Ethylene Exposure Exacerbates Botrytis Damage in Cut Roses[1]

Ben A. Bergmann and John M. Dole[2]

Abstract

Separate experiments showed that current cut rose (*Rosa* × *hybrida*) cultivars vary considerably in Botrytis (*Botrytis cinerea*) susceptibility and ethylene (ET) sensitivity. There was no pattern when considering these two traits together for the 26 cultivars used, and neither trait appeared predictive of the other. Four cultivars were identified that included one each that is Botrytis susceptible/ET sensitive, Botrytis non-susceptible/ET sensitive, Botrytis susceptible/ET insensitive, and Botrytis non-susceptible/ET insensitive. Exposing these cultivars to ET often resulted in reduced vase life and more pronounced negative Botrytis responses (flower Botrytis damage, leaf Botrytis incidence, and frequency of termination due to Botrytis). This was true for ET sensitive and insensitive cultivars, Botrytis susceptible and non-susceptible cultivars, Botrytis inoculated and non-inoculated stems, and stems exposed to ET either before or after Botrytis inoculation and incubation. Thus, even if ET is not thought to have a major influence on a given cut rose cultivar in terms of classic negative ET responses (leaf drop, petal wilt, altered opening rate), ET exposure could have a significant negative impact on that cultivar by exacerbating Botrytis damage. Shipping temperatures and methods can have a strong influence on Botrytis damage, with pre-cooling of shipping boxes before cold storage/transport being beneficial.

Index words: Botrytis blight, *Botrytis cinerea*, cut flowers, floriculture, gray mold, *Rosa* L. hybrids.

Species used in this study: Rose (*Rosa* × *hybrida*) focal cultivars 'Cuenca', 'Daphnee', 'Freedom', 'Vendela'; Botrytis (*Botrytis cinerea* Pers. ex. Fr.).

Chemicals used in this study: 1-methylcyclopropene (EthylBloc); ethylene; silver thiosulfate.

Significance to the Horticulture Industry

Botrytis damage to roses during storage and shipping continues to be a challenge for the cut flower industry, and decreased flower quality due to ethylene (ET) exposure can be a problem for some cut rose cultivars. This research confirmed that current cut rose cultivars vary considerably in Botrytis susceptibility and ET sensitivity. We found that Botrytis infection and development did not influence ET sensitivity, but exposure to ET increased Botrytis damage. This phenomenon occurred in both relatively Botrytis susceptible and non-susceptible rose cultivars and in both relatively ET sensitive and insensitive cultivars. The increased Botrytis damage was observed whether ET exposure was before or after *B. cinerea* infection. An anti-ET treatment may be warranted with cut roses to help prevent Botrytis damage during transport, even if the shipped cultivars are perceived to be Botrytis non-susceptible and/or ET insensitive.

Introduction

Damage caused by *Botrytis cinerea* has long been one of the floral industry's most significant postharvest challenges (Coyier 1985, Droby and Lichter 2007), and cut rose cultivars differ in Botrytis susceptibility (Pie and Brouwer 1993, Hammer and Evensen 1994). Ethylene (ET) exposure can also be problematic for sensitive species and cultivars of cut flowers during postharvest handling, and roses are known to be slightly to moderately sensitive to ET across cultivars (Dole et al. 2017) with demonstrated sensitivity differences among cultivars (Reid et al. 1989, Macnish et al. 2010). Temperatures and relative humidity (RH) during storage and shipping of cut flowers are often conducive to Botrytis infection (Williamson et al. 1993) and development (Zhang and Sutton 1994, Sosa-Alvarez et al. 1995) and a concomitant loss in flower quality (van der Sman et al. 1996). Closed shipping boxes allow ET from stressed plant tissue to accumulate (Muller et al. 2000), sometimes to a level shown to reduce vase life of cut roses (Macnish et al. 2010). This research with cut roses was conducted to better understand the relationship 1) between Botrytis susceptibility and ET sensitivity and 2) between temperature and RH within shipping boxes and Botrytis incidence. To accomplish these objectives, one experiment screened current cut rose cultivars for Botrytis susceptibility, and one experiment screened the same cultivars for ET sensitivity. Two experiments investigated the relationship between Botrytis susceptibility and ET sensitivity and the influence of ET on Botrytis infection and development. Two experiments investigated the influence of within-box temperature and RH during international transport on Botrytis incidence.

Materials and Methods

Plants. For each experiment, roses with ≥45 cm (17.7 in) stems were delivered overnight to our laboratory at North Carolina State University (NCSU) from a distribution center in Miami, FL, after arriving there from a commercial grower in Colombia, South America.

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[2]Research Associate and Associate Dean and Director of Academic Programs for the College of Agriculture and Life Sciences, respectively, North Carolina State University, Department of Horticultural Science, Campus Box 7609, Raleigh, NC 27695-7609, bergmann@ncsu.edu (corresponding author).

Rose isolate B. cinerea spore production. We established an axenic culture of *B. cinerea* collected from an infected rose petal. The sterile *B. cinerea* rose isolate was maintained vegetatively 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, Kansas, USA) 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. One plate was submitted to the NCSU Plant Disease and Insect Clinic, which used PCR to verify the identity of our newly isolated rose strain as *B. cinerea*.

Reproductive 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 petri dishes containing 20 ml (0.68 fl oz) oatmeal agar medium (Thermo Fisher Scientific, Lenexa, Kansas, USA) prepared per manufacturer instructions. After 3 weeks, sporulating plates were used to establish additional plates of reproductive fungus – by inverting a sporulating plate over a fresh, oatmeal agar plate and tapping it three times – and to harvest spores for inoculations. 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, St. Louis, MO, USA), 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. 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 spores. *Botrytis cinerea* 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 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 15 rose stems. Inoculation and imposition of control treatments was followed by incubating flowers for 24 h at constant 20 C (68 F) wrapped in black plastic bags separated by treatment.

Cut rose exposure to ET and anti-ET agents. Ethylene treatments were applied by cutting 2.5 cm (0.98 in) from stem bases and placing 15 stems per jar by cultivar into 500 ml (16.9 fl oz) tap water and placing the jars into 210-L (55.5-gal) gas-tight chambers into which ET was injected to achieve the desired concentration. Accuracy of ET treatments was verified one hour after injecting ET into a chamber and at the end of the exposure period by sampling chamber headspace gas and quantifying the ET concentration in a gas chromatograph (Varian 3400; Varian Inc., Walnut Creek, CA) fitted with a glass column (Porapak Q, 80–100 mesh, 183 cm (72.0 in) by 2 mm (0.08 in); Sigma-Aldrich, Inc., St. Louis, MO) running at 120 C (248 F) injector, 120 C (248 F) column, and 130 C (266 F) detector (flame ionization) temperatures. Flow rates for the He carrier, H_2, and O_2 were 30, 16, and 90 ml (1.01, 0.54, and 3.04 oz)$\cdot min^{-1}$, respectively. Sample injection volume was 1 ml (0.03 oz) of headspace gas drawn via a neoprene port in the chamber lid. Flowers were exposed to ET treatments for 24 h at 21 C (70 F) in darkness.

