydrangea (*Hydrangea macrophylla*) because of its attractive flower and variable sepal color (Kazaz et al., 2020). The sepal color of bigleaf hydrangea ranges from white to red, pink, blue, or purple with a diversity of hues (light to dark) depending upon the cultivar, soil pH, and availability of aluminum (McClintock, 1957; Yoshida et al., 2003).

The floriculture market depends upon the ornamental characteristics of flowers such as longevity, shape, size, color, form, and texture (Seglie et al., 2009). However, bigleaf hydrangea flowers are greatly affected by the plant pathogenic fungus *Botrytis cinerea* (Baysal-Gurel et al., 2016). Botrytis blight disease reduces the ornamental quality of cut flowers, which makes them unsalable and represents a huge economic burden to growers. This opportunistic fungus has a devastating impact in both greenhouse and field production, and in postharvest environments, including storage and transportation. The fungal pathogen *B. cinerea* causes petal specking, leaf and fruit rots, and flower blight on many important horticultural crops, including ornamentals (Bika et al., 2020; Darras et al., 2005; Salinas and Verhoeff, 1995; Tomas et al., 1995). Infection with the pathogen usually starts in the early growth and development of a plant under certain environmental conditions, but the pathogen may remain quiescent and inactive (Prusky, 1996), becoming aggressive when it senses certain physical and physiological changes in the host's tissue (Williamson et al., 2007). Disease symptoms due to latent infections are often expressed in postharvest conditions (Muñoz et al., 2019).

Routine fungicide application (multisite and/or site-specific) has been the primary tool for the management of botrytis blight in greenhouse and field production. There are numerous multisite fungicides such as captan [Fungicide Resistance Action Committee code (FRAC) M3] and thiram (FRAC M4), and site-specific fungicides such as anilinopyrimidines (FRAC 9), quinone outside inhibitors [QoI (FRAC 11)], succinate dehydrogenase inhibitors [SDHI (FRAC 7)], phenylpyrroles (FRAC 12), and sterol biosynthesis inhibitors class III (FRAC 17) that are available in the market (Fernández-Ortuño et al., 2015). In the past, benzimidazoles (FRAC 1) and dicarboximides (FRAC

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
7.8125	fl oz/gal	$mL \cdot L^{-1}$	0.1280
3.7854	gal	L	0.2642
0.5933	$lb/yard^3$	$kg \cdot m^{-3}$	1.6856
7.4892	oz/gal	$g \cdot L^{-1}$	0.1335
(°F − 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

2) were used for the management of *B. cinerea*. However, the efficacy of these fungicides was limited (Elad, 1988), and they are no longer recommended for botrytis blight management (Sun et al., 2010). In the mid-1990s, different new group compounds such as anilinopyrimidines (cyprodinil), phenylpyrrole (fludioxonil), and hydroxyanilide (fenhexamid) were introduced (Rosslenbroich and Stuebler, 2000). The anilinopyrimidines do not affect the germination of spores but do prevent the growth of germ tube and mycelium of the fungus. In addition, they also inhibit the secretion of cell wall degrading enzymes by *B. cinerea* (Milling and Richardson, 1995). Similarly, phenylpyrrole and hydroxyanilide materials inhibit spore germination and induce swelling, bursting, and debranching of mycelium and germ tubes (Debieu et al., 2001; Leroux, 1996). Recently, SDHI fungicides such as boscalid, isofetamid, and fluxapyroxad have been introduced that effectively manage botrytis blight in various crops, including woody ornamentals (Baysal-Gurel and Simmons, 2017; Sierotzki and Scalliet, 2013). This fungicide class inhibits *B. cinerea* growth and development by blocking cellular or mitochondrial respiration (Piqueras et al., 2014).

The invention and development of fungicides with new chemical compounds have played crucial roles in maintaining value and stability of production. However, the number of fungicides registered for environmental horticulture crops has been decreasing due to disease resistance development, as well as environmental and human health issues (Gullino and Garibaldi, 2007). *B. cinerea* resistance to single-site fungicides such as thiophanate-methyl (FRAC 1), iprodione (FRAC 2), boscalid (FRAC 7), cyprodinil (FRAC 9), fludioxonil (FRAC 12), and fenhexamid (FRAC 17) has been observed worldwide across greenhouse, storage, and shipment conditions (Muñoz et al., 2019; Rodríguez et al., 2014; Rupp et al., 2017). Therefore, new a.i. with different modes of action that are socio-environmentally friendly need to be sought.

The resistance of pathogens to fungicides has increased the interest in development and adoption of biorational products for fungal disease management (Fravel, 2005). "Biorational" refers to pesticides (botanicals, minerals, microorganisms, and minimum-risk chemicals) of natural origin that have low or no negative effect on beneficial organisms and the environment (Reddy, 2016). Numerous studies have shown that use of beneficial fungi, such as *Clonostachys rosea* (synonym *Gliocladium roseum*), *Ulocladium atrum,* and the *Trichoderma* species, has effectively provided botrytis blight management in cyclamen (*Cyclamen persicum*), geranium (*Pelargonium* sp.), rose (*Rosa* sp.), moth orchid (*Phalaenopsis* sp.), and begonia (*Begonia* sp.) (Morandi et al., 2000; Sutton et al., 1997; Zaldúa and Sanfuentes, 2010; Zhao et al., 2018). Similarly, some of the yeasts such as black yeast (*Exophiala jeanselmei*), and bacteria such as *Bacillus subtilis* substantially suppress *B. cinerea* in rose flowers (Redmond, 1987; Tatagiba et al., 1998). Botanical products [e.g., giant knotweed (*Reynoutria sachalinensis*) extract] and mineral salts (e.g., mono and dibasic salt of phosphorous) also have been used for botrytis blight management in various crops (Reddy, 2016). However, biorational products were limited in their efficacy when applied to wider field conditions (Hu et al., 2009). In addition, they typically cannot provide enough protection from *B. cinerea* infection when the inoculum loads are high, but they can be useful for rotation or combination with fungicides for improved management of preharvest and postharvest *B. cinerea* infection.

