Influence of Essential Oils on Post-infection Botrytis Damage in Cut Roses[1]

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Abstract

We assessed the degree to which 16 post-infection treatments controlled Botrytis (*Botrytis cinerea* Pers. ex. Fr.) damage in cut roses (*Rosa* × *hybrida*). Additional experiments examined whether essential oils (EO) of cinnamon (*Cinnamomum zeylanicum* Blume) leaf (CLO), clove (*Eugenia caryophyllata* Thunb.) bud (CBO), and thyme (*Thymus vulgaris* L.) (TO) could reduce damage in Botrytis-infected cut roses. The 16 treatments applied to 'Light Orlando' cut roses differed in reducing Botrytis damage and causing phytotoxicity damage. Only the synthetic fungicide fludioxonil [applied as 0.23 g · L^{-1} (0.00024 oz · fl oz^{-1}) Medallion®] resulted in the desirable combination of greatly reduced stem termination frequency due to Botrytis damage and relatively minor flower phytotoxicity. When applied to cut rose 'Freedom' or cultivars with light colored flowers ('Cool Water', 'Jessika', 'Polar Star', 'Tiffany'), all EO aqueous solutions caused pronounced phytotoxicity damage, but only TO reduced Botrytis damage significantly compared to untreated flowers. Roses exposed to EO vapor rather than an aqueous solution tended to exhibit less phytotoxicity. Vapors of CLO and CBO tended to reduce Botrytis damage less and caused greater flower phytotoxicity than TO vapor and aqueous fludioxonil. Thyme oil vapor exposures of 4.6 and 9.1 ppm warrant further investigation.

Index words: Botrytis blight, *Botrytis cinerea* Pers. ex. Fr., cut flowers, floriculture, fungicide, gray mold, *Rosa* × *hybrida*.

Chemicals used in this study: *Bacillus subtilis* (Cease®), bleach (Clorox®), chlorothalonil (Daconil®), copper sulphate (Phyton® 27), fenhexamide (Elevate®), fludioxonil (Medallion®), hydrogen peroxide (ZeroTol® 2.0), iprodione (Chipco® 26019 Flo), potassium bicarbonate (Milstop®), pyraclostrobin + boscalid (Pageant® Intrinsic®).

Species used in this study: Rose (*Rosa* × *hybrida*) 'Cool Water', 'Freedom', 'Jessika', 'Polar Star', 'Tiffany', Botrytis (*Botrytis cinerea* Pers. ex. Fr.).

Significance to the Horticulture Industry

Minimizing Botrytis damage to cut flowers during storage and shipping is an ongoing industry challenge. Management practices during cut rose production attempt to eradicate the causal organism *Botrytis cinerea* Pers. ex. Fr., but even the most rigorous greenhouse protocols cannot ensure elimination of the ubiquitous pathogen. Thus, safe, inexpensive, reliable methods are needed to reduce damage in Botrytis-infected cut roses. This work shows that the promise of essential oil treatments to protect against fungal damage that has been demonstrated in various horticultural crops might be applicable to cut roses. Thyme essential oil was shown to reduce Botrytis damage in Botrytis-infected roses nearly to the extent that the most effective synthetic fungicide fludioxonil did. The flower phytotoxicity damage problem observed with aqueous essential oil treatments was reduced considerably by switching to vapor application. This work indicates that further research is warranted with thyme oil vapor with treatments centered between 4.6 and 9.1 ppm.

Introduction

Botrytis cinerea Pers. ex. Fr. has a broad host range and can infect almost every plant part (Rosslenbroich and Stuebler 2000). Dole and Wilkins (2005) mention diseases due to Botrytis – nearly always *B. cinerea* – as occurring in more than two-thirds of 108 floriculture crop genera, and Botrytis is considered a major problem in nearly half of those. Rose has long been known as a species in which *B. cinerea* is widespread and able to cause significant damage to the crop (Coyier 1985). Classic Botrytis blight on rose flowers starts with the appearance of small lesions that can spread causing necrosis of whole petals (Pie and Brouwer 1993) or petal abscission or whole flower collapse if infection spreads to the receptacle (Droby and Lichter 2007). While it can negatively impact production, *B. cinerea* can also reduce quality and vase life of cut flowers (Tomas et al. 1995), and the economic losses caused by Botrytis can be particularly great in cut roses as one of the most important ornamental crops in the world (Vrind 2005).

Several aspects of handling and shipping cut flowers result in conditions conducive to *B. cinerea* growth such that latent *B. cinerea* infections in cut roses (Elad 1988a) often become known only after storage and transport. Densely packed shipping boxes cause breaking and bruising of stems, leaves, and flowers, which provides sites for the fungus to infect the plant tissue and leads to ethylene production which has been found to make host tissue more susceptible to infection (Elad 1988b). Efforts to maintain cut flowers at low, constant temperatures during transport are not always successful. Warm temperatures and temperature fluctuations causing condensation in shipping boxes result in optimal conditions for *B. cinerea* (Sosa-Alvarez et al. 1995, Zhang and Sutton 1994) spore germination and infection [13 to 24 C (55 to 75 F), >93%

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Table 1. Treatments and controls imposed on Botrytis-infected 'Light Orlando' cut roses in Experiment 1. Infection was accomplished by spraying rose flowers and leaves to run-off with $10^5 \cdot ml^{-1}$ (0.03 fl oz) *Botrytis cinerea* spores and incubating them in black plastic bags for 24 h at constant 20 C (36 F). Following incubation, cinnamon and thyme oil treatments were applied by spraying flowers and leaves to run-off with solution, and all other treatments were applied by dipping entire stems into 15 L (4.0 gal) solution for 20 s.

Treatment active ingredient			
Common name	**IUPAC Chemical name**	**%**	**FRAC class**
Bacillus subtilis	*Bacillus subtilis* strain QST 713	1.34	44
Batine	Trade secret	Trade secret	NC
Bleach	Sodium hypochlorite	6.00	NC
Chlorothalonil	2,4,5,6-tetrachloroisophthalonitrile	12.50	M5
Cinnamon leaf oil	*Cinnamomum zeylanicum* leaf essential oil	100	NC
Copper sulphate	Copper sulphate pentahydrate	21.36	4
Fenhexamide	N-(2,3-dichloro-4-hydroxyphenyl)-1-methyl cyclohexanecarboxamide	50.00	17
FloraDip R	Trade secret	Trade secret	NC
Fludioxonil	4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile	50.00	12
Hydrogen peroxide	Hydrogen peroxide	27.10	NC
Iprodione	3-(3,5-dichlorophenyl)-N- (1-methylethyl)-2,4-dioxo-1- imidazolidinecarboxamide	23.30	2
Potassium bicarbonate	Potassium bicarbonate	85.00	NC
Pyraclostrobin + Boscalid	Methyl {2-[1-(4-chlorophenyl)pyrazol-3-yloxymethyl]phenyl}(methoxy)carbamate + 2-chloro-N-(4′-chlorobiphenyl-2-yl)nicotinamide	12.80 + 25.20	11 + 7
Pyraclostrobin + Fluxapyroxad	Methyl {2-[1-(4-chlorophenyl)pyrazol-3-yloxymethyl]phenyl}(methoxy)carbamate + 3-(difluoromethyl)-1-methyl-N-(3′,4′,5′-trifluorobiphenyl-2-yl)pyrazole-4-carboxamide	21.26 + 21.26	11 + 7
Stylet oil	Paraffin oil	97.10	NC
Thyme oil	*Thymus vulgaris* and/or *Thymus zygis* essential oil	100	NC
Control	At *B. cinerea* inoculation	At treatment application	
No fungicide	Inoculation with *B. cinerea* spores	Tap water dip	
No spore	Spray with spore-free inoculation solution	Tap water dip	
Water	Spray with tap water	Tap water dip	
Absolute	Untreated	Untreated	

relative humidity, and 8 to 12 h of free water)] and disease development [15 to 20 C (59 to 68 F)].

The principal way to reduce incidence and severity of Botrytis damage in cut roses has been to treat them with synthetic fungicides before storage or transport, but interest in finding natural, organic compounds for control of microorganisms in postharvest crop handling has been fueled by several concerns. Control of Botrytis is increasingly difficult due to resistance of *B. cinerea* to synthetic fungicides (Hahn 2014), particularly the benzimidazoles (FRAC class 1). Findings that *B. cinerea* can develop fungicide resistance in one season (Redmond et al. 1987) and that *B. cinerea* strains may have multiple fungicide resistances (Elad et al. 1992) have increased interest in alternatives to synthetic fungicides. Additionally, restrictions on the use of conventional agricultural fungicides have become more common throughout the world (Dayan et al. 2009), and less toxic and biodegradable alternatives are preferred by retailers and consumers (Wisniewski et al. 2001).

Essential oils (EO) from plants have long been recognized as effective fungicides (Wilson et al. 1997). Cinnamon bark/leaf (*Cinnamomum zeylanicum* Blume), clove bud (*Eugenia caryophyllata* Thunb.), and common thyme (*Thymus vulgaris* L.) EOs have been shown to have strong antimicrobial activity (Davidson et al. 2013). Common thyme and cinnamon were found to be the most effective of 18 EOs tested in terms of inhibiting growth of five common postharvest fungal pathogens (Combrinck et al. 2011). Of the 49 EOs tested, *B. cinerea* spore germination was inhibited most by those from red thyme (*Thymus zygis* L.), palmarosa [*Cymbopogon martini* (Roxb.) J.F.Watson], clove bud and cinnamon leaf (Wilson et al. 1997).