Three anti-ET treatments were used in this work: 0 ppm ET with charcoal, 700 $nL \cdot L^{-1}$ (0.70 ppm) 1-methylcyclopropene (1-MCP), and 28.3 ppm silver thiosulfate (STS). As gaseous treatments, the first two of these were applied in chambers as described for ET, except instead of applying ET, activated charcoal was placed into the 0 ppm ET chamber to absorb ambient ET, and EthylBloc (Floralife Inc., Walterboro, SC) was placed into the 700 $nL \cdot L^{-1}$ (0.70 ppm) 1-MCP chamber. When used per manufacturer instructions, wetted EthylBloc releases known quantities of 1-MCP to get the concentration desired. Vase solution treatments were applied by cutting 2.5 cm (0.98 in) from stem bases and placing them 15 per jar by cultivar into 500 ml (16.9 fl oz) tap water with or without 28.3 ppm STS and tenting flowers in black plastic. Flowers were exposed to anti-ET and tap water only control treatments for 24 h at 21 C (70 F) in darkness.

Post-treatment handling of cut roses. After imposition of experiment treatments, all leaves were removed from each stem except the three uppermost that had at least three leaflets each. Flower stems were then recut to 40 cm (15.75 on) before placing them into vases containing 350 ml (11.83 fl oz) tap water. Flowers were held at constant 20 C (68 F) under 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (137 ft-c) fluorescent light for 12 $h \cdot d^{-1}$ at 40–60% RH for observation through flower termination.

Each stem was rated daily through termination for flower Botrytis damage starting 1 d after placing flowers into the postharvest environment. The 1 to 8 scale is a modified decay index of flower petal and receptacle as 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; 8, 76-100% disease or collapse of flower head at receptacle. Each stem was rated daily as 1 or 0 for presence or absence of at least one Botrytis lesion on the leaves.

Each stem was rated daily through termination for flower openness on a 0 to 3 scale: 0, tight (all petals upright, some outer petals slightly reflexed); 1, medium (most whorls beginning to reflex); 2, open (outer whorls completely reflexed); 3, fully open (stamens visible). At the end of each flower's vase life, it was noted if the principal reason for termination was Botrytis (Fig 1a) or some other reason: bent neck, discolored petals, failure to open, necrotic petal edges, petal drop, or petal wilt (Fig 1b).

Fig. 1a. Reasons for terminating cut roses due to Botrytis (cultivar): 1) petal tips (Impression), 2) petal bases (Jade), 3) entire outer petals (Bonanza), 4) entire middle petals (Domenica), 5) entire inner petals (Sandra – outer petals pulled back), 6) receptacle (Moon Walk).

Statistics. Each experiment was conducted as a randomized, complete block design with three roses for each treatment by cultivar combination within each of five blocks. Thus, 15 individual stems of each cultivar were subjected to each treatment in each experiment. Statistical analyses were conducted with JMP Pro 13 (SAS, Cary, NC). A standard least squares method was used to perform analysis of variance on three of the dependent variables assessed daily: flower Botrytis damage rating, flower openness rating, and days to termination (vase life). Treatment and cultivar were treated as fixed effects, and block and replicate within block were treated as random effects. Binary logistic regression was used to determine the influence of treatments on variables rated as 1 or 0:

Fig. 1b. Reasons for terminating cut roses not due to Botrytis (cultivar): 1) necrotic petal edges (Sandra), 2) discolored petals (Dark Lulu), 3) failure to open – either entirely or beyond outer petals (Marisa), 4) petal drop (Daphnee), 5) flower wilt (Domenica), 6) bent neck (Impression).

Table 1. In-vase response of 26 cut rose cultivars to inoculation with *Botrytis cinerea* as measured by flower botrytis damage rating after 5 d, vase life, and frequency of stem terminations due to flower Botrytis damage.

	Day 5 flower Botrytis damage rating[z]				Vase life (days)				Termination due to flower Botrytis damage (%)			
	Botrytis inoculation				Botrytis inoculation				Botrytis inoculation			
Cultivar	Control	Inoculated[y]		Significance[x]	Control	Inoculated[y]		Significance[x]	Control	Inoculated[y]		Significance[x]
Bonanza	1.3	5.5	abc	***	13.9	6.6	kl	***	0	80	abcd	NS
Caramba	1.1	6.7	cdefg	***	6.4	5.6	lm	NS	12	100	a	***
Citran	1.0	3.3	a	***	12.3	10.9	cd	NS	0	40	fgh	NS
Cool Water	1.0	4.9	abcd	***	9.1	5.4	m	**	0	73	bcde	NS
Cuenca	1.0	3.8	abcdefg	**	15.7	9.3	fgh	***	4	67	efg	***
Daphnee	1.2	1.2	g	NS	8.4	7.9	ij	NS	0	0	j	NS
Dark Lulu	1.0	4.5	abcde	***	14.1	8.4	ghi	***	0	73	bcde	NS
Domenica	1.4	4.9	abcd	***	16.7	9.1	fgh	***	60	87	abc	NS
Escimo	1.8	2.7	cdefg	NS	10.9	9.8	def	NS	20	67	cde	*
Freedom	1.0	1.0	g	NS	13.5	11.5	bc	NS	0	7	ij	NS
Hot Princess	1.0	2.1	defg	NS	8.9	9.5	efg	NS	0	27	hi	NS
Idole	1.1	4.6	abcde	***	17.2	10.1	def	***	7	80	abcd	***
Impression	1.2	1.6	efg	NS	14.9	12.9	a	NS	13	33	gh	NS
Jade	2.4	3.3	cdefg	NS	11.7	12.5	ab	NS	100	100	a	NS
Marilyn	1.6	5.5	abc	***	9.8	8.2	hij	**	21	87	abc	**
Marisa	1.6	6.5	ab	***	12.3	6.6	kl	***	0	87	abc	NS
Moon Walk	1.5	3.2	cdefg	*	11.4	10.9	cd	NS	7	60	def	**
Ocean Song	2.2	5.5	abc	***	13.1	6.7	kl	***	73	100	a	NS
Orange Crush	1.0	5.2	abc	**	13.8	7.5	ijk	***	8	91	ab	***
Punch	1.0	6.4	ab	***	14.0	6.5	klm	***	0	100	a	***
Queen Berry	1.0	1.3	fg	NS	13.0	10.6	cde	*	0	33	gh	NS
Sandra	1.0	3.3	cdefg	***	10.7	8.5	ghi	*	0	53	efg	NS
Satina	1.3	4.1	abcdef	***	10.5	8.3	hi	*	100	100	a	NS
Sprit	1.0	5.3	abc	***	13.6	7.1	jk	***	13	87	abc	***
Taiga	1.0	3.6	bcdefg	***	18.6	11.7	bc	***	7	60	def	**
Vendela	2.2	6.6	ab	***	18.1	9.3	fgh	***	40	87	abc	**
ALL	1.3	4.1		***	12.8	8.9		***	19	68		***

Botrytis inoculation: whole stems sprayed to run-off with $10^5 \cdot ml^{-1}$ (0.03 fl oz) spores in solution and incubated in black plastic bags for 24 h at 21 C (70 F). In-vase observations: after inoculation stems were placed into vases containing tap water under constant 20 C (68 F) with 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (137 ft-c) fluorescent light for 12 h $\cdot d^{-1}$ at 40 – 60% relative humidity.

n = 15 stems per cultivar × treatment combination.