An integrated management program including good sanitation practices, greenhouse environmental control, fungicides, and biorational products is required for the successful management of botrytis blight and for U.S. ornamental growers to remain competitive in the floriculture market. The objective of this study was to evaluate different fungicides and biorational products for the management of postharvest *B. cinerea* infection and postharvest vase life of bigleaf hydrangea cut flowers. The fungicides and biorational products were also assessed for phytotoxicity and application residue on cut flowers. The results of this study will provide growers of ornamentals with potential treatments for botrytis blight management.

Materials and methods

Plant material. 'Nikko Blue' bigleaf hydrangea plants were purchased from commercial nurseries in 2017 and maintained in 5-gal pots in a greenhouse for 2 years at the Tennessee State University Otis L. Floyd Nursery Research Center (TSUNRC), McMinnville, TN. Growing media consisted of 100% pine bark amended with 11.12 lb/yard^3 19N–2.1P–7.4K controlled-release fertilizer (Osmocote Pro; ICL Specialty Fertilizers, Dublin, OH), 1.01 lb/yard^3 micronutrient fertilizer (Micromax, ICL Specialty Fertilizers), 1.01 lb/yard^3 iron sulfate, and 0.34 lb/yard^3 Epsom salts. Irrigation was applied using micro bubbler emitters installed on short stakes in a greenhouse and maintained for 2, 3, and 4 min twice per day with the amount of 0.5, 0.75, and 1 L of water per day in May, June, and July, respectively. The bigleaf hydrangea plants did not receive any pesticides other than test products. 'Nikko Blue' bigleaf hydrangea has panicle-type inflorescences that contain small, fertile flowers and large, showy sepals. The term "flower" used in this article

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refers to a single harvested stem with its panicle of flowers and sepals. The bigleaf hydrangea flowers were harvested when sepals began showing color. Average temperatures in the greenhouse for May, June, and July were 23.5 °C [27.4/17.6 °C (maximum/minimum)], 23.9 °C (27.3/18.1 °C), and 24.1 °C (28.7/19.2 °C), respectively; average relative humidity (RH) was 94.5%, 95.7%, and 95.5%, respectively.

Fungal inoculum preparation. Isolate FBG2015-02 of *B. cinerea* was obtained from the culture collection of F. Baysal-Gurel at TSUNRC. The *B. cinerea* specimen was originally isolated from a diseased 'Nikko Blue' bigleaf hydrangea and maintained on potato dextrose agar (PDA) medium (Sigma-Aldrich, St. Louis, MO). Before this study, 'Nikko Blue' bigleaf hydrangea flowers and leaves were inoculated with a *B. cinerea* conidial suspension, and the pathogen was subsequently re-isolated from the diseased flowers and leaves to ensure virulence. For the preparation of inoculum, *B. cinerea* cultures were grown on PDA medium at 21 °C with 24 h fluorescent light. The conidial suspension was prepared by flooding a 10- to 14-d-old culture of *B. cinerea* with sterile distilled water, filtering it through cheesecloth, and then diluting it with sterile distilled water containing 0.1% (w/v) nonionic surfactant [polyoxyethylenesorbitan monolaurate (Tween 20; Sigma-Aldrich, St. Louis, MO)]. Conidial concentration was determined using a hemocytometer (Hausser Scientific, Horsahm, PA) under a light microscope (BX50; Olympus, Center Valley, PA). The final concentration was adjusted to 1×10^6 conidia/mL using $C_1V_1 = C_2V_2$, where C_1 is concentration of spores from hemocytometer count, V_1 is volume of suspension needed, C_2 is desired concentration of spores, and V_2 is the final desired volume of suspension.

Treatments and experimental design. The study was conducted at the TSUNRC, and 2-year-old 'Nikko blue' bigleaf hydrangea plants were used for the experiment. Five fungicides and five biorational products were evaluated for their ability to manage botrytis blight on bigleaf hydrangea cut flowers following the IR-4 Environmental Horticulture Program protocol 19-002 (IR-4 Project, 2019) (Table 1). Treatments were applied according to the recommended rates provided by registrants. Control treatments included nontreated, inoculated (positive control) and nontreated, noninoculated (negative control). Two treatment methods were used for this study: Preharvest whole-plant spray and postharvest dip applications. The rates of the treatments were the same for whole-plant spray and dip applications (Table 1). Even though fluxapyroxad + pyraclostrobin (Orkestra Intrinsic; BASF, Florham Park, NJ) and fluopyram + trifloxystrobin (Broadform; Bayer, Whippany, NJ) were recommended only for preharvest whole-plant spray application according to IR-4 protocol, they also were included in the dip application study to learn whether this method of application would be safe to the bigleaf hydrangea cut flowers and effective against botrytis blight. Multiple harvestings of flowers from the same set of bigleaf hydrangea plants were used for both experiments.

Whole-plant spray applications of fungicides or biorational products were applied as preventive preharvest treatments on bigleaf hydrangea plants (including foliage and flowers). The trial was carried out from 15–31 July 2019. Five single plant replications per treatment were arranged in a completely randomized design. Plants were sprayed with treatments until runoff, using a backpack carbon dioxide (CO_2)-pressurized sprayer (Bellspray, Opelousas, LA) with a tapered-edge flat-spray pattern stainless-steel nozzle (TP8002VS; TeeJet Technologies, Springfield, IL) at 40 psi, 3 d before harvesting flowers. The plants were left for about 3 h for drying of the applied treatment solution. Then the whole plant, including flowers and foliage, was artificially inoculated by uniformly spraying foliage and flowers with a *B. cinerea* spore suspension (10^6 conidia/mL) using a hand-held sprayer. The negative control plants were sprayed with only the sterile distilled water containing 0.1% (w/v) of nonionic surfactant (Tween 20). The bigleaf hydrangea plants were left in the greenhouse for 3 d, and then five single-flower replications per treatment were harvested.

Dip applications of conventional fungicides and biorational products were done as preventive postharvest treatments against botrytis blight. The first trial of dip application was conducted from 13–24 May 2019, and the second trial was conducted from 15–30 June 2019. Each treatment had six single-flower replications, which were arranged in a completely randomized design. The freshly harvested bigleaf hydrangea flowers were dipped for 10 s in prepared solutions of treatments according to protocol rates. The flowers were left for about 2 h for drying of the treatments, then they were artificially inoculated by uniformly spraying with the *B. cinerea* spore suspension (10^6 conidia/mL) using a hand-held sprayer. The negative control flowers were sprayed with only sterile distilled water containing 0.1% (w/v) nonionic surfactant (Tween 20), while positive control flowers were sprayed with the *B. cinerea* spore suspension.