Plant-derived EOs have shown potential as alternatives to synthetic fungicides to control postharvest diseases. Peaches inoculated with *Bacillus amyloliquefaciens* PPCB004 and stored in modified atmosphere packaging impregnated with lemongrass [*Cymbopogon citratus* Stapf) EO eliminated disease caused by the fungal pathogens *B. cinerea*, *Penicillium expansum* Link and *Rhizopus stolonifer* (Ehrenb.Fr.) Vuill. without diminishing fruit appearance and acceptability (Arrebola et al. 2010). Wrapping table grapes (*Vitis vinifera* L.) in modified atmosphere packaging impregnated with the EO constituents eugenol, thymol, and carvacrol dramatically decreased mold, yeast, and bacteria counts and lowered berry decay frequency (Guillén et al. 2007). In work focused on *B. cinerea* in grapes, Tripathi et al. (2008) demonstrated increased storage life of up to 6 d through ginger (*Zingiber officinale* Rosc.) and holy basil (*Ocimum sanctum* L.) EO treatments.

The first objective of our work was to evaluate the efficacy of 16 treatments, including commercial synthetic fungicides and non-synthetic products, for reducing post-infection disease damage caused by *B. cinerea* in cut roses. We then looked more closely at the potential to use three aqueous EOs as an alternative to an effective synthetic fungicide. Finally, we investigated the application of the same three EOs as a vapor rather than as an aqueous

Table 1. Extended.

Commercial product			
Name	**Formulation**	**Source**	**Amount per L**
Cease®	Liquid	BioWorks, Inc., Victor, NY, USA	15.1 ml
Batine (solutions 1 and 2)	Liquid	Chrysal International, Naarden, The Netherlands	1 mL each solution
Clorox® Regular-Bleach1	Liquid	The Clorox Company, Oakland, CA, USA	0.4 · mL, pH to 7.0 with 1N HCl
Daconil®	Liquid	Southern Agricultural Insecticides, Inc. Palmetto, FL, USA	9.7 ml
Cinnamon oil	Liquid	Sigma-Aldrich, Saint Louis, MO, USA	2.0 ml
Phyton® 27	Liquid	Phyton Corporation, Minneapolis, MN, USA	25.4 ml
Elevate®	Water dispersible granule	Arysta LifeScience, Cary, NC, USA	1.2 g
FloraDip R	Liquid	Floralife, Walterboro, SC, USA	4.0 ml
Medallion® WDG	Water dispersible granule	Syngenta Crop Protection, Greensboro, NC, USA	0.2 g
ZeroTol® 2.0	Liquid	BioSafe Systems, LLC, East Hartford, CT, USA	2.5 ml
Chipco® 26019 Flo	Liquid	Bayer Environmental Sciences, Research Triangle Park, NC, USA	2.5 ml
MilStop®	Water soluble granule	BioWorks, Inc., Victor, NY, USA	3.0 g
Pageant® Intrinsic®	Wetable granule	BASF Corporation, Research Triangle Park, NC, USA	1.1 g
BAS703 06F Experimental	Wetable granule	BASF Corporation, Research Triangle Park, NC, USA	0.8 g
JMS Stylet-Oil®	Liquid	JMS Flower Farms, Inc., Vero Beach, FL, USA	15.8 ml
Thyme oil	Liquid	Sigma-Aldrich, Saint Louis, MO, USA	2.0 ml

solution in an attempt to avoid their phytotoxic effects while still achieving their fungistatic benefits.

Materials and Methods

Plants. For each experiment roses with 45 cm (17.7 in) stems were delivered overnight to our laboratory at NCSU from a distribution center in Miami, FL, after arriving there from a commercial grower in Colombia, South America. Cultivar Light Orlando was used in the treatment screening trial (Experiment 1) because it is a popular cultivar in the industry that is known to be susceptible to *B. cinerea*. Experiments 2 and 3 were conducted with the red cultivar 'Freedom' and four light-colored (Light Mix) cultivars: 'Cool Water' (lavender), 'Jessika' (pink), 'Polar Star' (white), and 'Tiffany' (yellow).

Production of rose isolate B. cinerea spores. We used a rose petal with gray mold symptoms to establish a *B. cinerea* culture *in vitro*, from which a sterile rose isolate was obtained by initiating new culture plates three times in succession using a 9 mm^2 (0.014 in^2) plug taken from the advancing edge of mycelium that appeared free of microbial contaminants. All vegetative *B. cinerea* cultures were maintained in 15 by 90 mm (0.59 by 3.54 in) polystyrene petri dishes containing 20 mL (0.68 fl oz) potato dextrose agar (PDA) medium (Thermo Fisher Scientific, Lenexa, KS) prepared per manufacturer instructions and sealed with parafilm within an incubator held at constant 20 C (68 F) with 16 h daylight provided by fluorescent bulbs. Four vegetative subcultures preceded the establishment of reproductive cultures. One plate was submitted to the NCSU Plant Disease and Insect Clinic, which used a polymerase chain reaction to identity our rose isolate as *B. cinerea*. Identity of the isolate was further confirmed by two-direction sequencing of the G3PDH gene in the laboratory of Gary Chastagner at Washington State University – Puyallup.

Sporulating cultures were established by placing a 9 mm^2 (0.014 in^2) plug of culture medium with vegetative mycelium onto the center of each of several petri dishes containing 20 mL (0.68 fl oz) oatmeal agar medium (Thermo Fisher Scientific, Lenexa, KS) prepared per manufacturer instructions. After 3 weeks, sporulating plates were used to establish additional sporulating plates by inverting each over a fresh, oatmeal agar plate and tapping it three times. Spores were then harvested from the 3-week-old, sporulating plates for future inoculations of plant material. Spore harvest was accomplished by flooding sporulating plates with approximately 15 mL (0.51 fl oz) of sterile solution of deionized water with 15% glycerol and 0.01% Tween 80 (Sigma-Aldrich, Saint Louis, MO), rubbing the fungus for several minutes with a glass rod, and straining the liquid through four layers of sterile cheesecloth. The suspension spore concentration was quantified for each plate by counts made under 40x magnification with a Neubauer hemacytometer (LW Scientific, Lawrenceville, GA). Spore suspensions, typically between $5 \times 10^6 \cdot mL^{-1}$ (0.03 fl oz) and $5 \times 10^7 \cdot mL^{-1}$ (0.03 fl oz) spores, were stored in 40 mL (1.35 fl oz) aliquots at -80 C (-112 F).

Cut rose inoculation with B. cinerea. *Botrytis cinerea* spore inoculum was prepared by thawing a frozen spore suspension and diluting it with tap water to yield a final spore concentration of $10^5 \cdot mL^{-1}$ (0.03 fl oz). Inoculation suspension spore concentration was verified by a hemacytometer count, and viability of spores was confirmed by placing 1.0 mL (0.03 fl oz) spore suspension onto PDA medium and counting spores with an emergent germination tube after 4 h at room temperature. Roses were inoculated by spraying all sides of leaves and flowers with constantly agitated inoculum with a hand-held, household spray bottle to the point of run-off. Total delivered inoculum volume was approximately 40 mL (1.35 fl oz) per dozen roses. Inoculation and imposition of control treatments was followed by holding flowers for 24 h at 20 C (68 F) wrapped in bunches of 12 in black plastic bags separated by treatment. Experiment treatments were imposed after the 24-h incubation and before storing them at 2 C (36 F) for 48 h.

Post cold storage handling. Once removed from the cooler, flower stems were recut before placing them in groups of three into vases containing 350 mL (11.8 fl oz) tap water. All leaves were removed except the three uppermost that had at least three leaflets each. Flowers were held at constant 20 C (68 F) under 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light for 12 $h \cdot d^{-1}$ at 40 – 60% relative humidity (RH) for observation through flower termination.

Assessment of Botrytis damage and phytotoxicity damage. Disease development on each individual was assessed 1 d after placing flowers into the postharvest environment and every day thereafter through the termination of each stem. Flowers were rated using a modified decay index of flower petals and receptacles described by Hazendonk et al. (1995) and Meir et al. (1998): 1, no symptoms; 2, 1% disease or 1-4 pinpoint lesions; 3, 2-5% disease or 5-19 pinpoint lesions; 4, 6-12% disease or >20 pinpoint lesions; 5, 13-25% disease; 6, 26-50% disease; 7, 51-75% disease; and 8, 76-100% disease or collapse of flower head at receptacle. Stems were rated as 0 or 1 for the presence of ≥1 Botrytis leaf lesions. At the end of each flower's vase life it was noted if the principal reason for termination was classic Botrytis damage or some other reason: bent neck, petal discoloration, petal drop, petal marginal necrosis, petal wilt, failure to open, phytotoxicity.

Flower and leaf phytotoxicity was assessed for each individual 1 and 3 d after placing stems into the postharvest environment using a three-point, subjective scale: 0, no apparent damage; 1, slight damage; 2, pronounced damage. For flowers, slight damage was margin damage penetrating up to approximately 2 mm (0.08 in) into petal tissue at any point and pronounced damage was margin damage penetrating ≥2 mm (0.08 in) into petal tissue at any point and/or browning of petal creases. For leaves, slight damage was minor yellowing and/or darkened margins, and pronounced damage was significant yellowing and/or darkened areas present on more than just leaf margins.

Experiment 1: Efficacy of 16 treatments. Upon arrival at the laboratory 'Light Orlando' roses were inoculated with *B. cinerea* spores, sprayed with spore-free inoculation solution, or left untreated. Four controls and 16 treatments (Table 1) were imposed following *B. cinerea* inoculation and incubation. Most treatments were applied by inverting and dipping rose stems into buckets containing 15 L (4.0 gal) treatment solution made with tap water to within 10 cm (3.9 in) of stem bases such that the flower and all foliage was submerged. Treatment imposition took 20 s: 5 s to slowly submerge, 5 s to slowly swirl through solution, 5 s to raise and drain upside down, 5 s for 5 downward pulsing shakes. Cinnamon leaf oil (CLO, *Cinnamomum zeylanicum*, Ceylon leaf type, Sigma-Aldrich, Saint Louis, MO) and thyme oil (TO, *Thymus vulgaris* and/or *Thymus zygis*, Sigma-Aldrich, Saint Louis, MO) were applied by spraying all sides of leaves and flowers with constantly agitated 0.2% EO in tap water with a hand-held, household spray bottle to the point of run-off.