[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 followed by the same letter are not significantly different at $P = 0.05$ using Student's t-test.

[x]NS = not significant, and *, **, and *** indicate significant differences at the $P<0.05$, $P<0.01$, and $P<0.001$, respectively, for within cultivar pair-wise tests.

presence of Botrytis on flowers, presence of Botrytis on leaves, leaf drop, and whether Botrytis was the principle cause for termination. Student's t-test was used to identify significant differences among means.

Experiment 1. Susceptibility to Botrytis was assessed for 26 cut rose cultivars (Table 1). Upon arrival, stems were either inoculated with *B. cinerea* spores and incubated or were controls that were treated the same apart from there being no spores in the inoculation solution. They were then placed in jars for observation through termination.

Experiment 2. Sensitivity to ET was assessed for the same 26 cut rose cultivars as in Experiment 1. Upon arrival, stems were exposed to 0, 0.1, or 1.0 ppm ET. They were then placed in jars for observation through termination.

Experiment 3. Four cultivars were selected for this experiment based on results from Experiments 1 and 2 which showed that within our system they were relatively Botrytis susceptible (B+) or not (B–) and relatively ET sensitive (E+) or not (E–): 'Cuenca' (B+E+), 'Vendela' (B+E–), 'Daphnee' (B–E+), and 'Freedom' (B–E–). Upon arrival, stems were treated with 0 ppm ET (charcoal in treatment chamber), 1.0 ppm ET, 700 $nl \cdot L^{-1}$ (0.70 ppm) 1-MCP, 28.3 ppm STS, or tap water. Subsequently half of the stems in each of these treatments were inoculated with *B. cinerea* spores and incubated, and half were controls treated the same apart from there being no spores in the inoculation solution. They were then placed in jars for observation through termination. In addition to the observed variables noted above, stems were scored on days 3, 6 and 9 as 1 or 0 for presence or absence of leaf drop.

Experiment 4. The same four cut rose cultivars as in Experiment 3 were used. Upon arrival, stems were inoculated with *B. cinerea* spores and incubated or were controls that were treated the same apart from there being no spores in the inoculation solution. Subsequently 15 inoculated and 15 non-inoculated stems of each cultivar were treated with 0 ppm ET with charcoal in the chamber, 1.0 ppm ET, 700 $nl \cdot L^{-1}$ (0.70 ppm) 1-MCP, 28.3 ppm STS,

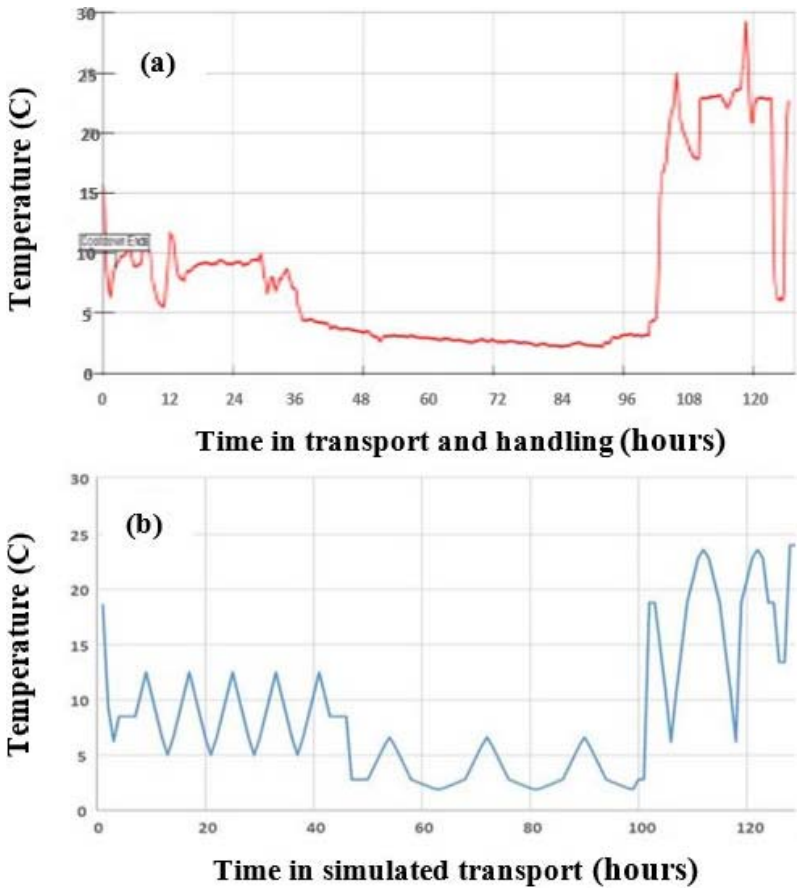

Fig. 2a-b. Temperature timeline for a single shipment of cut roses from a production facility near Bogotá, Colombia to Raleigh, NC, USA (a) and for simulated shipping temperatures in the laboratory.

or tap water. They were then placed in jars for observation through termination. In addition to the observed variables noted above, stems were scored on days 3, 6 and 9 as 1 or 0 for presence or absence of leaf drop.

Experiment 5. Prior to the experiment, temperatures were recorded every 10 m within standard, 200-rose, cardboard shipping boxes on eight occasions (twice each in August, September, October, and April) for the duration of transport from a production farm near Bogotá, Colombia to the laboratory in Raleigh, NC. Temperature data (e.g. Fig 2a) were analyzed to provide ranges and fluctuations for shipping simulations (Fig 2b). Cut rose cultivars Cuenca, Freedom, Idole, and Vendela were held for 5 d in standard, cardboard shipping boxes and exposed to constant 3.5 C (38 F) or to fluctuating temperatures simulating shipping conditions. There were two periods of greatest temperature fluctuation: the first 40 h with five cycles alternating between 12 C (54 F) for 2 h and 5 C (41 F) for 6 h similar to transport from a producer in Colombia to the Miami, FL airport and the last 28 h with four cycles alternating between 24 C (75 F) for 6 h and 10 C (50 F) for 1 h similar to ground transport from a freight forwarding company in Miami, FL to Raleigh, NC. Roses were then placed in jars for observation through termination.

Experiment 6. Cut rose cultivars 'Sweet Unique' and 'Hot Party' were used to test the effects of flower packing density and pre-cooling of packed boxes prior to placement in coolers on temperature and RH within shipping boxes and subsequent influence on Botrytis incidence and vase life. Packing density was either 200 stems per box (completely full = densely packed) or 100 stems per box (= loosely packed). Boxes were moved from room temperature (= ambient) to a 3.5 C (38 F) cooler, and half the boxes were subjected to pre-cooling by pulling 3.5 C (38 F) air through their vent holes with a high-speed exhaust fan. Two RHT 20 data loggers (Extech Instruments Corp, Waltham, MA) within each box, one placed at the edge of a flower bunch and one placed in the center of a flower bunch, recorded temperature and RH every 10 min for 24 h. Flower boxes were then held in storage an additional 4 d at constant 3.5 C (38 F) before stems were placed in jars for observation through termination.