The cut flowers were maintained in 900-mL beakers filled with tap water and placed in cold storage for 3 d at 4 °C, using a completely randomized design. Flowers were then displayed in normal room conditions, using a completely randomized design. The average maximum and minimum temperatures and RH were monitored using a weather station (WatchDog 2700; Spectrum Technologies, Aurora, IL). The average minimum and maximum temperatures of room condition for preharvest whole-plant spray (21–31 July 2019), postharvest dip Trial I (16–24 May 2019), and postharvest dip Trial II (18–30 June 2019) were 19 and 23 °C, 18 and 24 °C, and 18.5 and 23 °C, respectively; and average RH was 66%, 68%, and 62%, respectively.

Data recording. Evaluations were made on botrytis blight disease severity and phytotoxicity using a 1 to 5 ordinal scale [1 = no symptom (0%), 2 = 1% to 25% sepals affected, 3 = 26% to 50% sepals affected, 4 = 51% to 75% sepals affected, 5 = 76% to 100% sepals affected], and marketability of cut flowers using a 1 to 5 ordinal scale (1 = dead, 2 = poor quality, 3 = commercially acceptable, 4 = good quality, 5 = healthy). Evaluations were done every 2 d until the negative control flowers began to degrade in appearance. The ordinal scale values were converted into percentages using the midpoint value as described

Table 1. List of fungicides and biorational products and application rates according to IR-4 protocol for management of botrytis blight on bigleaf hydrangea cut flowers. All treatments were used for both dip application and whole-plant spray application.

Treatment[z]	Product a.i. (%)	Product application rate[y]	Product group[x]	FRAC code[w]
Aureobasidium pullulans strains DSM 14940 and DSM 14941	40 + 40	0.75 g·L^{-1}	BCA	NC
Fludioxonil	11.8	0.30 mL·L^{-1}	Phenylpyrrole	12
Fluopyram + trifloxystrobin	21.4 + 21.4	0.60 mL·L^{-1}	SDHI + QoI	7 + 11
Fluxapyroxad + pyraclostrobin	21.26 + 21.26	0.60 mL·L^{-1}	SDHI + QoI	7 + 11
Giant knotweed extract	5	10 mL·L^{-1}	Plant extract	P 05
Iprodione	23.3	1.25 mL·L^{-1}	Dicarboximide	2
Isofetamid	36	1.05 mL·L^{-1}	SDHI	7
Mono- and di-potassium salts of phosphorus acid + hydrogen peroxide	27.1 + 14	10 mL·L^{-1}	Phosphonate	P 07
SP2480 experimental extract[v]	unknown	2.34 mL·L^{-1}		NC
Ulocladium oudemansii strain U3[u]	45	3.60 g·L^{-1}	BCA	NC

[z]*A. pullulans* strains DSM 14940 and DSM 14941 (Botector; Westbridge Agriculture Products, Vista, CA), fludioxonil (Medallion; Syngenta, Greensboro, NC), fluopyram + trifloxystrobin (Broadform; Bayer, Whippany, NJ), fluxapyroxad + pyraclostrobin (Orkestra Intrinsic; BASF, Florham Park, NJ), giant knotweed extract (Regalia; Marrone Bio Innovations, Davis, CA), iprodione (Chipco 26019, Bayer), isofetamid (Astun; OHP, Bluffton, SC), mono and di-potassium salts of phosphorus acid + hydrogen peroxide (OxiPhos; BioSafe Systems, Harford, CT), SP2480 (SePRO, Carmel, IN), *U. oudemansii* strain U3 (BW165N; BioWorks, Victor, NY).
[y]1 g·L^{-1} = 0.1335 oz/gal, 1 mL·L^{-1} = 0.1280 fl oz/gal.
[x]SDHI = succinate dehydrogenase inhibitor, QoI = quinone outside inhibitor, BCA = biological control agent.
[w]Fungicide Resistance Action Committee code; NC = not classified.
[v]Nonionic surfactant (CapSil; Aquatrols, Paulsboro, NJ) used with SP2480 at a rate of 0.3 mL·L^{-1}.
[u]Nonionic surfactant (Brandt Organics Ag Aide; Brandt Consolidated, Springfield, IL) used with *U. oudemansii* strain U3 at a rate of 0.6 mL·L^{-1}.

by Bock et al. (2009). The area under disease progress curve (AUDPC) was calculated using formula $\sum\{[(x_i + x_i - 1)/2](t_i - t_i - 1)\}$, where x_i is botrytis blight disease severity rating midpoint value on each evaluation date, and $(t_i - t_i - 1)$ is the number of days between evaluations. Floral longevity (vase life) is the period (days) from harvest until flower marketability was no longer rated 3 or higher. Floral longevity was calculated using the formula $\sum(x_1 + x_2 + x_3 + \ldots\ldots + x_n)$, where x_1, x_2, x_3, $\ldots x_n$ were the marketability ratings on each evaluation date, and $x_1 = 1$ if $x_1 \geq 3$ on the marketability scale, otherwise $x_1 = 0$; $x_2 = 3$ if $x_2 \geq 3$ on the marketability scale, otherwise $x_2 = 2$; $x_3 \ldots x_n = 2$ if $x_3 \ldots x_n \geq 3$ on the marketability scale, otherwise $x_3 \ldots x_n = 0$. The absence or presence of application residue was also recorded for each treatment.

Statistical analysis. Botrytis blight disease severity, AUDPC, and floral longevity were compared among treatments for two types of application methods. SAS software (version 9.4 for Windows; SAS Institute, Cary, NC) was used to run statistical analysis of data. SigmaPlot software (version 14 for Windows; Systat Software, San Jose, CA) was used for graphical representation of data. One-way analysis of variance was performed using the general linear model procedure (PROC GLM) and Welch's *t* test to partition variance in disease severity index, AUDPC, and longevity into sources attributable to treatment and error. Welch's *t* test is a modification of Student's *t* test that does not assume equal variances (Welch, 1947; Zheng et al., 2013). Treatment means were separated using Tukey's Studentized range test at the 5% level of significance.