The experiment included four controls: 1) "no fungicide control" consisting of roses sprayed the same as spore-inoculated flowers at the time of inoculation and then dipped in tap water at the time of treatment imposition, 2) "no spore control" consisting of roses sprayed with spore-free inoculation solution (diluted with tap water the same as the spore-containing inoculum) at the time of inoculation and then dipped in tap water at the time of treatment imposition, 3) "water control" consisting of roses sprayed with tap water at the time of inoculation and then dipped in tap water at the time of treatment imposition, and 4) "absolute control" consisting of roses that were neither sprayed at the time of inoculation nor dipped/sprayed at the time of treatment imposition.

After treatments and controls were applied, roses were wrapped in black plastic, separated by treatment, and stored at 2 C (36 F) for 48 h. After cold storage stems were cut to 40 cm (15.8 in).

The experiment was conducted as a randomized complete block design with one vase with three roses for each treatment within each of four blocks, meaning that 12 flowers were subjected to each of the 20 treatments. Blocks were used to remove spatial variation across the laboratory. The entire experiment was repeated so that a total of 24 flowers were subjected to each treatment. Statistical analyses were conducted with JMP Pro 12 (SAS, Cary, NC). A standard least squares method was used to perform analyses of variance on flower Botrytis damage ratings and flower and leaf phytotoxicity ratings. Treatment and experiment repetition were treated as fixed effects, and block was treated as a random effect. Binary logistic regression was used to determine the influence of treatments on variables rated as yes/no: presence of Botrytis damage on flowers, presence of Botrytis damage on leaves, and whether the stem was terminated for flower Botrytis damage. Tukey's HSD test was used to identify significant differences among means.

Experiment 2: Efficacy of EOs applied in aqueous solution. Upon arrival to the laboratory 'Freedom' and Light Mix (a mix of cultivars 'Cool Water', 'Jessika', 'Polar Star', and 'Tiffany') roses were inoculated with *B. cinerea* spores or sprayed with tap water as controls. Four controls and 12 treatments (Table 5) were imposed

Table 2. Influence of 16 treatments on Botrytis-infected 'Light Orlando' cut roses as measured by frequency of flowers with Botrytis after 5 d, flower Botrytis damage rating after 5 d, frequency of leaves with Botrytis after 7 d, and frequency of flowers terminated due to Botrytis damage. Infection was accomplished by spraying flowers and leaves to run-off with $10^5 \cdot ml^{-1}$ (0.03 fl oz) *Botrytis cinerea* spores and incubating them in black plastic bags for 24 h at 20 C (68 F). Following incubation, cinnamon leaf and thyme oil treatments were applied by spraying flowers and leaves to run-off with solution, and all other treatments were applied by dipping entire stems into 15 L (4.0 gal) solution for 20 s. Following treatment, roses were stored in black plastic bags for 48 h at 2 C (36 F) before cutting stems to 40 cm (15.8 in) and placing them into the postharvest environment [constant 20 C (68 F) with 20 µmol $\cdot m^{-2} \cdot s^{-1}$ light for 12 h $\cdot d^{-1}$ at 40 to 60% relative humidity] in vases containing tap water for daily observation through stem termination (13 d). n = 24 flowers per treatment and control.

Treatment	Day 5 flower Botrytis frequency (%)	Day 5 flower Botrytis damage rating[z]	Day 7 leaf Botrytis frequency (%)	Termination due to flower Botrytis damage (%)
Bacillus subtilis	70.8 abc[y]	5.29 abcd[y]	41.7 abcde[y]	83.3 ab[y]
Batine	41.7 def	4.42 bcde	54.2 abcde	75.0 ab
Bleach	72.0 abc	5.40 abc	40.0 abcde	80.0 ab
Chlorothalonil	40.0 def	4.32 bcde	44.0 abcde	88.0 a
Cinnamon leaf oil	36.0 def	4.36 bcde	44.0 abcde	48.0 bcde
Copper sulphate	76.0 ab	5.60 abc	76.0 a	96.0 a
Fenhexamide	76.0 ab	5.72 ab	60.0 abcd	88.0 a
FloraDip R	52.0 bcde	4.40 bcde	64.0 abc	64.0 abcd
Fludioxonil	28.0 efg	3.24 efg	16.7 cde	36.0 cde
Hydrogen peroxide	52.0 bcde	4.88 abcd	44.0 abcde	80.0 ab
Iprodione	40.0 def	4.00 cdef	32.0 abcde	76.0 ab
Potassium bicarbonate	68.0 abc	5.08 abcd	70.8 ab	92.0 a
Pyraclostrobin + Boscalid	48.0 cde	4.06 bcdef	52.0 abcde	72.0 abc
Pyraclostrobin + Fluxapyroxad	32.0 defg	4.12 bcdef	41.7 abcde	64.0 abcd
Stylet oil	92.0 a	6.20 a	68.0 ab	100.0 a
Thyme oil	28.0 efg	3.72 def	28.0 bcde	48.0 bcde
Control				
No fungicide	56.0 bcd	5.04 abcd	52.0 abcde	92.0 a
No spore	7.7 g	2.00 g	15.4 de	11.5 e
Water	8.3 g	1.83 g	8.3 e	16.7 e
Absolute	16.7 fg	2.54 fg	16.7 cde	25.0 de
Source	ANOVA *P*-values, $\alpha = 0.05$			
Treatment	<0.0001	<0.0001	<0.0001	<0.0001
Repetition	<0.0001	<0.0001	0.0264	0.0166
Treatment x Repetition	<0.0001	<0.0001	0.2632	0.1636
Block(Repetition)	0.0848	0.0993	0.1423	0.4580

[z]Eight-point scale: 1, no symptoms; 2, 1% disease or 1-4 pinpoint lesions; 3, 2-5% disease or 5-19 pinpoint lesions; 4, 6-12% disease or >20 pinpoint lesions; 5, 13-25% disease; 6, 26-50% disease; 7, 51-75% disease; and 8, 76-100% disease or collapse of flower head at receptacle.

[y]Values within a column and independent variable followed by the same letter are not significantly different at $P = 0.05$ using Tukey's HSD test.

following *B. cinerea* inoculation and incubation. Cinnamon leaf oil, clove bud oil (CBO, *Eugenia* spp., Sigma-Aldrich, Saint Louis, MO) and TO aqueous solutions were prepared by combining 0.1, 0.2 or 0.4 mL (0.0034, 0.0068, or 0.0135 fl oz) EO with 0.8 mL (0.0271 fl oz) 95% ethanol and concussing the mixed solution several times before slowly adding 100 mL (3.38 fl oz) tap water with constant agitation to yield treatment concentrations of 0.1, 0.2, and 0.4% v/v for each oil. Fludioxonil solutions were made by dissolving the commercial fungicide Medallion® WDG (Syngenta Crop Protection, Greensboro, NC) into water to achieve the desired concentrations of active ingredient: 0.125, 0.250, and 0.500 g $\cdot L^{-1}$ (0.00013, 0.00026, and 0.00052 oz $\cdot$ fl oz^{-1}). Oil treatments were applied as a spray and fludioxonil treatments were applied as a dip as described for Experiment 1.

The experiment included four controls: 1) "infected + water control" consisting of roses inoculated and later dipped in tap water as previously described, and 2) "non-infected + water control" consisting of roses sprayed with spore-free inoculation solution and dipped in tap water at the time of EO and fludioxonil treatments as previously described, 3) "infected + ethanol control" consisting of roses inoculated as previously described and then sprayed with 0.8% ethanol in tap water at the time of EO and fludioxonil treatments, and 4) "non-infected + ethanol control" consisting of roses sprayed with spore-free inoculation solution as previously described and then sprayed with 0.8% ethanol in tap water at the time of EO and fludioxonil treatments.

After treatments and controls were applied, roses were wrapped in black plastic, separated by treatment, and stored at 2 C (36 F) for 48 h. After cold storage stems were cut to 40 cm (15.8 in) before placing them into vases. Each vase held either three 'Freedom' roses or three Light Mix roses. Three different cultivars were assigned randomly to each Light Mix vase.

The experiment was conducted as a randomized complete block design with one vase with three roses for each treatment by cultivar ('Freedom' versus Light Mix) combination within each of four blocks, meaning that 12 'Freedom' and 12 Light Mix flowers were subjected to each of the 16 treatments. Blocks were used to remove spatial variation across the laboratory. The entire experiment was repeated so that a total of 48 flowers, 24 of which were 'Freedom' and 24 of which were Light Mix, were subjected to each treatment. Statistical analyses were conducted with JMP Pro 12 (SAS, Cary, NC). A standard

Table 3. Influence of 16 treatments on Botrytis-infected 'Light Orlando' cut roses as measured by flower and leaf phytotoxicity ratings after 3 d. Infection was accomplished by spraying rose flowers and leaves to run-off with $10^5 \cdot ml^{-1}$ (0.03 fl oz) *Botrytis cinerea* spores and incubating them in black plastic bags for 24 h at 20 C (68 F). Following incubation, cinnamon leaf and thyme oil treatments were applied by spraying flowers and leaves to run-off with solution, and all other treatments were applied by dipping entire stems into 15 L (4.0 gal) solution for 20 s. Following treatment, roses were stored in black plastic bags for 48 h at 2 C (36 F) before cutting stems to 40 cm (15.8 in) and placing them into the postharvest environment [constant 20 C (68 F) with 20 µmol $\cdot m^{-2} \cdot s^{-1}$ light for 12 h $\cdot d^{-1}$ at 40 to 60% relative humidity] in vases containing tap water for daily observation through stem termination (13 d). n = 24 stems per treatment and control.