Results and Discussion

Experiment 1. For inoculated stems, 19 of 26 cultivars (73%) had greater flower Botrytis damage after 5 d in vase compared to non-inoculated stems (Table 1). Three of the 26 cultivars (12%) had a greater frequency of Botrytis lesions on leaves after 5 d in vase when inoculated, and all three ('Marilyn', 'Marisa', and 'Satina') were among the 19 that exhibited greater flower Botrytis damage after 5 d (data not shown). Three cultivars were highly Botrytis susceptible based on 100% of flowers terminated due to

Table 2. In-vase response of 26 cut rose cultivars to ethylene (ET) exposure as measured by flower openness after 4 d in vase, flower openness at stem termination, and vase life.

	Day 4 flower openness[z]					Flower openness[z] at termination					Vase life (d)				
	ET (ppm)					ET (ppm)					ET (ppm)				
Cultivar	0	0.1	1.0[y]		0 vs 1.0[x]	0	0.1	1.0[y]		0 vs 1.0[x]	0	0.1	1.0[y]		0 vs 1.0[x]
Bonanza	1.5	1.9	2.2	abcd	**	2.9	2.9	3.0	a	NS	12.8	12.8	11.9	bcde	NS
Caramba	1.8	2.3	2.4	ab	***	2.3	2.8	2.9	ab	***	7.0	7.1	5.8	h	NS
Citran	1.3	1.3	1.1	hij	NS	2.3	2.4	2.5	abcde	NS	12.7	13.5	12.1	bcde	NS
Cool Water	1.9	2.1	1.5	efgh	*	2.5	2.5	2.3	cde	NS	8.6	9.3	7.1	fgh	NS
Cuenca	0.9	1.2	1.7	defg	***	2.0	2.4	2.4	bcde	**	17.3	14.7	13.1	bc	***
Daphnee	1.9	2.1	2.3	abc	**	2.9	2.9	3.0	a	NS	8.6	6.9	6.6	gh	*
Dark Lulu	1.6	1.5	1.8	cdef	NS	2.0	2.0	2.1	de	NS	14.7	12.7	12.0	bcde	**
Domenica	1.5	1.3	1.2	ghij	NS	2.3	2.6	2.1	de	NS	16.5	16.7	13.6	b	**
Escimo	2.1	2.0	2.1	abcde	NS	3.0	2.9	2.8	abc	NS	11.5	10.7	10.1	def	NS
Freedom	1.3	1.3	1.1	hij	NS	2.3	2.2	2.1	de	NS	13.9	13.7	12.7	bcd	NS
Hot Princess	1.6	1.7	1.7	defg	NS	2.6	2.7	2.7	abc	NS	9.7	10.5	10.5	bcde	NS
Idole	0.4	0.9	0.8	ij	*	1.9	2.0	2.0	e	NS	17.8	18.5	17.5	a	NS
Impression	1.1	1.3	1.2	ghij	NS	2.7	2.5	2.5	abcde	NS	13.3	13.7	13.3	bc	NS
Jade	1.4	1.2	0.9	ij	***	2.0	2.1	2.5	abcde	**	14.5	12.6	12.2	bcde	*
Marilyn	0.7	0.9	1.0	ij	NS	1.3	1.5	1.3	f	NS	11.5	10.9	9.8	def	NS
Marisa	2.0	2.0	1.9	bcde	NS	2.5	2.0	2.1	de	**	10.5	11.5	11.1	bcde	NS
Moon Walk	1.6	1.7	1.7	defg	NS	2.0	2.3	2.1	de	NS	11.0	12.1	11.6	bcde	NS
Ocean Song	1.5	1.3	1.3	fghi	NS	2.0	2.1	2.0	e	NS	13.5	13.1	11.6	bcde	*
Orange Crush	1.0	1.1	0.9	ij	NS	2.1	2.5	2.6	abcd	***	14.0	14.8	11.9	bcde	***
Punch	1.6	1.6	1.6	efgh	NS	2.1	2.1	2.1	de	NS	12.9	12.9	12.6	bcd	NS
Queen Berry	1.4	1.6	1.6	efgh	NS	2.1	2.2	2.3	cde	NS	14.1	13.5	12.5	bcd	NS
Sandra	0.9	1.1	0.7	j	NS	1.9	2.0	1.2	f	***	11.1	10.9	10.3	cde	NS
Satina	1.3	1.3	1.3	fghi	NS	2.0	2.0	2.0	e	NS	11.2	10.4	9.3	efg	*
Sprit	2.5	2.6	2.5	a	NS	3.0	3.0	3.0	a	NS	12.0	11.9	11.1	bcde	NS
Taiga	1.6	1.8	1.7	defg	NS	2.7	2.8	2.7	abc	NS	17.1	17.3	17.4	a	NS
Vendela	2.3	2.2	2.1	abc	NS	2.4	2.5	2.5	abcde	NS	19.1	11.5	18.6	a	NS
ALL	1.6	1.6	1.5		NS	2.3	2.4	2.6		NS	13.0	12.5	11.8		***

Ethylene exposure: whole stems in vases with water were exposed 0, 0.1, or 1.0 ppm ET for 24 h at 21 C (70 F) in darkness.

In-vase observations: after ET treatment stems were placed into vases containing tap water under constant 20 C (68 F) with 20 μmol · m^{-2} · s^{-1} (137 ft-c) fluorescent light for 12 h · d^{-1} at 40 – 60% relative humidity.

n = 15 stems per cultivar × treatment combination.

[z]Four-point scale: 0, tight (all petals upright, some outer petals slightly reflexed); 1, medium (most whorls beginning to reflex); 2, open (outer whorls completely reflexed, all whorls reflexing to a high degree); and 3, fully open (stamens visible).

[y]Values within the 1.0 ppm ET column followed by the same letter are not different at P=0.05 using Student's t-test.

[x]NS = not significant, and *, **, and *** indicate significant differences at the $P<0.05$, $P<0.01$, and $P<0.001$, respectively, for within cultivar pair-wise tests of 0 versus 1.0 ppm ET exposure.

Botrytis when inoculated and very high Botrytis frequency when not inoculated: 'Jade', 'Ocean Song', and 'Satina' (Table 1). Eleven cultivars were susceptible based on ≥54% termination due to Botrytis and vase life reduction of ≥40% when comparing inoculated to non-inoculated flowers: 'Bonanza', 'Cool Water', 'Cuenca', 'Dark Lulu', 'Domenica', 'Idole', 'Marisa', 'Orange Crush', 'Punch', 'Sprit', and 'Vendela '(Table 1). Three cultivars were non-susceptible based on inoculated flowers having ≤27% termination due to Botrytis and vase life not significantly reduced for inoculated flowers compared to untreated flowers: 'Daphnee, 'Freedom', and 'Hot Princess' (Table 1).

Experiment 2. Of the 26 cultivars, flower openness was influenced by ET exposure in seven (27%) after 4 d in vase, with five opening more with ET exposure and two opening less, and in six (22%) at termination, with four opening more with ET exposure and two opening less (Table 2). Our finding that 38% of the rose cultivars tested were affected by ET exposure as measured by flower openness after 4 d in vase and/or at termination was lower than the 62% reported in 2010 (Macnish et al.), which was lower than the 83% reported in 1989 (Reid et al.). Eight cultivars exhibited ET sensitivity by having a significantly shorter vase life when treated with 1.0 ppm ET compared to no-ET controls: Caramba, Cool Water, Cuenca, Daphnee, Dark Lulu, Domenica, Ocean Song, and Satina (Table 2). In three cultivars, the reduction in vase life was seen when exposed to 1.0 ppm but not 0.1 ppm ET: Domenica, Ocean Song, and Orange Crush (Table 2).