Results

Efficacy of preventive preharvest whole-plant spray application of fungicides and biorational products. The first botrytis blight symptom on bigleaf hydrangea flower was observed on the day 4 of the trial (immediately after removing plants from 3 d of cold storage). The negative control flowers had the lowest botrytis blight disease severity and disease progress, whereas the positive control had the highest disease severity ($F = 29.29$, $P < 0.001$) and disease progress ($F = 22.67$, $P < 0.001$) (Table 2). The longest postharvest vase life (13 d) of bigleaf hydrangea cut flowers was observed in the negative control ($F = 6.82$, $P < 0.001$) (Fig. 1).

Preharvest whole-plant spray application of fluxapyroxad + pyraclostrobin (7.8%) and isofetamid (13%) effectively reduced postharvest botrytis blight disease severity compared with the positive control flowers (88%) (Table 2). Fluopyram + trifloxystrobin, iprodione, and mono and di-potassium salts of phosphorus acid + hydrogen peroxide were not effective in reducing postharvest botrytis blight severity and disease progression. Preharvest whole-plant spray application of fungicides fluxapyroxad + pyraclostrobin and isofetamid significantly lowered disease progress compared with the positive control and were statistically equivalent to the negative controls. The remaining treatments (fludioxonil, SP2480, *Ulocladium oudemansii* strain U3, *Aureobasidium pullulans* strains DSM 14940 and DSM 14941, and giant knotweed extract) numerically reduced postharvest disease progress; however, they were not statistically different from the positive control flowers.

The positive control had the shortest postharvest vase life of bigleaf hydrangea cut flowers (4 d) (Fig. 1). Whole-plant spray applications of fluxapyroxad + pyraclostrobin (12 d) and isofetamid (10 d) effectively extended the postharvest vase life of cut flowers and were statistically like the negative control flowers. No other treatment was effective in extending postharvest vase life compared with the positive control flowers.

Phytotoxicity was not observed in any of the treated bigleaf hydrangea

Table 2. Efficacy of preventive postharvest dip and preharvest whole plant spray application of fungicides and biorational products (Table 1) in reducing postharvest botrytis blight severity and disease progress [area under disease progress curve (AUDPC)] on bigleaf hydrangea cut flowers inoculated with *Botrytis cinerea*, where control treatments included the nontreated, noninoculated (negative control) and nontreated, inoculated (positive control) flowers.

	Botrytis blight (mean ± SE)											
	Postharvest dip application								Preharvest whole-plant spray application			
	Trial I				Trial II							
Treatment	Disease severity (%)[z]		Disease progress (AUDPC)[y]		Disease severity (%)		Disease progress (AUDPC)		Disease severity (%)		Disease progress (AUDPC)	
Aureobasidium pullulans strains DSM 14940 and DSM 14941	42.2 ± 10.0	c-e[x]	219.4 ± 40.8	c-e[x]	58.8 ± 7.7	a-c[x]	439.6 ± 53.8	b-d	55.0 ± 16.7	a-c[x]	377.2 ± 114.3	ab[x]
Fludioxonil	46.3 ± 5.3	b-e	263.9 ± 39.7	c-e	25.5 ± 5.6	c-e	225.2 ± 41.2	de	63.0 ± 15.8	ab	322.7 ± 77.1	a-c
Fluopyram + trifloxystrobin[w]	83.8 ± 4.2	a	431.8 ± 33.3	ab	33.8 ± 11.9	b-e	322.1 ± 88.9	c-e	83.0 ± 5.0	a	461.3 ± 63.1	a
Fluxapyroxad + pyraclostrobin[w]	33.8 ± 4.2	de	180.8 ± 22.4	d-f	40.0 ± 16.0	b-d	402.5 ± 138.1	cd	7.8 ± 3.18	c	77.8 ± 34.9	bc
Giant knotweed extract	83.8 ± 4.2	a	366.8 ± 17.5	b-c	67.2 ± 4.2	ab	601.0 ± 25.6	a-c	58.0 ± 18.3	a-c	376.2 ± 109.3	ab
Iprodione	79.7 ± 5.3	ab	347.7 ± 18.3	bc	67.2 ± 7.7	ab	571.6 ± 54.2	a-c	68.0 ± 9.3	ab	463.0 ± 93.7	a
Isofetamid	33.8 ± 7.7	de	166.8 ± 38.3	ef	2.2 ± 2.2	de	80.8 ± 8.5	e	13.0 ± 0.0	bc	90.2 ± 8.1	bc
Mono- and di-potassium salts of phosphorus acid + hydrogen peroxide	79.7 ± 8.3	ab	378.1 ± 32.8	a-c	71.3 ± 8.3	ab	549.5 ± 41.8	a-c	83.0 ± 5.0	a	520.5 ± 55.3	a
SP2480 (experimental extract)	63.0 ± 12.9	a-d	311.4 ± 57.3	b-e	63.0 ± 11.2	a-c	509.5 ± 79.7	b-d	48.0 ± 16.9	a-c	323.0 ± 77.0	a-c
Ulocladium oudemansii strain U3	75.5 ± 12.5	a-c	332.3 ± 37.3	b-d	83.3 ± 4.2	a	705.3 ± 41.7	ab	53.0 ± 10.0	a-c	342.8 ± 49.6	a-c
Positive control	88.0 ± 0.0	a	531.8 ± 24.7	a	88.0 ± 0.0	a	808.8 ± 20.1	a	88.0 ± 0.0	a	634.5 ± 55.7	a
Negative control	17.2 ± 4.2	e	55.8 ± 16.8	f	0.0 ± 0.0	e	68.2 ± 4.2	e	7.8 ± 3.2	c	28.6 ± 6.4	c
F	10.71		14.79		42.06		151.02		29.29		22.67	
df	11		11		11		11		11		11	
P	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	

[z]Disease severity evaluated on day 10 of dip application (Trial I), day 12 of dip application (Trial II), and day 12 of whole-plant spray application, when all replications of positive control were high in disease severity scale (i.e., 5). Botrytis blight severity on each bigleaf hydrangea flower was evaluated using a 0 to 5 disease severity scale [1 = no symptom (0%); 2 = 1% to 25% sepals affected; 3 = 26% to 50% sepals affected; 4 = 51% to 75% sepals affected; 5 = 76% to 100% sepals affected] and changed to a percentage basis by taking the midpoint value as described by Bock et al. (2009). Values are the mean of six single-flower replications in the dip application trial and five single-flower replications in the whole-plant spray application trial.