	Day 3 phytotoxicity rating	
Treatment	**Flower[z]**	**Leaf[y]**
Bacillus subtilis	1.17 ab[x]	0.38 ab[x]
Batine	0.42 cde	0.29 ab
Bleach	0.49 cde	0.13 ab
Chlorothalonil	0.69 bc	0.12 ab
Cinnamon leaf oil	0.74 abc	0.19 ab
Copper sulphate	1.22 a	0.41 ab
Fenhexamide	0.76 abc	0.37 ab
FloraDip R	0.76 abc	0.25 ab
Fludioxonil	0.21 de	0.00 b
Hydrogen peroxide	0.70 bc	0.33 ab
Iprodione	0.53 cd	0.08 b
Potassium bicarbonate	1.24 a	0.51 a
Pyraclostrobin + Boscalid	0.63 cd	0.29 ab
Pyraclostrobin + Fluxapyroxad	0.91 abc	0.34 ab
Stylet oil	1.18 ab	0.50 a
Thyme oil	0.78 abc	0.21 ab
Control		
No fungicide	0.00 e	0.00 b
No spore	0.00 e	0.08 b
Water	0.00 e	0.00 b
Absolute	0.00 e	0.00 b
Source	ANOVA *P*-values, $\alpha = 0.05$	
Treatment	<0.0001	<0.0001
Repetition	<0.0001	<0.0001
Treatment x Repetition	<0.0001	<0.0001
Block(Repetition)	0.3088	0.8545

[z]Three-point, subjective scale: 0, no apparent damage; 1, margin damage penetrating up to approximately 2 mm (0.08 in) into petal tissue at any point; 2, margin damage penetrating ≥2 mm (0.08 in) into petal tissue at any point and/or browning of petal creases.

[y]Three-point subjective scale: 0, no apparent damage; 1, minor yellowing and/or darkened margins; 2, significant yellowing and/or darkened areas present on more than just leaf margins.

[x]Values within a column and independent variable followed by the same letter are not significantly different at $P = 0.05$ using Tukey's HSD test.

least squares method was used to perform analyses of variance on flower Botrytis damage ratings and flower and leaf phytotoxicity damage ratings. Treatment, cultivar, and experiment repetition were treated as fixed effects, and block was treated as a random effect. Binary logistic regression was used to determine the influence of treatments on variables rated as yes/no: presence of Botrytis damage on flowers, presence of Botrytis damage on leaves, and whether the stem was terminated for flower Botrytis damage. Tukey's HSD test was used to identify significant differences among means.

Experiment 3 - Efficacy of EOs applied as a vapor. This experiment was similar to Experiment 2 with the exceptions of applying EO treatments as a vapor and the altered methods required to accommodate that change. After *B. cinerea* inoculation and incubation of 'Freedom' and Light Mix roses, fludioxonil treatments were applied as a dip as previously described. Twelve flowers for each treatment by cultivar combination and each control had stems cut to 30 cm (11.8 in) and put into tap water in a vase which was placed into a sealable, plastic, 17-L (0.60 ft^3) bucket. Roses in the EO treatments were exposed to EO vapor by placing 0.1, 0.2, or 0.4 mL (0.0034, 0.0068, or 0.0135 fl oz) of an EO onto a filter paper disc and placing it into the bucket before sealing it. Complete volatilization of these volumes of EO in the bucket volume would provide atmospheres of 4.6, 9.1, and 18.2 ppm, respectively.

The experiment included four controls: 1) "infected + water control" consisting of roses inoculated and later dipped in tap water as previously described, and 2) "non-infected + water control" consisting of roses sprayed with spore-free inoculation solution and dipped in tap water at the time of EO and fludioxonil treatments as previously described, 3) "infected + 0 control" consisting of roses inoculated as previously described and then left untreated at the time of EO and fludioxonil treatments, and 4) "non-infected + 0 control" consisting of roses sprayed with spore-free inoculation solution as previously described and then left untreated at the time of EO and fludioxonil treatments.

Buckets containing flowers for all treatments (Table 7) were stored in the dark at 2 C (36 F) for 48 h before flowers were placed into vases as in Experiment 2. Numbers of flowers, experiment design, and statistical analyses were the same as for Experiment 2.

Results and Discussion

Experiment 1: Efficacy of 16 treatments. The high rates of Botrytis infection we observed in inoculated and incubated 'Light Orlando' cut roses was expected because $10^5 \cdot mL^{-1}$ (0.03 fl oz) is a high concentration of *B. cinerea* spores compared to typical naturally occurring pathogen density, and we provided optimum conditions for spores to germinate and infect plant tissue (Sosa-Alvarez et al. 1995, Zhang and Sutton 1994) prior to imposing treatments. The observation of Botrytis damage in non-inoculated control flowers was not surprising given that 'Light Orlando' is a rose cultivar known to be susceptible to Botrytis and that approximately 10% of flowers evidenced Botrytis infection upon receipt (not used in the experiment).

Treatment influenced ($P<0.001$) incidence of flowers and leaves having Botrytis and flower Botrytis damage on every day observations were made. The greatest separation among treatments in incidence and severity of Botrytis on flowers and leaves was found on day five and seven, respectively (Table 2). All stem terminations due to flower Botrytis damage (typically a score ≥6 on the scale described and/or petal drop due to Botrytis damage at

Table 4. Influence of treatment with aqueous solution of thyme oil, cinnamon leaf oil, clove bud oil, or fludioxonil at three concentrations on Botrytis-infected 'Freedom' and Light Mix cut roses as measured by flower Botrytis damage rating after 1 and 6 d, flower phytotoxicity rating after 3 d, leaf Botrytis frequency after 5 d, and termination due to flower Botrytis damage. Infection was accomplished by spraying flowers and leaves to run-off with $10^5 \cdot mL^{-1}$ (0.03 fl oz) *Botrytis cinerea* spores and incubating them in black plastic bags for 24 h at 20 C (68 F). Following incubation, oil treatments were applied by spraying flowers and leaves to run-off with solution, and fludioxonil treatments were applied by dipping entire stems into 15 L (4.0 gal) solution or water for 20 s. Following treatment, roses were stored in black plastic bags for 48 h at 2 C (36 F) before cutting stems to 40 cm (15.8 in) and placing them into the postharvest environment [constant 20 C (68 F) with 20 µmol $\cdot m^{-2} \cdot s^{-1}$ light for 12 h $\cdot d^{-1}$ at 40 to 60% relative humidity] in vases containing tap water for daily observation through stem termination (14 d). n = 144 roses per treatment, 192 per concentration, and 288 per cultivar ('Freedom' and Light Mix).

Independent variable	Day 1 flower Botrytis damage rating[z]	Day 3 flower phytotoxicity rating[y]	Day 5 leaf Botrytis frequency (%)	Day 6 flower Botrytis damage rating[z]	Termination due to flower Botrytis damage (%)
Treatment					
Cinnamon leaf oil	2.31 ab[x]	0.80 a[x]	27.5 a[x]	5.43 a[x]	36.5 b[x]
Clove bud oil	2.63 a	0.63 ab	26.8 a	5.35 a	52.8 a
Thyme oil	1.87 c	0.38 c	6.6 b	3.26 b	26.3 bc
Fludioxonil	2.14 bc	0.50 bc	12.3 b	3.17 b	16.7 c
Concentration[w]					
Low	2.21 a	0.51 a	19.3 a	4.20 a	40.0 a
Medium	2.35 a	0.64 a	15.3 a	4.18 a	30.9 a
High	2.13 a	0.57 a	19.5 a	4.28 a	27.8 a
Cultivar					
Freedom	1.58 b	0.81 a	1.4 b	3.61 b	19.7 b
Light mix[v]	2.88 a	0.32 b	43.5 a	5.33 a	46.3 a

[z]Eight-point scale: 1, no symptoms; 2, 1% disease or 1-4 pinpoint lesions; 3, 2-5% disease or 5-19 pinpoint lesions; 4, 6-12% disease or >20 pinpoint lesions; 5, 13-25% disease; 6, 26-50% disease; 7, 51-75% disease; and 8, 76-100% disease or collapse of flower head at receptacle.

[y]Three-point subjective scale: 0, no apparent damage; 1, margin damage penetrating up to approximately 2 mm (0.08 in) into petal tissue at any point; 2, margin damage penetrating ≥2 mm (0.08 in) into petal tissue at any point and/or browning of petal creases.

[x]Values within a column and independent variable followed by the same letter are not significantly different at $P = 0.05$ using Tukey's HSD test.

[w]Low = 0.10% or 0.125 g $\cdot L^{-1}$ (0.00013 oz $\cdot$ fl oz^{-1}), medium = 0.20% or 0.250 g $\cdot L^{-1}$ (0.00026 oz $\cdot$ fl oz^{-1}), high = 0.40% or 0.500 g $\cdot L^{-1}$ (0.00052 oz $\cdot$ fl oz^{-1}) for oils and fludioxonil, respectively.

[v]Mix of four light colored cultivars: 'Cool Water' (lavender), 'Jessika' (pink), 'Polar Star' (white), 'Tiffany' (yellow).