Experiments 3 and 4. Stems of all cultivars had increased flower Botrytis damage after 3 d in vase and higher frequency of termination due to Botrytis when inoculated with *B. cinerea* compared to non-inoculated controls whether ET and anti-ET treatments came before or after inoculation (Table 3). As in Experiment 1, cultivars Cuenca and Vendela were relatively more Botrytis susceptible while Daphnee and Freedom were less so. Looking at *B. cinerea* inoculated stems across ET and anti-ET treatments, termination due to Botrytis was lower for the two non-susceptible than for the two susceptible cultivars whether they received ET and anti-ET treatments

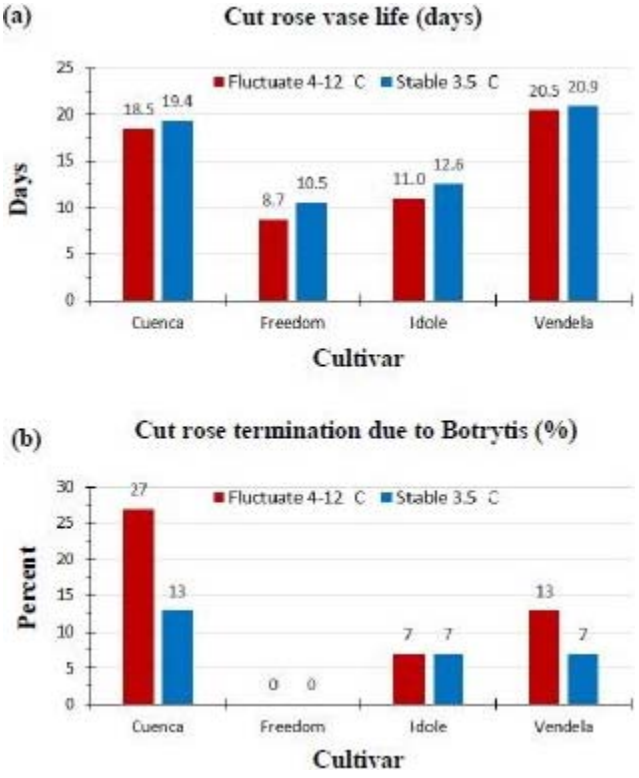

Fig. 3a-b. Vase life (a) and frequency of stem termination due to Botrytis (b) in four cut rose cultivars after 5 d simulated transport with stable 3.5 C (38 F) or with temperatures fluctuating as shown in Fig 2b.

before or after inoculation (Table 3). Freedom had less flower Botrytis damage than other cultivars across all treatments, whether flowers received ET and anti-ET treatments before or after *B. cinerea* inoculation (Table 3). Cuenca had more leaf Botrytis than other cultivars across all treatments when flowers received ET and anti-ET treatments before *B. cinerea* inoculation (Table 3).

Compared to non-inoculated controls, inoculation with *B. cinerea* resulted in shorter vase life for the two Botrytis susceptible but not the two Botrytis non-susceptible cultivars when ET and anti-ET treatments were applied before inoculation (Table 3), and shortened vase life was seen in all four cultivars if ET and anti-ET treatments were applied after inoculation (Table 3). Vase life for flowers inoculated with *B. cinerea* compared to non-inoculated flowers was reduced more for Botrytis susceptible than non-susceptible cultivars. When ET exposure was before *B. cinerea* inoculation, vase life changes compared to non-inoculated flowers were Vendela -41% > Cuenca -28% >> Daphnee -7% = Freedom -2% ($P < 0.0001$). When ET exposure was after *B. cinerea* inoculation changes in vase life were Vendela -65% = Cuenca -62% >> Daphnee -41% = Freedom -37% ($P < 0.0001$).

As in Experiment 2, cultivars Cuenca and Daphnee were relatively ET sensitive while Vendela and Freedom were not. Compared to 1-MCP and charcoal treatments, ET sensitive cultivars treated with ET had higher leaf drop frequency after 3 d in vase and increased flower openness after 5 d in vase, but these responses did not differ between STS treated and water control flowers (Table 3). In no case did ET or anti-ET treatments influence leaf drop or flower openness in ET insensitive cultivars (Table 3). Leaf drop and flower openness responses were similar within cultivar whether ET and anti-ET agents were applied before or after *B. cinerea* inoculation (Tables 3).

Compared to controls, ET exposure resulted in shorter vase life for ET sensitive but not ET insensitive cultivars when ET was applied before (Cuenca and Daphnee, Table 3) or after (Cuenca, Table 3) inoculation. Vase life for flowers exposed to 1 ppm ET compared to non-exposed flowers was reduced more for ET sensitive than insensitive cultivars. When ET exposure was before *B. cinerea* inoculation, vase life changes compared to 0 ppm ET with charcoal control flowers were Cuenca -27% = Daphnee -26% > Freedom -18% = Vendela -13% ($P = 0.0086$). When ET exposure was after *B. cinerea* inoculation changes in vase life were Cuenca -19% > Daphnee -7% = Freedom +1% > Vendela +11% ($P = 0.0109$).

Connection between Botrytis and ET responses. Inoculation with *B. cinerea* did not influence the ET sensitivity variables of leaf drop and flower openness on any observation day in any cultivar. This was the case whether ET and anti-ET treatments were applied before or after inoculation (e.g. Table 3).

Ethylene and anti-ET treatments influenced the Botrytis susceptibility variables of flower Botrytis damage, leaf Botrytis frequency, and flower termination due to Botrytis on all observation days in at least some cultivars whether they were applied before or after inoculation (e.g. Table 3). The ET insensitive and Botrytis non-susceptible cultivar Freedom had more flower Botrytis damage, higher incidence of leaf Botrytis, and higher frequency of termination due to Botrytis when flowers were exposed to ET compared to 1-MCP treated and charcoal control flowers (Table 3). Treatment of Freedom flowers with STS before Botrytis inoculation reduced flower Botrytis damage and frequency of termination due to Botrytis but had no effect when applied after inoculation (Table 3).

Ethylene and anti-ET agents applied before versus after B. cinerea *inoculation*. Influence of *B. cinerea* inoculation across ET and anti-ET treatments on Botrytis response was similar whether the treatments were imposed before or after inoculation (Table 3). Influence of ET and anti-ET treatments across inoculation treatments on Botrytis response differed in some cases between pre- and post-inoculation application. Treatment with STS before inoculation significantly affected flower Botrytis damage in all four cultivars and termination due to Botrytis in three cultivars (Table 3), but the influence was not observed when STS was applied after inoculation (Table 3). The STS pre-treatment had a negative influence on these variables in Cuenca, Daphnee, and Vendela but a positive effect in Freedom (Table 3). Vase life was influenced in three cultivars when STS was applied pre-inoculation but in no cultivars when applied post-inoculation (Table 3). Timing of ET exposure affected leaf Botrytis differently – ET applied before inoculation affected Daphnee only and did not affect leaf Botrytis frequency across cultivars (Table 3), but ET applied after inoculation affected all four cultivars

Table 3. Response of cut rose cultivars Cuenca, Daphnee, Vendela and Freedom when treated with ethylene (ET) or anti-ET agents before or after inoculation with *Botrytis cinerea* as measured by flower Botrytis damage rating after 3 d in vase, leaf Botrytis frequency after 3 d in vase, vase life, leaf drop frequency after 3 d in vase, and flower openness after 5 d in vase.