[y]Disease progress was the mean progression of disease during the entire experiment period and was calculated on a percentage basis for each treatment by using the formula $\sum\{[(x_i + x_i - 1)/2](t_i - t_i - 1)\}$, where x_i is botrytis blight disease severity rating midpoint value on each evaluation date and $(t_i - t_i - 1)$ is number of days between evaluations. Values are the mean of six single-flower replications that were evaluated for 12 d in the dip application (Trial I) and 16 d in the dip application (Trial II), and five single-flower replications that were evaluated for 14 d in the whole-plant spray application trial.

[x]Treatment means within columns followed by different letters were significantly different ($P < 0.05$). One-way analysis of variance was used to partition variance in disease severity and disease progress. Means of each treatment were compared using Tukey's honestly significant difference test at $\alpha = 0.05$.

[w]Treatment is not labeled for dip application.

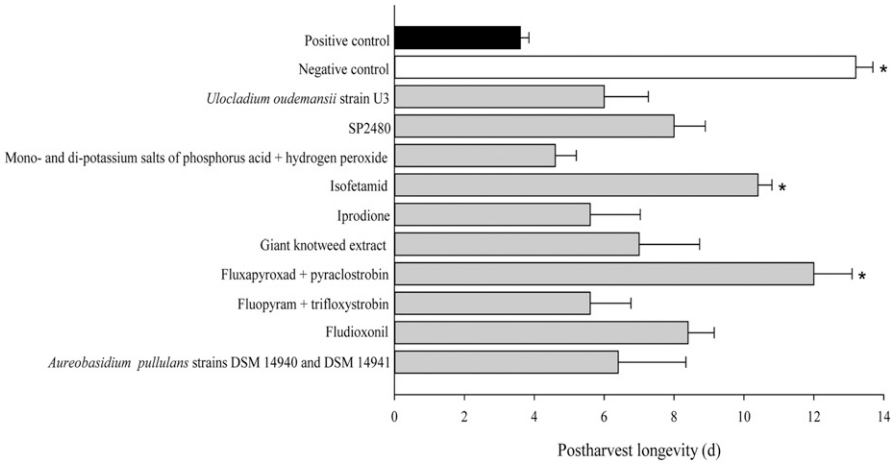

Fig. 1. Postharvest longevity (mean ± SE) of bigleaf hydrangea cut flowers after application of preharvest whole-plant spray of fungicides and biorational products (Table 1) and inoculation with *Botrytis cinerea*. The fungicides and biorational products were sprayed until runoff 3 d before the harvesting of the bigleaf hydrangea flowers. Longevity is the period from harvest until flowers were rated 3 or more in marketability scale. Marketability of cut flowers was evaluated using a 1 to 5 ordinal scale (1 = dead, 2 = poor quality, 3 = commercially acceptable, 4 = good quality, 5 = healthy). Control treatments included the nontreated, noninoculated (negative control) and nontreated, inoculated with *B. cinerea* (positive control). Values are the mean of five single-flower replications that were evaluated for 14 d, and asterisks beside the bar indicate significant differences in the longevity of cut flowers within the treatments compared with the positive control ($F = 6.82$, df = 11, $P < 0.001$).

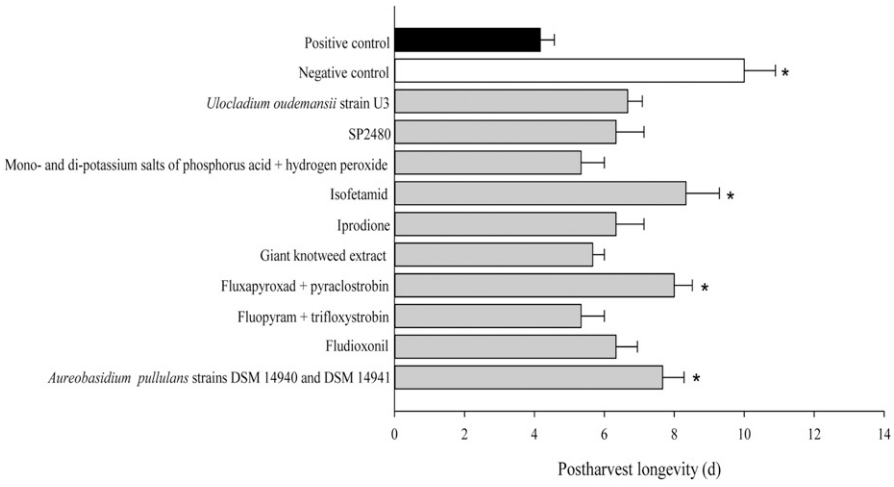

Fig. 2. Postharvest longevity (mean ± SE) of bigleaf hydrangea cut flowers after postharvest dip application of fungicides and biorational products (Table 1) and inoculation with *Botrytis cinerea* (dip Trial I). The bigleaf hydrangea flowers were dipped for 10 s in treatment solution immediately after harvesting, and then cold stored for 3 d and displayed in a normal room condition. Longevity is the period from harvest until flowers were rated 3 or more in marketability scale. Marketability of cut flowers was evaluated using a 1 to 5 ordinal scale (1 = dead, 2 = poor quality, 3 = commercially acceptable, 4 = good quality, 5 = healthy). Control treatments included the nontreated, noninoculated (negative control) and nontreated, inoculated with *B. cinerea* (positive control). Values are the mean of six single-flower replications that were evaluated for 12 d, and asterisks beside the bar indicate significant differences in longevity of cut flowers within the treatments compared with the positive control ($F = 5.66$, df = 11, $P < 0.001$).

flowers. However, black application residue of *U. oudemansii* strain U3 was observed in all treated flowers, whereas pink residue of giant knotweed extract was observed in two out of five treated flowers.