Table 5. Influence of treatment with aqueous solution of thyme oil, cinnamon leaf oil, clove bud oil, or fludioxonil at three concentrations on Botrytis-infected roses across cultivars 'Cool Water', 'Freedom', 'Jessika', 'Polar Star', and 'Tiffany' as measured by flower phytotoxicity rating after 3 d, vase life, Botrytis damage rating ≥6 at termination, and termination due to Botrytis damage. Infection was accomplished by spraying flowers and leaves to run-off with $10^5 \cdot mL^{-1}$ (0.03 fl oz) *Botrytis cinerea* spores and incubating them in black plastic bags for 24 h at 20 C (68 F). Following incubation, oil treatments were applied by spraying flowers and leaves to run-off with solution, and fludioxonil treatments were applied by dipping entire stems into 15 L (4.0 gal) solution or water for 20 s. Following treatment, roses were stored in black plastic bags for 48 h at 2 C (36 F) before cutting stems to 40 cm (15.8 in) and placing them into the postharvest environment [constant 20 C (68 F) with 20 µmol $\cdot m^{-2} \cdot s^{-1}$ light for 12 h $\cdot d^{-1}$ at 40 to 60% relative humidity] in vases containing tap water for daily observation through stem termination (14 d). n = 48 roses per treatment by concentration combination and control.

Treatment	Concentration	Day 3 flower phytotoxicity rating[z]	Vase life(d)	Flower Botrytis damage rating ≥6[y] at termination (%)	Termination due to flower Botrytis damage (%)
Cinnamon leaf oil	0.10 %	0.57 bcde[x]	5.8 cdef[x]	60.0 abc[x]	41.7 abc[x]
Cinnamon leaf oil	0.20 %	1.00 a	4.7 f	60.0 abc	36.0 bc
Cinnamon leaf oil	0.40 %	0.84 ab	5.8 cdef	54.2 abcd	32.0 bcd
Clove bud oil	0.10 %	0.46 cdef	5.3 ef	79.2 a	66.7 a
Clove bud oil	0.20 %	0.76 abc	5.4 def	78.3 ab	45.8 abc
Clove bud oil	0.40 %	0.68 abcd	6.4 bcde	75.0 ab	45.8 abc
Thyme oil	0.10 %	0.23 fg	6.9 abcd	46.4 cde	28.6 bcde
Thyme oil	0.20 %	0.38 def	7.4 abc	33.3 def	20.8 cde
Thyme oil	0.40 %	0.52 cdef	7.0 abc	37.5 cde	29.2 bcde
Fludioxonil[w]	0.125 g $\cdot L^{-1}$	0.43 cdef	7.0 abcd	29.2 defg	25.0 bcde
Fludioxonil	0.250 g $\cdot L^{-1}$	0.44 cdef	7.3 abc	25.0 efg	20.8 cde
Fludioxonil	0.500 g $\cdot L^{-1}$	0.65 abcd	7.5 ab	8.3 fg	4.2 e
Control					
Infected + water		0.13 fg	5.0 ef	73.1 ab	65.4 a
Non-infected + water		0.00 g	8.1 a	8.3 fg	8.3 de
Infected + ethanol		0.32 efg	5.9 cdef	53.9 bcd	46.2 ab
Non-infected + ethanol		0.14 fg	7.3 abc	8.0 g	8.0 de

[z]Three-point subjective scale: 0, no apparent damage; 1, margin damage penetrating <2 mm (0.08 in) into petal tissue at any point; 2, margin damage penetrating ≥2 mm (0.08 in) into petal tissue at any point and/or browning of petal creases.

[y]≥26% flower tissue diseased and/or collapse of flower head at receptacle.

[x]Values within a column followed by the same letter are not significantly different at $P = 0.05$ using Tukey's HSD test.

[w]Concentration equivalents: 0.00013, 0.00026, and 0.00052 oz $\cdot$ fl oz^{-1}.

Table 6. Influence of treatment with vapor of thyme oil, cinnamon leaf oil, or clove bud oil or aqueous fludioxonil at three concentrations on Botrytis-infected 'Freedom' and Light Mix cut roses as measured by flower Botrytis damage rating after 1 and 6 d, flower phytotoxicity rating after 3 d, leaf Botrytis frequency after 5 d, and termination due to Botrytis damage. Infection was accomplished by spraying flowers and leaves to run-off with $10^5 \cdot mL^{-1}$ (0.03 fl oz) *Botrytis cinerea* spores and incubating them in black plastic bags for 24 h at 20 °C. Following incubation, stems were cut to 30 cm (11.8 in) and placed in vases containing tap water, and oil vapor treatments were applied by placing EO onto filter paper placed with flowers within a sealed bucket, and fludioxonil treatments were applied by dipping entire stems into 15 L (4.0 gal) solution or water for 20 s. Vases of treated flowers were then stored in 17-L (0.60 ft^3), sealed, plastic buckets in the dark for 48 h at 2 C (36 F) before placing flowers into the postharvest environment [constant 20 C (68 F) with 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light for 12 h $\cdot$ d^{-1} at 40 to 60% relative humidity] in vases containing tap water for daily observation through stem termination (14 d). n = 144 roses per treatment, 192 per concentration, and 288 per cultivar ('Freedom' and Light Mix).

Independent variable	Day 1 flower Botrytis damage rating[z]	Day 3 flower phytotoxicity rating[y]	Day 5 leaf Botrytis frequency (%)	Day 6 flower Botrytis damage rating[z]	Termination due to flower Botrytis damage (%)
Treatment					
Cinnamon leaf oil	1.77 ab[x]	0.32 a[x]	26.0 a[x]	4.13 a[x]	27.9 ab[x]
Clove bud oil	2.28 a	0.26 a	16.8 a	4.65 a	46.1 a
Thyme oil	1.28 c	0.20 a	16.8 a	2.25 b	18.2 b
Fludioxonil	2.01 bc	0.35 a	13.2 a	2.92 b	15.3 b
Concentration[w]					
Low	2.47 a	0.21 b	28.0 a	4.06 a	37.0 a
Medium	2.27 a	0.20 b	18.2 ab	3.56 ab	25.9 ab
High	0.79 b	0.44 a	8.4 b	2.78 b	17.8 b
Cultivar					
Freedom	1.33 b	0.38 a	4.1 b	2.79 b	15.5 b
Light mix[v]	2.39 a	0.18 b	32.3 a	4.18 a	38.4 a

[z]Eight-point scale: 1, no symptoms; 2, 1% disease or 1-4 pinpoint lesions; 3, 2-5% disease or 5-19 pinpoint lesions; 4, 6-12% disease or >20 pinpoint lesions; 5, 13-25% disease; 6, 26-50% disease; 7, 51-75% disease; and 8, 76-100% disease or collapse of flower head at receptacle.

[y]Three-point subjective scale: 0, no apparent damage; 1, margin damage penetrating <2 mm (0.08 in) into petal tissue at any point; 2, margin damage penetrating ≥2 mm (0.08 in) into petal tissue at any point and/or browning of petal creases.

[x]Values within a column and independent variable followed by the same letter are not significantly different at $P = 0.05$ using Tukey's HSD test.

[w]Low = 4.6 ppm or 0.125 g $\cdot$ L^{-1} (0.00013 oz $\cdot$ fl oz^{-1}), medium = 9.1 ppm or 0.250 g $\cdot$ L^{-1} (0.00026 oz $\cdot$ fl oz^{-1}), high = 18.2 ppm or 0.500 g $\cdot$ L^{-1} (0.00052 oz $\cdot$ fl oz^{-1}) for oils and fludioxonil, respectively.

[v]Mix of four light colored cultivars: 'Cool Water' (lavender), 'Jessika' (pink), 'Polar Star' (white), 'Tiffany' (yellow).

petal bases) occurred by 13 d, and influence of treatment was highly significant for frequency of stem termination due to flower Botrytis damage ($P<0.001$, Table 2). Flower Botrytis damage was the cause for stem termination for >50% of individuals in the no fungicide control and 13 of the 16 treatments and for 12 to 25% of stems even in the non-inoculated stems – the no spore, water, and absolute controls (Table 2). Reduction in frequency of stem termination due to flower Botrytis damage was 25% or more for five of the 16 treatments (fludioxonil, CLO, TO, FloraDip R, and pyraclostrobin + fluxapyroxad) compared to no fungicide control flowers that were inoculated with *B. cinerea* spores and then dipped in tap water after the 24-hr incubation period (Fig. 1D).

Differences among synthetic fungicides in ability to reduce Botrytis damage in roses infected with *B. cinerea* has been reported previously (Elad 1988a). Chlorothalonil and iprodione were common between this and that previous work, and our results were similar in showing that these compounds were minimally and moderately effective, respectively, compared to the other tested synthetic fungicides for reducing Botrytis damage. Macnish et al. (2010) showed that chlorine bleach can greatly reduce Botrytis incidence in non-inoculated roses when they are dipped just before putting them into vases or prior to shipment. However, we found that bleach treatment did not reduce Botrytis damage in inoculated and incubated 'Light Orlando', perhaps because the previous study demonstrated protection against infection primarily whereas our work was with post-infection flowers. We did find that the bleach treatment resulted in minimal phytotoxicity damage as found previously (Macnish et al. 2010). We found that flowers treated with fludioxonil had a significantly lower frequency of termination due to Botrytis than those treated with bleach, opposite to a previous report (Macnish et al. 2010), indicating that fludioxonil was more effective than bleach in reducing disease symptoms in flowers that had been infected through inoculation and incubation.

The 15% glycerol and 0.01% Tween 80 in the inoculation solution did not contribute to the frequency of Botrytis in flowers or severity of Botrytis damage in flowers or leaves based on evaluations of control plants (Table 2, no spore control versus water control). Likewise, simply wetting flowers and leaves with water did not contribute to the frequency of Botrytis in flowers or severity of Botrytis damage in flowers or leaves (Table 2, water control versus absolute control).