	ET and anti-ET treatments before *B. cinerea* inoculation					ET and anti-ET treatments after *B. cinerea* inoculation				
	Cultivar[z]					Cultivar[z]				
	Cuenca	Daphnee	Vendela	Freedom	All	Cuenca	Daphnee	Vendela	Freedom	All
	(B+E+)[y]	(B−E+)	(B+E−)	(B−E−)		(B+E+)[y]	(B−E+)	(B+E−)	(B−E−)	
Treatment	**Day 3 flower Botrytis damage rating[y]**					**Day 3 flower Botrytis damage rating[y]**				
B. cinerea:										
Yes	4.1 A[x] a[w]	4.5 A a	4.2 A a	2.7 B a	3.9 a	6.1 A[x] a[w]	5.9 A a	5.8 A a	4.3 B a	5.5 a
No	3.3 A b	2.9 A b	3.0 A b	2.1 B b	2.8 b	2.7 A b	1.8 B b	2.3 AB b	2.1 AB b	2.2 b
Gas:										
ET	3.8 A NS	4.4 A a	4.3 A a	2.8 B a	3.8 a	4.6 A NS	4.4 A a	4.6 A NS	3.9 A a	4.4 a
1-MCP	4.7 A	4.6 A a	3.6 B a	2.4 C b	3.8 a	4.0 A	3.6 A b	4.0 A	2.5 B c	3.5 b
Charcoal	4.4 A	3.0 B b	3.1 B b	2.2 C b	3.2 b	3.9 AB	3.5 AB b	4.3 A	3.2 B b	3.7 b
Solution:										
STS	3.5 A a	4.0 A a	4.3 A a	1.5 B b	3.3 NS	4.0 A NS	3.6 AB NS	3.5 AB NS	3.1 B NS	3.6 NS
Water	2.1 B b	2.6 A b	2.7 A b	2.9 A a	2.6	4.3 A	3.9 AB	3.8 AB	3.3 B	3.8
All	3.7 A	3.7 A	3.6 A	2.4 B	3.3	4.2 A	3.8 A	4.0 A	3.2 B	3.8
	Day 3 leaf Botrytis frequency (%)					**Day 3 leaf Botrytis frequency (%)**				
B. cinerea:										
Yes	53 A a	22 B NS	5 C NS	11 C NS	23 NS	39 A a	26 AB NS	18 B NS	28 AB a	28 NS
No	29 A b	21 AB	2 C	12 B	16	27 A b	20 A	24 A	9 Bb	20
Gas:										
ET	57 A NS	31 B a	10 C NS	10 C NS	27 NS	56 A a	30 Ba	31 Ba	40 AB a	39 a
1-MCP	41 A	25 A ab	0 B	5 B	18	28 A b	14 AB b	7 AB b	14 B b	16 b
Charcoal	48 A	14 B b	7 BC	0 C	17	31 A b	35 Aa	13 Bb	10 B b	22 b
Solution:										
STS	31 A NS	14 B NS	14 B NS	3 C NS	16 NS	31 A NS	27 AB NS	23 AB NS	13 B NS	24 NS
Water	35 A	24 A	26 A	0 B	21	18 AB	10 B	30 A	17 AB	19
All	42 A	22 B	10 BC	6 C	20	33 A	23 A	21 A	19 A	24
	Termination due to Botrytis (%)					**Termination due to Botrytis (%)**				
B. cinerea:										
Yes	55 B a	27 D a	68 Aa	43 Ca	48 a	95 Aa	80 Ba	97 Aa	83 Ba	89 a
No	36 A b	12 B b	12 Bb	15 Bb	19 b	5 Ab	0 Ab	157 Ab	12 Ab	8 b
Gas:										
ET	41 AB NS	30 B a	53 ANS	43 AB a	42 a	43 BNS	47 B NS	59 AB NS	70 Aa	55 NS
1-MCP	72 A	27 C a	47 B	14 C b	40 a	55 AB	40 B	60 A	33 Bb	47
Charcoal	62 A	3 D b	30 BC	17 C b	28 b	47 AB	37 B	57 A	40 AB b	45
Solution:										
STS	38 AB a	28 BCNS	52 A a	17 C b	34 NS	53 A NS	30 B NS	57 A NS	57 AN	49 NSS
Water	14 A b	11 B	19 B b	50 A a	24	53 A	47 A	50 A	47 A	49
All	46 A	20 B	40 A	28 B	33	50 A	40 B	56 A	49 AB	49
	Vase life (days)					**Vase life (days)**				
B. cinerea:										
Yes	6.6 B b	4.1 C NS	8.1 AB b	8.3 A NS	6.8 b	4.0 C b	4.1 C b	5.6 B b	7.1 A b	5.2 b
No	9.1 B a	4.4 C	13.8 AB a	8.5 B	9.0 a	10.6 B a	7.0 C a	16.0 A a	11.2 B a	11.2 a
Gas:										
ET	5.4 C b	3.4 D b	10.7 A NS	6.6 B NS	6.7 b	6.3 B b	5.9 B NS	9.7 A NS	8.7 A NS	7.7 NS
1-MCP	6.9 B a	4.2 C a	10.2 A	7.2 B	7.3 b	8.2 B a	4.7 C	9.9 A	9.9 A	8.2
Charcoal	7.4 B a	4.6 C a	12.3 A	8.0 B	8.1 a	7.8 B a	5.3 C	10.4 A	8.6 B	8.0
Solution:										
STS	10.3B a	4.8 D NS	8.8 C b	12.7A a	9.2 a	7.2 B NS	6.0 B NS	11.7 A NS	10.1 A NS	8.8 NS
Water	9.2 B b	4.6 D	12.8 A a	7.4 C b	8.5 b	7.0 BC	6.0 C	11.9 A	8.8 B	8.4
All	7.9 BC	4.3 C	11.0 A	8.4 B	7.9	7.3 C	5.6 D	10.7 A	9.2 B	8.2
	Day 3 leaf drop (%)					**Day 3 leaf drop (%)**				
B. cinerea:										
Yes	16.4A NS	0.0 B NS	0.0 B NS	1.3 B NS	4.4 NS	17 A NS	2 B NS	0 B NS	01 B NS	5 NS
No	15.3A	2.7 B	1.3 B	0.0 B	4.8	15 A	0 B	1 B	0 B	4
Gas:										
ET	41.4A a	6.7 B a	0.0 C NS	0.0 C NS	12.0 a	34 A a	9 B a	0 C NS	0 C NS	11 a
1-MCP	3.5 A b	0.0 A b	0.0 A	0.0 A	0.9 b	2 A b	0 A b	0 A	0 A	0 b
Charcoal	3.5 A b	0.0 A b	0.0 A	0.0 A	0.9 b	0 A b	0 A b	0 A	0 A	0 b

Table 3. Continued.