Efficacy of preventive postharvest dip application of fungicides and biorational products. Botrytis blight symptom development was observed in 3 d after artificial inoculation on bigleaf hydrangea flowers. Negative control and positive control hydrangea flowers had the lowest and highest disease severity (Trial I: $F = 10.71$, $P < 0.001$; Trial II: $F = 42.06$, $P < 0.001$) and AUDPC (Trial I: $F = 14.79$, $P < 0.001$; Trial II: $F = 151.02$, $P < 0.001$) in either of the dip application trials, respectively (Table 2). The negative control flowers maintained the longest postharvest vase life (Trial I: 10 d, $F = 5.66$, $P < 0.001$; Trial II: 13 d, $F = 65.45$, $P < 0.001$) (Figs. 2 and 3).

In the first dip trial, isofetamid (33.8%), fluxapyroxad + pyraclostrobin (33.8%), *A. pullulans* strains DSM 14940 and DSM 14941 (42.2%), and fludioxonil (46.3%) significantly reduced postharvest botrytis blight disease severity compared with the positive control flowers (88.0%) and were statistically equivalent to the negative control (17.2%) (Table 2). Isofetamid and fluxapyroxad + pyraclostrobin substantially lowered disease progress and were not statistically different from the negative control. *A. pullulans* strains DSM 14940 and DSM 14941, *U. oudemansii* strain U3, iprodione, fludioxonil, giant knotweed extract, and SP2480 reduced the botrytis blight postharvest progression compared with the positive control flowers, but they were not statistically equivalent to the negative control. Fluopyram + trifloxystrobin and mono and di-potassium salts of phosphorus acid + hydrogen peroxide were ineffective in reducing disease progress. Isofetamid, fluxapyroxad + pyraclostrobin, *A. pullulans* strains DSM 14940 and DSM 14941, and fludioxonil significantly reduced botrytis blight disease severity during the entire experiment period, whereas iprodione, SP2480, mono and di-potassium salts of phosphorus acid + hydrogen peroxide, giant knotweed extract, and *U. oudemansii* strain U3 were effective up to

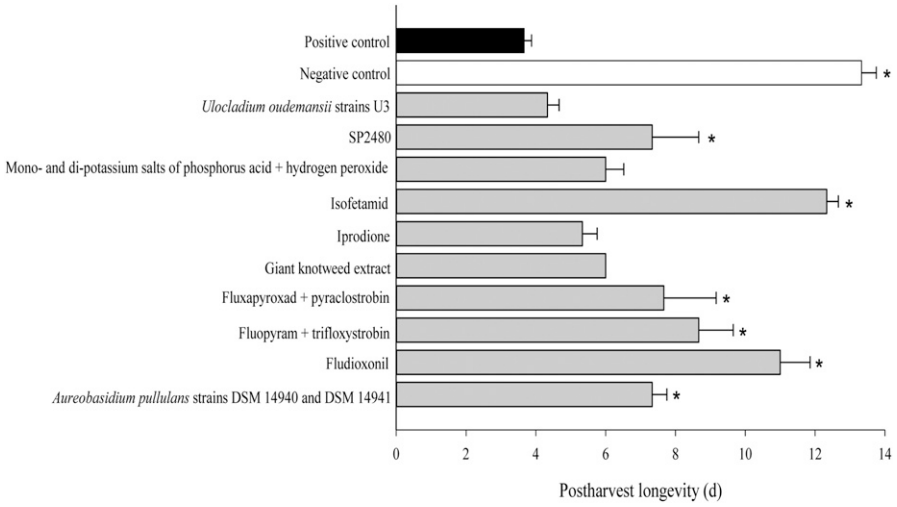

Fig. 3. Postharvest longevity (mean ± SE) of bigleaf hydrangea cut flowers after postharvest dip application of fungicides and biorational products (Table 1) and inoculation with *Botrytis cinerea* (dip Trial II). The bigleaf hydrangea flowers were dipped for 10 s in treatment solution immediately after harvesting, and then cold stored for 3 d and displayed in a normal room condition. Longevity is the period from harvest until flowers were rated 3 or more in marketability scale. Marketability of cut flowers was evaluated using a 1 to 5 ordinal scale (1 = dead, 2 = poor quality, 3 = commercially acceptable, 4 = good quality, 5 = healthy). Control treatments included the nontreated, noninoculated (negative control) and nontreated, inoculated with *B. cinerea* (positive control). Standard error = 0 for the mean postharvest longevity of giant knotweed extract. Values are the mean of six single-flower replications that were evaluated 16 d, and asterisks beside the bar indicate significant differences in longevity of cut flowers within the treatments compared with the positive control ($F = 65.45$, df = 11, $P < 0.001$).

8 d of postharvest life in this trial (data not shown). The fungicides isofetamid (8 d) and fluxapyroxad + pyraclostrobin (8 d), and the biorational product *A. pullulans* strains DSM 14940 and DSM 14941 (8 d) significantly maintained longer postharvest vase life of bigleaf hydrangea cut flowers than the positive control (4 d) (Fig. 2).

In the second dip trial, isofetamid (2.2%), fludioxonil (25.5%), fluopyram + trifloxystrobin (33.8%), and fluxapyroxad + pyraclostrobin (40.0%) significantly reduced postharvest botrytis blight symptom development and disease severity compared with the positive control flowers (88.0%), with isofetamid, fludioxonil, and fluopyram + trifloxystrobin statistically equivalent to the negative control (Table 2). Isofetamid, fludioxonil, and fluopyram + trifloxystrobin reduced disease progress significantly and were statistically equivalent to the negative control, while fluxapyroxad + pyraclostrobin, SP2480, and *A. pullulans* strains DSM 14940 and DSM 14941 lowered disease incidence but were not equivalent to the negative control. *U. oudemansii* strain U3, iprodione, mono and di-potassium salts of phosphorus acid + hydrogen peroxide, and giant knotweed extract were equivalent to the positive control. The fungicide isofetamid significantly reduced postharvest botrytis blight disease severity during the entire experiment period, followed by fluxapyroxad + pyraclostrobin (14 d), fluopyram + trifloxystrobin (14 d), and fludioxonil (14 d), whereas the biorational product *A. pullulans* strains DSM 14940 and DSM 14941 effectively lowered the disease severity for 10 d (data not shown). The shortest postharvest vase life of cut flowers was observed in the positive control (4 d) (Fig. 3). Isofetamid (12 d) and fludioxonil (11 d) significantly maintained the longest vase life of bigleaf hydrangea cut flowers, and the vase life was not statistically different from the negative control flowers (13 d). Other treatments that extended the postharvest vase life of cut flowers were fluopyram + trifloxystrobin (9 d), *A. pullulans* strains DSM 14940 and DSM 14941 (7 d), and SP2480 (7 d) (Fig. 3).