Phytotoxicity manifested as petal margin necrosis (Fig. 1B) was common, and flower and leaf phytotoxicity ratings differed among treatments after 3 d in the vases (Table 3). It was clear that treatments were responsible for the damage, because non-treated control roses did not exhibit the same damage whether Botrytis-infected or not (Table 3). Thus, neither the 15% glycerol and 0.01% Tween 80 in the inoculation solution (Table 3, no spore control versus water control) nor simply wetting flowers and leaves with water (Table 3, water control versus absolute control) caused phytotoxicity in flowers or leaves.

Table 7. Influence of treatment with vapor of thyme oil, cinnamon leaf oil, clove bud oil, or aqueous fludioxonil at three concentrations on Botrytis-infected roses across cultivars 'Cool Water', 'Freedom', 'Jessika', 'Polar Star', and 'Tiffany' as measured by flower phytotoxicity rating after 3 d, vase life, Botrytis damage rating ≥6 at termination, and termination due to Botrytis damage. Infection was accomplished by spraying flowers and leaves to run-off with $10^5 \cdot mL^{-1}$ (0.03 fl oz) *Botrytis cinerea* spores and incubating them in black plastic bags for 24 h at 20 C (68 F). Following incubation, stems were cut to 30 cm (11.8 in) and placed in vases containing tap water, and oil vapor treatments were applied by placing EO onto filter paper placed with flowers within a sealed bucket, and fludioxonil treatments were applied by dipping entire stems into 15 L (4.0 gal) solution or water for 20 s. Vases of treated flowers were then stored in 17-L (0.60 ft^3), sealed, plastic buckets in the dark for 48 h at 2 C (36 F) before placing flowers into the postharvest environment [constant 20 C (68 F) with 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light for 12 $h \cdot d^{-1}$ at 40 to 60% relative humidity] in vases containing tap water for daily observation through stem termination (14 d). n = 48 roses per treatment by concentration combination and control.

Treatment	Concentration	Day 3 flower phytotoxicity rating [z]	Vase life(d)	Flower Botrytis damage rating ≥6[y] at termination (%)	Termination due to Botrytis damage (%)
Cinnamon leaf oil	4.6 ppm	0.22 cde[x]	6.3 def[x]	55.4 cd[x]	32.0 cde[x]
Cinnamon leaf oil	9.1 ppm	0.25 def	5.3 fg	58.1 bcd	32.0 cde
Cinnamon leaf oil	18.2 ppm	0.50 a	7.1 bcd	46.2 de	20.0 defg
Clove bud oil	4.6 ppm	0.22 cde	5.6 fg	76.1 a	62.7 a
Clove bud oil	9.1 ppm	0.13 def	5.9 ef	75.0 ab	41.8 bc
Clove bud oil	18.2 ppm	0.44 ab	7.8 b	67.2 abc	33.8 cd
Thyme oil	4.6 ppm	0.10 def	7.4 bc	44.5 de	24.6 def
Thyme oil	9.1 ppm	0.10 def	7.9 b	30.6 ef	16.8 efg
Thyme oil	18.2 ppm	0.40 abc	9.0 a	33.3 ef	13.2 fgh
Fludioxonil[w]	0.125 $g \cdot L^{-1}$	0.20 cde	8.0 ab	22.7 fgh	29.0 cdef
Fludioxonil	0.250 $g \cdot L^{-1}$	0.23 cde	6.7 cde	27.4 efg	12.8 fgh
Fludioxonil	0.500 $g \cdot L^{-1}$	0.63 a	9.0 a	3.8 h	4.0 gh
Control					
Infected + water		0.04 ef	5.8 efg	64.0 abcd	53.4 ab
Infected + no treat		0.04 ef	4.8 g	62.3 abcd	54.2 ab
Non-infected + water		0.04 ef	8.1 ab	3.8 h	8.3 gh
Non-infected + no treat		0.00 f	7.8 b	3.8 h	0.0 h

[z]Three-point subjective scale: 0, no apparent damage; 1, margin damage penetrating <2 mm (0.08 in) into petal tissue at any point; 2, margin damage penetrating ≥2 mm (0.08 in) into petal tissue at any point and/or browning of petal creases.

[y]≥26% flower tissue diseased and/or collapse of flower head at receptacle.

[x]Values within a column followed by the same letter are not significantly different at $P = 0.05$ using Tukey's HSD test.

[w]Concentration equivalents: 0.00013, 0.00026, and 0.00052 $oz \cdot fl\ oz^{-1}$.

There was no consistent difference between the seven synthetic fungicides and the other nine treatments when looking at the desirable combined responses of greatly reduced frequency of flower terminations due to Botrytis damage and low flower phytotoxicity damage (Fig. 1D). Only treatment with fludioxonil yielded these two responses.

Botrytis frequencies and phytotoxicity ratings observed in leaves paralleled what was observed in flowers. Leaf phytotoxicity was almost never greater than the slight damage of minor yellowing and/or darkened margins in any treatment. In no case did leaf observations for roses treated with a given compound contradict the flower observations for that compound. The fact that leaf phytotoxicity was almost always much lower than that for flowers of the same treatment suggests that future experiments can be streamlined by collecting data from flowers only.

While the effect of block within experiment repetition was not significant for any variable, repetitions of the experiment differed from each other in several instances (Tables 2 and 3). Across all treatments the second repetition of the experiment had higher infection rates, more rapidly developing Botrytis damage, and ultimately more severe Botrytis damage than the first. For example, frequency of flowers with Botrytis after 5 d in the first repetition was 32% while it was 63% in the second, and flower Botrytis damage rating after 5 d was 3.78 for the first repetition and 4.92 for the second, highly significantly different in both cases ($P<0.001$). Differences in Botrytis infection frequency and ultimate disease severity between the two repetitions point to the need for future experiments to be repeated in time. One explanation for differences between repetitions is the RH in the trial laboratory. Though maintained between 40% and 60%, the first repetition was conducted while RH was closer to 40% for the duration of the experiment while for the second RH was closer to 60%. The flowers in each repetition may have also been differentially amenable to post-infection Botrytis damage because of their dissimilar production histories and/or shipping and handling conditions.

Inclusion of repeating an experiment in time in future research will be particularly important given the observation that interaction between repetition and treatment was significant for some variables: flower and leaf phytotoxicity ratings after 3 d (Table 3) and flower Botrytis frequency and damage rating after 5 d (Table 2).

Experiment 2: Efficacy of EOs applied in aqueous solution. Treatment and cultivar ('Freedom' versus Light Mix) significantly influenced most dependent variables on most days, but treatment concentration did not. Five of these variables are shown in Table 4. That inoculation with *B. cinerea* had a less negative effect on 'Freedom' compared to Light Mix flowers was not surprising as the former is known to be relatively Botrytis resistant