	ET and anti-ET treatments before *B. cinerea* inoculation					ET and anti-ET treatments after *B. cinerea* inoculation				
	Cultivar[z]					Cultivar[z]				
	Cuenca	Daphnee	Vendela	Freedom	All	Cuenca	Daphnee	Vendela	Freedom	All
	(B+E+)[y]	(B−E+)	(B+E−)	(B−E−)		(B+E+)[y]	(B−E+)	(B+E−)	(B−E−)	
Treatment	Day 3 leaf drop (%)					Day 3 leaf drop (%)				
Solution:										
STS	13.8A NS	0.0 B NS	0.0 B NS	0.0 B NS	3.5 b	19 A NS	0 B NS	0 B NS	0 B NS	5 NS
Water	17.2A	0.0 B	3.2 B	3.3 B	5.9 a	18 A	0 B	2 B	0 B	5
All	15.9A	1.3 B	0.6 B	0.7 B	4.6	17 A	2 B	0 B	0 B	5
	Day 5 flower openness[y]					Day 5 flower openness[y]				
B. cinerea:										
Yes	1.2 C NS	2.6 A NS	1.9 B NS	1.3 B NS	1.8 NS	1.2 C NS	2.8 A NS	1.9 B NS	0.9 D NS	1.7 NS
No	1.2 C	2.6 A	2.1 B	1.4 C	1.8	1.2 C	2.9 A	2.0 B	1.1 C	1.8
Gas:										
ET	1.4 B a	2.9 A a	1.9 B NS	1.1 B NS	1.8 NS	1.6 C a	3.0 A a	1.9 B NS	0.9 D NS	1.9 NS
1-MCP	1.2 A b	2.4 A b	2.1 A	1.4 A	1.8	1.2 C b	2.6 A b	1.8 B	1.1 C	1.7
Charcoal	1.2 B b	2.4 A b	2.2 A	1.1 B	1.7	1.0 C b	2.8 A ab	1.9 B	0.9 C	1.7
Solution:										
STS	1.2 B NS	2.5 A NS	2.2 A NS	1.5 AB NS	1.9 NS	1.0 C NS	2.9 A NS	2.1 B NS	1.0 C NS	1.8 NS
Water	1.1 B	2.7 A	2.0 AB	1.4 B	1.8	1.2 C	3.0 A	1.9 B	1.0 D	1.8
All	1.2 C	2.6 A	2.1 B	1.3 C	1.8	1.2 C	2.9 A	1.9 B	1.0 D	1.8

Whole stems were treated with 1 ppm ET or an anti-ET agent [700 $nL \cdot L^{-1}$ (0.70 ppm) 1-methylcyclopropene (1-MCP), activated charcoal in chamber, or 28.3 ppm silver thiosulfate (STS)] or were untreated for 24 h at 21 C (70 F) in darkness **before** they were sprayed to run-off with $10^5 \cdot ml^{-1}$ (0.03 fl oz) spore solution or the same solution without spores and incubated in black plastic bags for 24 h at 21 C (70 F).

In-vase observations: after inoculation stems were placed into vases containing tap water under constant 20 C (68 F) with 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (137 ft-c) fluorescent light for 12 $h \cdot d^{-1}$ at 40 – 60% relative humidity.

[z]Cultivars are identified as relatively Botrytis susceptible (B+) or not (B–) and ET sensitive (E+) or not (E–) based on our previous experiments.

[y]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; 8, 76-100% disease or collapse of flower head at receptacle.

[x]Values for cultivars within a treatment row and inoculation timing followed by the same upper case letter are not different at $P = 0.05$ according to Student's t-test.

[w]Values within a column and treatment group followed by NS or the same lower case letter are not different at $P = 0.05$ according to Student's t-test.

[v]Four-point scale: 0, tight (all petals upright, some outer petals slightly reflexed); 1, medium (most whorls beginning to reflex); 2, open (outer whorls completely reflexed, all whorls reflexing to a high degree); and 3, fully open (stamens visible).

and approximately doubled leaf Botrytis frequency across cultivars (Table 3). In all cases, the effect of ET exposure was to increase leaf Botrytis frequency (Tables 3).

That we observed increased Botrytis damage in many instances when higher levels of ET were present is not unexpected as exposure to ET can induce germination of *B. cinerea* spores (Kepczynska 1989), and increased levels of ET influences disease progression in plants infected by *B. cinerea* (Elad 1990). This influence varies with the organisms involved, timing of the exposure, and environmental conditions such that presence of ET can promote, reduce, or have no effect on disease progression in the host plant (Tudzynski and Sharon 2002). Previous research suggests that ET may directly affect both the host plant and *B. cinerea* in fungus-plant interactions (Chagué et al. 2006).

Experiment 5. No matter the time of year of the shipment, temperatures within shipping boxes during international cut rose transport followed a similar pattern that matched the stages of the shipping process. This pattern was similar to, but took less total time than, the single temperature recording published by Macnish et al. (2010). Our simulated shipping conditions (Fig. 2b) were based on the eight recorded temperature logs from actual shipments (e.g. Fig. 2a). Compared to flowers stored at constant 3.5 C (38 F) for 5 days, flowers subjected to the fluctuating temperatures of simulated shipping exhibited a shorter subsequent vase life ($P = 0.05$) in two of the four cultivars, Freedom and Idole, which also had significantly shorter vase lives ($P = 0.05$) than Cuenca and Vendela (Fig 3a). This is consistent with a previous finding that even a single, 4-h temperature spike [1 to 20 C (34 to 68 F)] can reduce vase life of Freedom (Clark and Dole 2015). Cultivars Cuenca and Vendela had greater frequency of stems terminated due to Botrytis ($P = 0.05$) when exposed to 5 d of fluctuating temperatures compared to stable 3.5 C (38 F) (Fig 3b), interesting because we had previously found those two cultivars to be relatively highly Botrytis susceptible.

Experiment 6. Compared to roses packed at typical, commercial density and placed into a 3.5 C (38 F) cooler, loosely packed flowers cooled more rapidly, particularly at the center of flower bunches (Fig 4 a and c). As expected, pre-cooling allowed flowers at both edges and centers of bunches to attain $7/8^{th}$ the target temperature [6.3 C and 3.5 C (43 and 38 F), respectively] more rapidly than the