No phytotoxicity was observed in any of the treated bigleaf hydrangea cut flowers in either of the dip trials. However, noticeable residues occurred after applications of *U. oudemansii* strain U3 (black) and giant knotweed extract (pink) in all treated hydrangea flowers.

Discussion

In this study, efficacy of preharvest whole-plant spray and postharvest dip applications of conventional fungicides and biorational products were screened for efficacy against botrytis blight, caused by *B. cinerea*, on bigleaf hydrangea cut flowers. Inoculated, nontreated flowers (positive controls) consistently showed the highest postharvest botrytis blight disease severity and disease progress. The disease symptoms in positive controls were observed immediately after removal from cold storage (4 °C), and positive control flowers were severely impacted by botrytis blight within 5 d. Similarly, *B. cinerea* infection was observed in freesia (*Freesia ×hybrida*) flowers when stored in 5 °C; the symptom development was slow but ceaseless (Darras et al., 2006). *Botrytis cinerea* is an opportunistic pathogen that can even cause infection at low temperatures [i.e., <5 °C (Couey and Follstad, 1996)], and it mostly affects stressed and senescent plant tissues (Williamson et al., 2007). The cut flowers are under stressful conditions during postharvest life, which makes them vulnerable to *B. cinerea* infection. Botrytis blight management in bigleaf hydrangea cut flowers is thus important for better postharvest vase life.

Isofetamid (Astun; OHP, Bluffton, SC) and fluxapyroxad + pyraclostrobin (Orkestra Intrinsic), fungicides that interfere with fungal cellular respiration, effectively reduced the postharvest botrytis blight disease severity and disease progress in all trials (whole-plant spray and dip applications). This finding was like other studies where isofetamid significantly reduced botrytis blight disease severity in bigleaf hydrangea, grape (*Vitis vinifera*), and strawberry (*Fragaria ×ananassa*) (Baysal-Gurel et al., 2018; Cavotto et al., 2018; Piqueras et al., 2014). Similarly, fluxapyroxad and pyraclostrobin, when applied alone or in combination, effectively managed botrytis blight and other fungal diseases (Chen et al., 2014; Min et al., 2014; Rebollar-Alviter and

Ellis, 2005; Shi et al., 2020; Vea and Palmer, 2020). The mixture of two chemical classes may also add flexibility (multiple target sites) in their action against phytopathogens (Pscheidt et al., 2017). Isofetamid and fluxapyroxad belong to the SDHI class of fungicides, and pyraclostrobin belongs to the QoI class of fungicides (FRAC, 2020). The SDHI and QoI fungicides block the electron transport system in fungal mitochondria, which interrupts the cellular energy cycle and, ultimately, results in the death of the fungi (Veloukas and Karaoglanidis, 2012; Zeng et al., 2015). Fluxapyroxad + pyraclostrobin is not labeled for dip application, so growers must manage botrytis blight preharvest when using this product. Fluopyram + trifloxystrobin (Broadform) did not effectively reduce postharvest botrytis blight severity and disease progress or maintain vase life of cut flowers in the current study. Disease severity increased after cold storage, reaching a maximum within 7 d of postharvest vase life. A similar result was reported by Vea and Palmer (2020), that the mixture of fluopyram and trifloxystrobin did not provide effective control of botrytis blight in geranium. However, fluopyram + trifloxystrobin effectively managed *B. cinerea* infection only in dip Trial II. The increased efficacy of fluopyram + trifloxystrobin (as well as isofetamid in dip Trial II) might be due to lower relative humidity (62%) in the flower display room, which might have slowed the growth of *B. cinerea* compared with conditions during the dip application Trial I (RH = 68%). Eden et al. (1996) observed a low level of *B. cinerea* infection on tomato (*Solanum lycopersicum*) flowers at 56% RH. However, a significant positive correlation between RH and *B. cinerea* infection was observed (i.e., flower infection increased under higher RH).

Fludioxonil (Medallion; Syngenta, Greensboro, NC), a phenylpyrrole class fungicide, substantially managed the postharvest botrytis blight of bigleaf hydrangea cut flowers during the entire experiment period. The preventive postharvest dip application of fludioxonil was effective in reducing botrytis blight disease severity and extending postharvest vase life of cut flowers. Fludioxonil interferes with the osmoregulatory pathway of fungi (Hahn, 2014), inhibiting the germination of spores and growth and development of mycelium (Kim et al., 2016). In tulip (*Tulipa* sp.) flowers, application of fludioxonil significantly reduced the number of blighted flowers and disease severity (Chastagner and DeBauw, 2017). However, preharvest whole-plant spray application of fludioxonil did not effectively reduce botrytis blight disease severity and disease progress in this study. This result may have been due to the conducive environmental conditions in the greenhouse and a higher inoculum level (i.e., 10^6 conidia/mL) that have facilitated *B. cinerea* infection, so that any treatment benefit was overcome. Further, fludioxonil is a nonsystemic fungicide and is not absorbed by the plant, so its efficacy is based on contact. Fludioxonil may break down too quickly in the environment after application, which would limit its efficacy; and sometimes the spraying method only provides fungicide coverage on exposed surface, leaving the unexposed side of sepals without treatment. Any pathogen like *B. cinerea* that can initially infect plants and reside there until suitable conditions occur for greater colonization can avoid subsequent surface treatments. Thus, the greater efficacy seen with the dipping method in our study may have been due to better surface coverage on flowers compared with the whole-plant spray method.

Iprodione (Chipco 26019, Bayer), a fungicide in the dicarboximide chemical group, was not effective in controlling postharvest botrytis blight management in bigleaf hydrangea cut flowers. Similar inconsistent results for iprodione were observed in strawberry and grapevine in controlling postharvest *B. cinerea* infection (Blacharski et al., 2001; Kim et al., 2016). In contrast, postharvest spray of iprodione with fan drying and dip application in rose flower and strawberry transplants showed good efficacy in managing botrytis blight severity (Goss and Mazarura, 2013; Oliveira et al., 2018). The poor performance of iprodione in this study could have been due to the use of only a single application, or the applied concentration was not high enough, or perhaps the isolate of *B. cinerea* used is insensitive to iprodione. Increases in antifungal activity of iprodione inhibiting spore germination and mycelium growth of *B. cinerea* have been observed with increases in concentration (Kim et al., 2016).