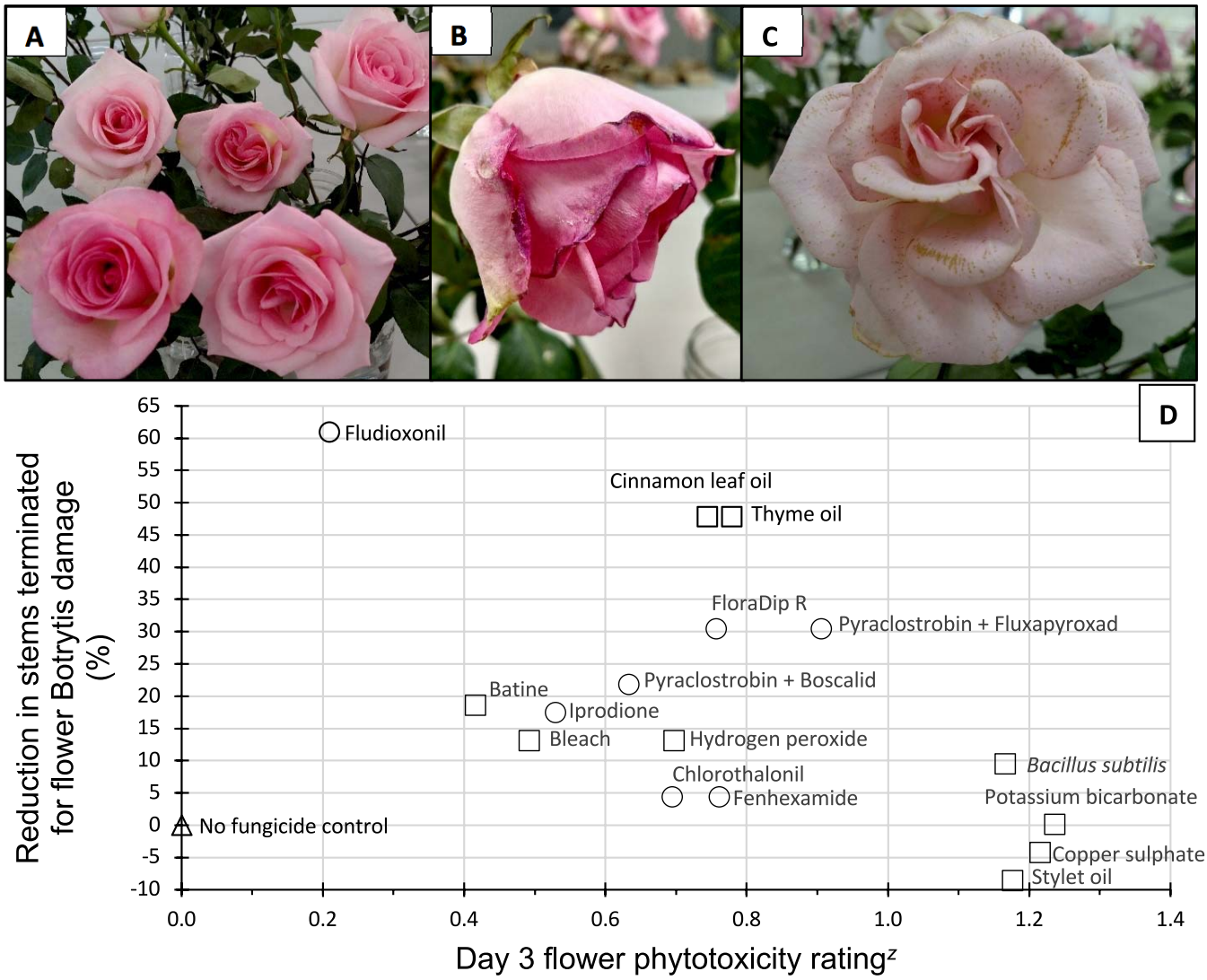

Fig. 1. **Influence of treatment with synthetic fungicides (○), non-synthetic compounds (□), or water as a no-fungicide control (△) on Botrytis-infected 'Light Orlando' cut roses, as measured by reduction in stems terminated for flower Botrytis damage and flower phytotoxicity rating after 3 d, subsequent to Botrytis infection, treatment, and storage. Infection was accomplished by spraying flowers and leaves to run-off with $10^5 \cdot ml^{-1}$ (0.03 fl oz) *Botrytis cinerea* spores and incubating them in black plastic bags for 24 h at 20 C (36 F). Following incubation, cinnamon leaf and thyme oil treatments were applied by spraying flowers and leaves to run-off with solution, and all other treatments were applied by dipping entire stems into 15 L (4.0 gal) solution or water for 20 s. Following treatment, roses were stored in black plastic bags for 48 h at 2 C (36 F) before cutting stems to 40 cm (15.8 in) and placing them into the postharvest environment [constant 20 C (68 F) with 20 µmol $\cdot m^{-2} \cdot s^{-1}$ light for 12 h $\cdot d^{-1}$ at 40 to 60% relative humidity] in vases containing tap water for daily observation through stem termination (13 d). A) No fungicide control flowers after 3 d, B) Phytotoxicity manifest as petal margin damage after 3 d, C) High number of Botrytis infection points after inoculation and 2 d storage, D) Phytotoxicity rating after 3 d in vase and reduction in frequency of flower terminations due to flower botrytis damage compared to no fungicide control flowers. Significant differences among treatments for phytotoxicity rating after 3 d and frequency of flowers terminated due to botrytis are shown in Tables 2 and 3, respectively. n = 24 stems per treatment and control.**
[z]Three-point, subjective scale: 0, no apparent damage; 1, margin damage penetrating up to approximately 2 mm (0.08 in) into petal tissue at any point; 2, margin damage penetrating ≥2 mm (0.08 in) into petal tissue at any point and/or browning of petal creases.

compared to the later. 'Freedom' had greater sensitivity to oil and fludioxonil applications compared to Light Mix flowers as observed in flower phytotoxicity rating after 1 d (0.53 versus 0.22, respectively, P=0.0138) and 3 d (Table 4). The trend for roses treated with medium and high oil concentrations to exhibit greater flower phytotoxicity than roses treated with the low concentration of the same oil was not accompanied by a trend for the higher concentrations to have less flower Botrytis damage (Fig. 2). Leaf phytotoxicity damage after 1 and 3 d was negligible and never differed among treatments (data not shown).

In general, roses treated with CLO or CBO exhibited more Botrytis damage and more phytotoxicity damage than those treated with TO or fludioxonil (Table 4). In almost no case was a significant difference among concentrations within an EO or fludioxonil treatment observed (Table 5). Stems treated with 0.10% TO were most similar to infected + water control stems in terms of flower phytotoxicity damage rating after 3 d, and flowers treated with fludioxonil at the highest concentration [0.50 g $\cdot L^{-1}$ (0.00052 oz $\cdot$ fl oz^{-1})] were most similar to non-infected + water and non-infected + ethanol control flowers in terms

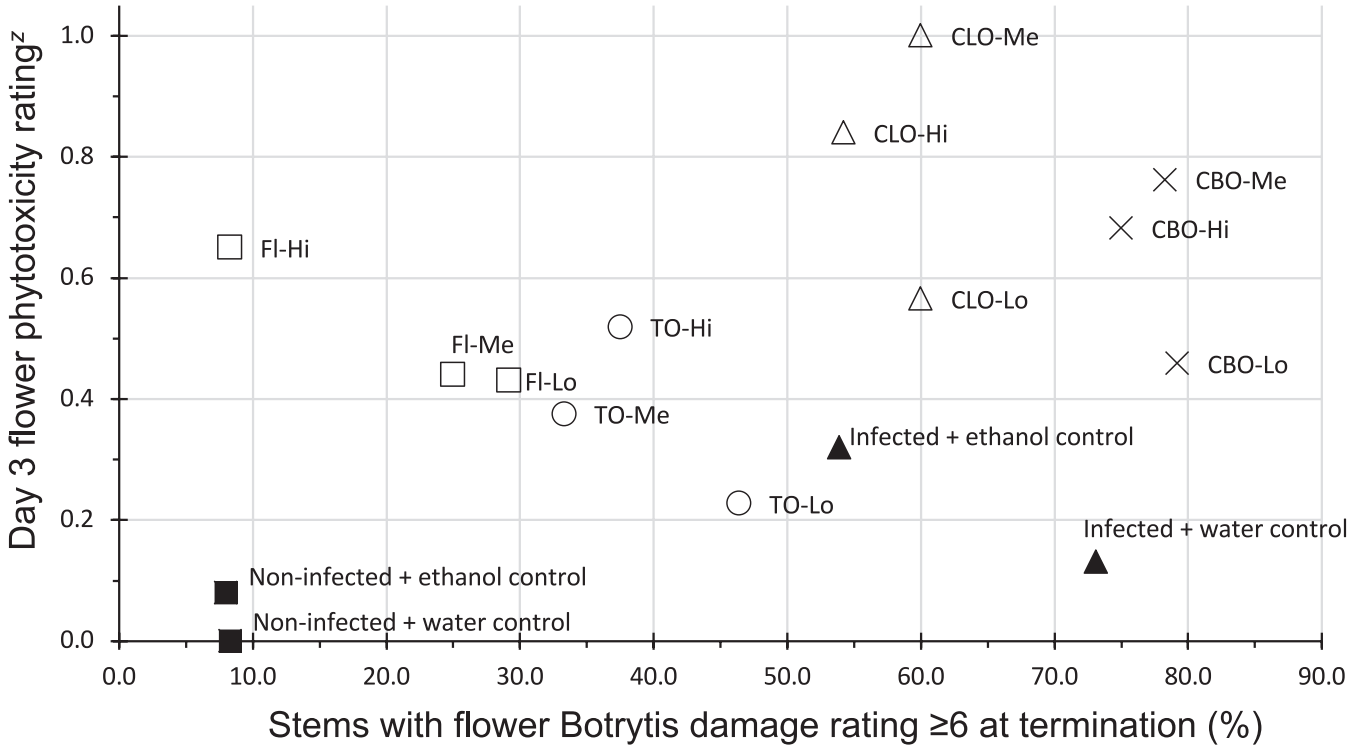

Fig. 2. **Influence of treatment with aqueous solution of thyme oil (○ TO), cinnamon leaf oil (△ CLO), clove bud oil (× CBO), or fludioxonil (□ Fl) at low (Lo), medium (Me), or high (Hi) concentration on Botrytis-infected cut roses across cultivars ('Cool Water', 'Freedom', 'Jessika', 'Polar Star', 'Tiffany'), as measured by flower phytotoxicity damage rating after 3 d and frequency of stems with flower Botrytis damage rating ≥6 (i.e. ≥26% flower tissue diseased and/or collapse of flower head at receptacle) at termination, subsequent to Botrytis infection, treatment, and storage. Infection was accomplished by spraying flowers and leaves to run-off with $10^5 \cdot ml^{-1}$ (0.03 fl oz) *Botrytis cinerea* spores and incubating them in black plastic bags for 24 h at 20 C (36 F). Following incubation, oil treatments were applied by spraying flowers and leaves to run-off with solution, and fludioxonil treatments were applied by dipping entire stems into 15 L (4.0 gal) solution or water for 20 s. Concentrations for oils or fludioxonil, respectively: Lo = 0.10% or 0.125 g · L^{-1} (0.00013 oz · fl oz^{-1}); Me = 0.20% or 0.250 g · L^{-1} (0.00026 oz · fl oz^{-1}); Hi = 0.40% or 0.500 g · L^{-1} (0.00052 oz · fl oz^{-1}). Following treatment, roses were stored in black plastic bags for 48 h at 2 C (36 F) before cutting stems to 40 cm (15.8 in) and placing them into the postharvest environment [constant 20 C (68 F) with 20 µmol · m^{-2} · s^{-1} light for 12 h · d^{-1} at 40 to 60% relative humidity] in vases containing tap water for daily observation through stem termination (14 d). Significant differences among treatments are indicated for the two dependent variables in Table 5. n = 24 stems per treatment by concentration combination and control. [z]Three-point, subjective scale: 0, no apparent damage; 1, margin damage penetrating up to approximately 2 mm (0.08 in) into petal tissue at any point; 2, margin damage penetrating ≥2 (0.08 in) mm into petal tissue at any point and/or browning of petal creases.**

of frequency of stems with severe flower Botrytis damage rating at termination (Fig. 2).