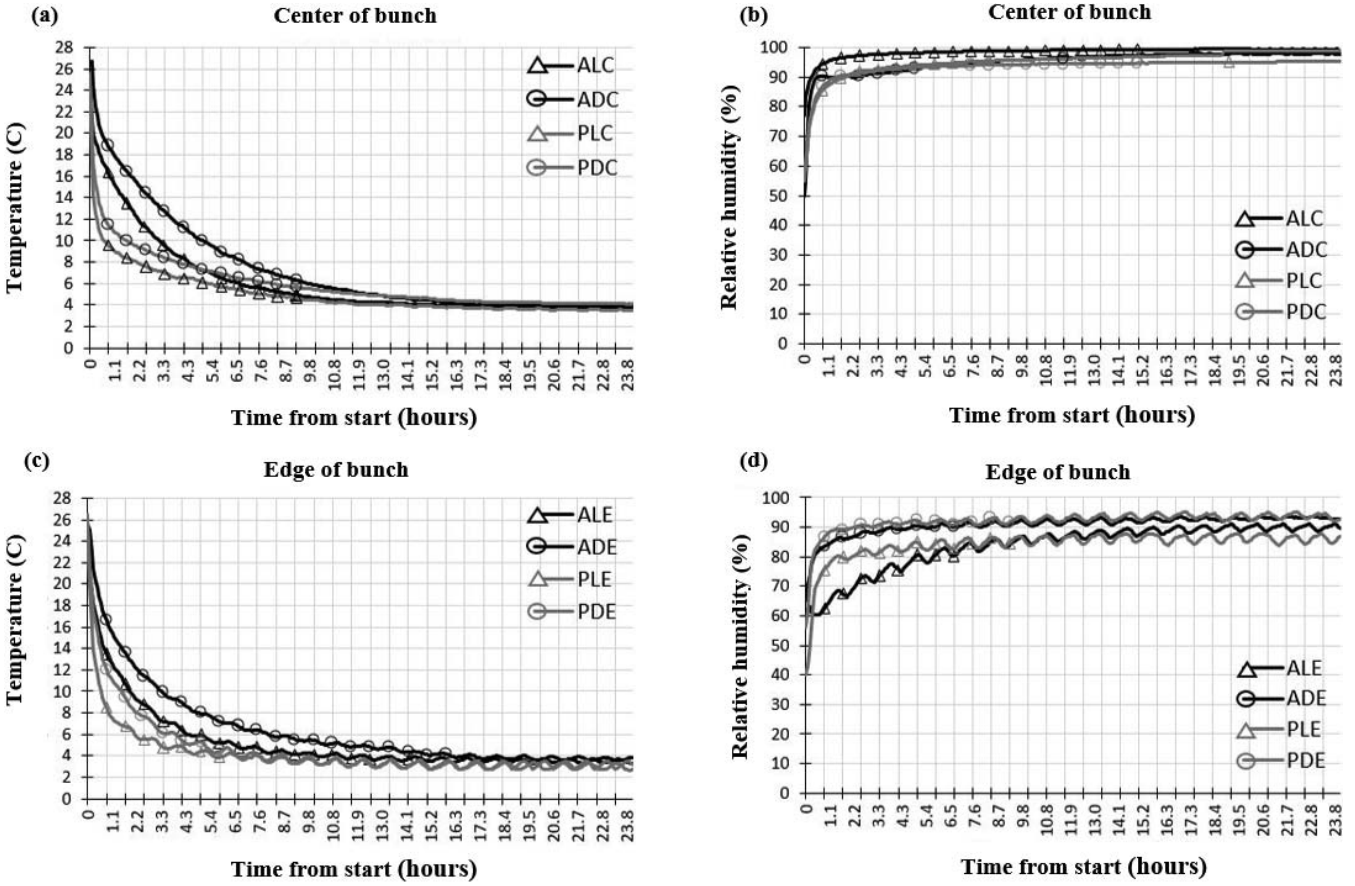

Fig. 4a-d. Temperature (a and c) and relative humidity (RH, b and d) at the center (a and b) or edge (c and d) of a bunch of roses within a standard cardboard shipping box kept in a 3.5 C (38 F) cooler for 24 h. Temperature and RH were recorded every 10 min during the 24 h, and the trial was repeated four times. Legend: box started at ambient temperature ("A") or was pre-cooled with forced 3. 5 C (38 F) air for 30 min ("P"), box was packed loosely ("L") or densely ("D").

flowers that were not pre-cooled (Fig 4 a and c). Bunch edges cooled more quickly but had more temperature fluctuation both while cooling down and once at the lowest storage temperature as compared to bunch centers (Fig 4c). Densely packed flowers remained warmer than loosely packed flowers during the cool-down process at both the center of edge of bunches (Fig 4 a and c). The RH within the center of bunches was $\geq$90% nearly the entire duration of the trial regardless of packing density or pre-cooling (Fig 4b). The RH at bunch edges was higher throughout the cooling period and duration of storage when boxes were densely packed (Fig 4d).

Because cultivars Sweet Unique and Hot Party were found to respond statistically the same to treatments, and there was no case of significant interaction between cultivar and the influences of pre-cooling or packing density, data for the two cultivars were combined in the analyses of the effects of pre-cooling and packing density on post-storage performance in vase. Following 5 d in a 3.5 C (38 F) cooler, the benefit of pre-cooling was seen in flowers once placed in vases. Compared to flowers from boxes that were at ambient temperature when placed into the 3.5 C (38 F) cooler, flowers from boxes that were pre-cooled had much longer vase life (9.5 vs 6.3 d, respectively, $P < 0.0001$), slightly less open flowers at termination (1.9 vs 2.2, respectively, $P = 0.0234$), lower ratings for flower Botrytis damage at termination (2.3 vs 4.2, respectively, $P < 0.0001$), and much lower frequency of Botrytis on leaves (15% vs 70%, respectively, $P < 0.0001$). Influence of packing density was not as pronounced with densely packed and loosely packed flowers having statistically the same flower openness (2.2 vs 2.1, respectively) and flower Botrytis damage rating at termination (3.5 vs 3.0, respectively). However, densely packed flowers had longer vase life (8.8 vs 7.0 d, respectively, $P = 0.0088$) and lower frequency of Botrytis on leaves (33% vs 52%, respectively, $P = 0.0208$) than did loosely packed flowers.

In summary, experiments with 26 cut rose cultivars showed that cultivars currently in production vary greatly in Botrytis susceptibility and ET sensitivity. Botrytis susceptibility was not predictive of ET sensitivity. Sensitivity to ET was not predictive of Botrytis susceptibility. The relationship between Botrytis susceptibility and ET sensitivity in cut roses was examined more closely in four cultivars by exposing them to ET and anti-ET agents either before or after inoculation with *B. cinerea* spores. Pre-inoculation and incubation treatments might influence infection and/or development of Botrytis while post-inoculation and incubation treatments could influence Botrytis development after infection had already occurred. Sensitivity to ET was not influenced by *B. cinerea*

inoculation in any cultivar whether ET and anti-ET treatments were applied before or after inoculation. However, Botrytis susceptibility was influenced by ET and anti-ET treatments. Exposing cut roses to ET resulted in significantly more negative Botrytis responses in many cases. This effect was observed in cultivars that we had found in the first two experiments to be relatively Botrytis susceptible or to be non-susceptible and in cultivars we had found to be relatively ET sensitive or to be insensitive. More pronounced negative Botrytis damage was observed in ET-exposed flowers whether they were Botrytis inoculated or not and whether the ET exposure was before or after *B. cinerea* inoculation and incubation.

The temperature pattern observed within cut rose shipping boxes from time of packing in Colombia, SA through unpacking in Raleigh, NC was similar during different months of the year and included two periods of greatly fluctuating temperatures. Temperature and RH were in a range conducive to Botrytis germination and development most of the time during transit. Treating cut roses with a temperature regime that mimicked transit conditions resulted in significantly reduced vase life in two of four cultivars and exacerbated Botrytis damage in two of four cultivars compared to flowers held for the same time in steady low temperature. Pre-cooling packed boxes before placement into coolers resulted in longer vase life, less rapid bud opening, less flower Botrytis damage, and lower frequency of Botrytis on leaves. Flowers that were in densely packed shipping boxes had flower openness and flower Botrytis damage rating at termination similar to those that were loosely packed, but densely packed flowers had longer vase life and lower frequency of Botrytis on leaves than loosely packed flowers.

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