Saprophytic ascomycete *A. pullulans* strains have been found effective in reducing postharvest botrytis blight (Castoria et al., 2001; Weiss et al., 2014). The antagonist activity of *A. pullulans* against pathogens is due to competition for nutrients and niches as well as production of some enzymes such as proteases and chitinases (Freimoser et al., 2019). In this study, *A. pullulans* strains DSM 14940 and DSM 14941 (Botector; Westbridge Agriculture Products, Vista, CA) showed slightly ineffective (preharvest whole-plant spray application) to effective (postharvest dip application) results in controlling the postharvest botrytis blight disease progression. The moderate performance of *A. pullulans* strains DSM 14940 and DSM 14941 in preharvest whole-plant spray application might have been due to less conducive greenhouse conditions for colonization of flower tissues. Bigleaf hydrangea plants were sprayed with *A. pullulans* DSM 14940 and DSM 14941 three days before the harvest and remained in the greenhouse under a condition of lower humidity and warmer temperature than the condition following dip applications, where flowers were taken to cold storage after treatment. According to Rotolo et al. (2018), high-disease pressure spraying with *A. pullulans* strains DSM 14940 and DSM 14941 alone (up to 11 sprays) did not give satisfactory results in managing *B. cinerea* infection in grapevine; however, application of these strains was effective when integrated with SDHI fungicides. Overall, *A. pullulans* strains DSM 14940 and DSM 14941 show good potential for use in combination or rotation with reduced-risk fungicides.

Another biocontrol agent, *U. oudemansii* strain U3 (BW165N; BioWorks, Victor, NY), showed inconsistent performance in reducing the postharvest botrytis blight disease severity and disease progress as well as in maintaining postharvest vase life. This finding contradicts previous work (Calvo-Garrido et al., 2014; Thomidis et al., 2015), where applications of *U. oudemansii* strain U3

substantially reduced botrytis blight incidence and disease severity in grape and strawberry. Time for establishment of biological control agents on host tissue before the pathogen is crucial for proper disease suppression. According to Kessel et al. (2002), sporulation of *B. cinerea* was not significantly reduced when *U. atrum* was applied 12 h or less before *B. cinerea* inoculation; however, when *U. atrum* was given 48 h head-start time, it completely suppressed the sporulation of *B. cinerea* in cyclamen leaf tissue. In this study, plants or flowers were inoculated with *B. cinerea* conidial suspension about 2 to 3 h after treatment with *U. oudemansii* strain U3, which might not have been enough time for *U. oudemansii* strain U3 to become well established on host tissue. Therefore, further inquiries are needed to elucidate the length of time required for *U. oudemansii* strain U3 to be established before *B. cinerea* challenge for optimal suppression. In addition, more study is needed to determine the concentration and number of additional applications required for *U. oudemansii* strain U3 to produce satisfactory postharvest botrytis blight reduction.

Except for *A. pullulans* strains DSM 14940 and DSM 14941, the biorational products tested under these conditions showed ineffective results. The biorational product *U. oudemansii* strain U3, mono and dipotassium salts of phosphorus acids + hydrogen peroxide (OxiPhos; Bio-Safe Systems, Harford, CT), giant knotweed extract (Regalia; Marrone Bio Innovations, Davis, CA), and SP2480 (experimental extract; SePRO, Carmel, IN) were not effective in reducing botrytis blight severity and maintaining the postharvest vase life of cut flowers. It is not uncommon for biorationals to perform differently in different test systems or across experiments in the same test system. For example, variable efficacy results were observed with giant knotweed extract when screening for botrytis blight disease efficacy in tulip and asiatic hybrid lilies [*Lilium* ×*asiatica* (Chastagner and DeBauw, 2014, 2017)]; however, it was found to be effective in protecting pruning wounds and leaves of tomato against botrytis blight (Bardin et al., 2008). Further studies are warranted looking at rate ranges and application of biorational products multiple times during production in addition to preharvest or postharvest treatments, endeavors outside the scope of these screening experiments.

The postharvest vase life of cut flowers is one of the most important quality determinants in the cut flower market because flowers often are subjected to lengthy handling, storage, and transportation processes. The increased postharvest vase life shown here from application of conventional fungicides and biorational products likely is due to reduction in postharvest botrytis blight disease severity, which facilitates better physiological functioning of bigleaf hydrangea cut flowers.

In ornamental production, curative fungicide applications for botrytis blight are not considered an appropriate strategy because any slight incidence of disease can ruin the ornamental characteristics of flowers. Thus, preventive application of fungicides/biorational products should be a major strategy for botrytis blight management to maintain the quality of the product and remain competitive in the market. The efficacy of fungicides and biorational products depends upon several factors related to the plant and application of the product, as well as the environmental conditions. This study used a single, diploid cultivar of bigleaf hydrangea, whereas triploid cultivars of hydrangea have thicker sepals and altered stomatal structure (Alexander, 2017). Disease severity, disease progress, and efficacy of fungicides and biorational products should be evaluated in more cultivars to gain a more complete picture of postharvest botrytis blight management in bigleaf hydrangea flowers. Application variables that may affect efficacy include concentration, timing and frequency, methods of application, and stage of flower development. This study tested the single application of fungicides and biorational products as preharvest and postharvest treatments. Only isofetamid and fluxapyroxad + pyraclostrobin consistently reduced the postharvest botrytis blight disease severity, and these two products also maintained the longer vase life of bigleaf hydrangea cut flowers in both preventive application methods. Fludioxonil and *A. pullulans* strains DSM 14940 and DSM 14941 significantly lowered the disease severity and improved postharvest vase life when applied as a postharvest dip treatment. All other treatments showed variable efficacy (slightly effective to ineffective) for botrytis blight management. Results of this study will provide growers with improved strategies for postharvest botrytis blight management to protect the quality and value of cut flowers.

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