Though differences were not significant for any variable assessed, trends suggested that the 0.8% final ethanol concentration used to solubilize the oils may have reduced Botrytis damage and/or contributed to the phytotoxicity we observed. For example, ethanol-treated control flowers, whether *B. cinerea* infected or not, tended to have higher flower phytotoxicity ratings than their water control counterparts after 3 d (Table 5). When infected flowers were subsequently treated with the ethanol control solution rather than with tap water as a control, frequency of significant Botrytis damage at termination and frequency of Botrytis being the cause for stem termination were about 20 percentage points numerically lower, though not statistically distinguishable (Table 5). We speculate that direct contact with ethanol or EO at higher concentrations could actually result in greater Botrytis damage to flowers because the necrotic tissue from the phytotoxic response is subsequently invaded after the ethanol and EO have volatilized. This may explain why the trend for roses treated with medium and high EO concentrations to exhibit greater flower phytotoxicity damage than roses treated with the low concentration of the same EO was not accompanied by a trend for the higher concentrations to have less flower Botrytis damage.

Experiment 3 - Efficacy of EOs applied as vapor. Treatment, concentration and cultivar ('Freedom' versus Light Mix) significantly influenced most dependent variables on most days (Table 6). The greater impact of concentration when oils were applied as vapor compared to aqueous solution is evidenced in the greater separation between responses to high concentration compared to low and medium concentrations of the same oil, shown graphically in Fig. 3 compared to Fig. 2. As in Experiment 2, inoculation with *B. cinerea* affected 'Freedom' consistently less negatively compared to Light Mix flowers (Table 6), and 'Freedom' was more sensitive to oil and fludioxonil applications compared to Light Mix flowers as observed in flower phytotoxicity rating after 1 d (0.26 versus 0.12, respectively, P=0.0091) and 3 d in vase (Table 6). Leaf phytotoxicity after 1 and 3 d was negligible and never differed among treatments (data not shown).

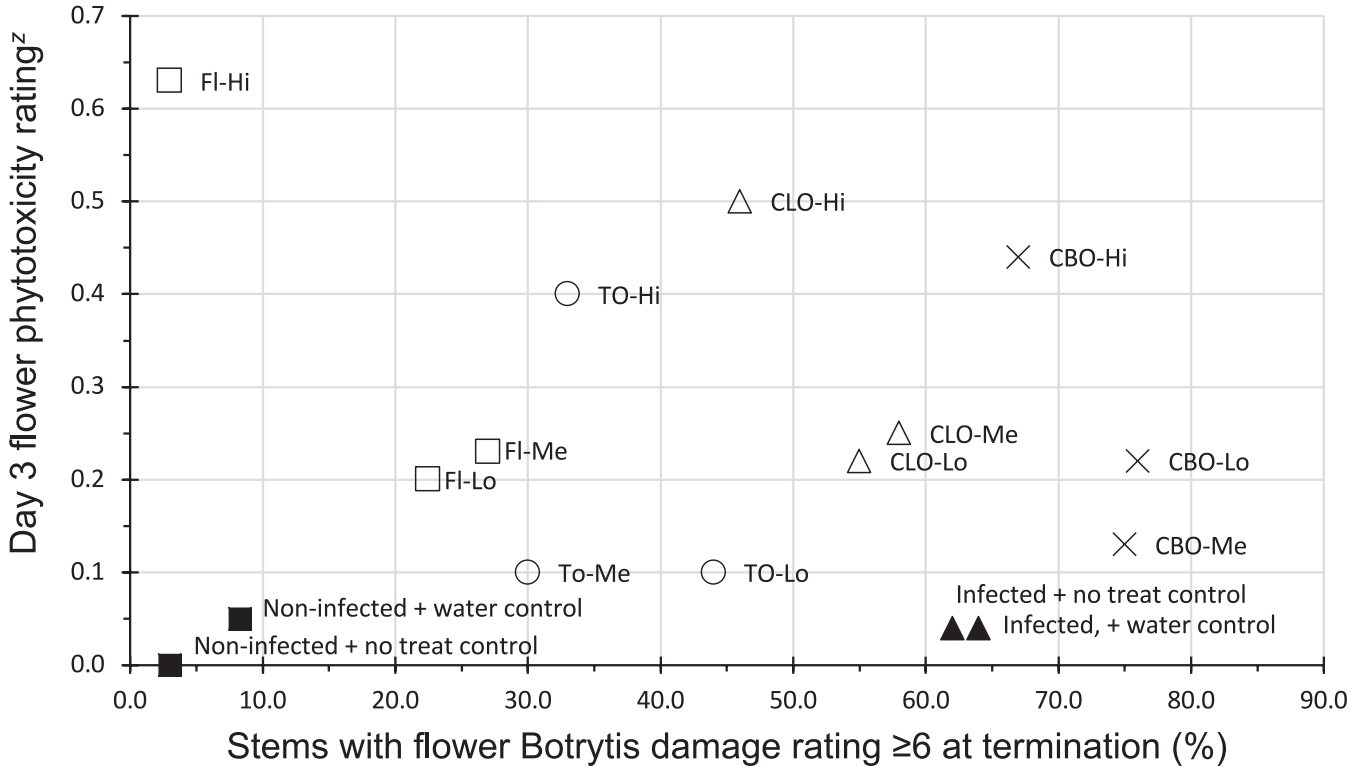

Fig. 3. Influence of treatment with vapor of thyme oil (○ TO), cinnamon leaf oil (△ CLO), or clove bud oil (× CBO) or aqueous fludioxonil (□ Fl) at low (Lo), medium (Me), or high (Hi) concentration on Botrytis-infected cut roses across cultivars ('Cool Water', 'Freedom', 'Jessika', 'Polar Star', 'Tiffany'), as measured by flower phytotoxicity rating after 3 d and frequency of stems with flower Botrytis damage rating ≥6 (i.e. ≥26% flower tissue diseased and/or collapse of flower head at receptacle) at termination, subsequent to Botrytis infection, treatment, and storage. Infection was accomplished by spraying flowers and leaves to run-off with $10^5 \cdot ml^{-1}$ (0.03 fl oz) *Botrytis cinerea* spores and incubating them in black plastic bags for 24 h at 20 C (36 F). Following incubation, stems were cut to 30 cm (11.8 in) and placed in vases containing tap water, and oil vapor treatments were applied by placing EO onto filter paper placed with flowers within a sealed bucket, and fludioxonil treatments were applied by dipping entire stems into 15 L (4.0 gal) solution or water for 20 s. Concentrations for oils or fludioxonil, respectively: Lo = 4.55 ppm or 0.125 g · L^{-1} (0.00013 oz · fl oz^{-1}); Me = 9.09 ppm or 0.250 g · L^{-1} (0.00026 oz · fl oz^{-1}); Hi = 18.18 ppm or 0.500 g · L^{-1} (0.00052 oz · fl oz^{-1}). Vases of treated flowers were then stored in 17-L (0.60 ft^3), sealed, plastic buckets in the dark for 48 h at 2 C (36 F) before placing flowers into the postharvest environment [constant 20 C (68 F) with 20 µmol · m^{-2} · s^{-1} light for 12 h · d^{-1} at 40 to 60% relative humidity] in vases containing tap water for daily observation through stem termination (14 d). Significant differences among treatments are indicated for the two dependent variables in Table 7. n = 24 stems per treatment x concentration combination and control. [z]Three-point, subjective scale: 0, no apparent damage; 1, margin damage penetrating up to approximately 2 mm (0.08 in) into petal tissue at any point; 2, margin damage penetrating ≥2 mm (0.08 in) into petal tissue at any point and/or browning of petal creases.

Roses treated with TO or fludioxonil exhibited less Botrytis damage than those treated with CLO or CBO, but flower phytotoxicity after 3 d did not differ among treatment compounds (Table 6). A difference among concentrations within an oil or fludioxonil was seen for some dependent variables observed on every day of the experiment. Vase life was longer for the high concentration of all compounds compared to one or both of the lower concentrations, but only CLO or CBO at the high concentration caused concomitantly greater flower phytotoxicity after 3 d (Table 7).

Control flowers that were not infected with *B. cinerea* and not treated with oil or fludioxonil had low frequency of severe Botrytis damage at termination and low phytotoxicity ratings after 3 d (Fig. 3). Among infected flowers, those treated with fludioxonil at the highest concentration [0.50 g · L^{-1} (0.00052 oz · fl oz^{-1})] exhibited the greatest reduction in frequency of severe Botrytis damage at termination compared to infected + water control flowers, but the reduced Botrytis damage was accompanied by the highest phytotoxicity damage rating after 3 d (Fig. 3). Exposure to 9.1 ppm TO vapor after *B. cinerea* inoculation was the only treatment in which flowers were similar to infected + water and infected + no treat control flowers in terms of flower phytotoxicity rating after 3 d and had a large reduction in frequency of severe Botrytis damage at termination (Fig. 3).

While data from Experiments 2 and 3 cannot be statistically compared, treatments of all three EOs at all three concentrations appeared to be less phytotoxic to flowers when applied as vapor rather than as aqueous solution (Tables 5 and 7).

In summary, we found in Experiment 1 that fludioxonil applied at 0.23 g · L^{-1} (0.00024 oz · fl oz^{-1}) was the one treatment exhibiting the desired combination of substantially reduced post-infection Botrytis damage and relatively low phytotoxicity in cut roses. In Experiment 2 we found that in some cases EOs worked as well as fludioxonil in terms of reducing Botrytis damage, but the reduced Botrytis damage generally came with greater

flower phytotoxicity, and flowers treated with TO or fludioxonil responded similarly in terms of having less post-infection flower Botrytis damage and less phytotoxicity compared to flowers treated with CLO or CBO. Experiment 3 showed that changing the EO delivery method from aqueous solution to a vapor reduced phytotoxicity in treated flowers, but the reduced phytotoxicity was not associated with a change in Botrytis damage. Further research is warranted with TO vapor with treatments centered on 9.1 ppm.

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