

# Effect of continuous exposure to exogenous ethylene during cold storage on postharvest decay development and quality attributes of stone fruits and table grapes

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## Abstract

The influence of continuous exposure to exogenous ethylene on fruit quality and on the development of postharvest brown rot of stone fruits and gray mold of table grapes during long-term cold storage was investigated using selected cultivars of table grapes (nonclimacteric) and climacteric (peach, plum, nectarine, and apricot) and nonclimacteric (sweet cherry) stone fruits. Depending on the experiment, climacteric stone fruits were exposed to concentrations of ethylene of 0, 0.1, 1, 3, 10, or 100  $\mu\text{l l}^{-1}$  during storage at 0, 5, or 10 °C for up to 28 days; sweet cherries were exposed to 0, 0.01, 0.1, or 1  $\mu\text{l l}^{-1}$  ethylene during storage at 0 or 5 °C for 21 days; and table grapes were exposed to 0, 0.125, 0.25, 0.5, or 1  $\mu\text{l l}^{-1}$  ethylene during storage at 0 or 5 °C for up to 60 days. Neither incidence nor severity of brown rot were affected by constant ethylene exposure on stone fruits wound-inoculated with *Monilinia fructicola*. Similarly, ethylene did not affect gray mold nesting ability on table grapes artificially inoculated with *Botrytis cinerea*. Furthermore, ethylene exposure neither influenced external quality attributes (skin color on peaches and cherries, skin pitting and stem browning on cherries, and rachis browning on table grapes) nor internal quality attributes (flesh firmness, soluble solids concentration, and titratable acidity on all fruit, and flesh color and internal breakdown on climacteric stone fruits). The only exceptions were flesh softening of apricots, which in every test was significantly enhanced by exogenous ethylene, and flesh mealiness in experiments with ‘Elegant Lady’ peaches, the appearance of which was delayed by ethylene exposure in one case. In conclusion, no general commercial benefit could be expected from actively removing ethylene from cold storage rooms or transport containers containing peaches, plums, nectarines, sweet cherries, or table grapes.

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## 1. Introduction

Ethylene is a gaseous plant hormone that at very low concentrations plays a major role in the regulation of the metabolism of harvested horti-

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cultural crops. The responses of harvested fruits, vegetables, and ornamental crops to endogenously produced and exogenously applied ethylene are numerous and varied, and they can be beneficial or detrimental depending on each case (Saltveit, 1999). In general, ethylene can influence the postharvest life of both climacteric and nonclimacteric fruit by affecting their quality attributes and the development of physiological disorders and postharvest diseases (Kader, 1985). Effects of ethylene on fruit external appearance, texture, flavor, and nutritive value have been extensively reviewed (Kader, 1985; Watada, 1986; Saltveit, 1999). Frequently, the action of ethylene results in promotion of fruit softening, acceleration of deterioration, and consequent abbreviation of postharvest life. An extreme case is kiwifruit, in which even a concentration of ethylene as low as  $0.01 \mu\text{l l}^{-1}$  (parts per million) induces flesh softening, limiting long-term cold storage (Mitchell, 1990). Recently, Wills et al. (1999) reported that ethylene removal to very low levels ( $0.005 \mu\text{l l}^{-1}$ ) during storage was beneficial for a variety of nonclimacteric fruits and vegetables. They suggested that the threshold level of ethylene action on nonclimacteric produce is well below  $0.005 \mu\text{l l}^{-1}$ , lower than the actual level in most postharvest commercial situations, as indicated by ethylene surveys such as those performed by Fraser et al. (1999) or Wills et al. (2000).

The influence of ethylene on postharvest decay development has been found to be different depending on the host-pathogen system. Lack of influence and both beneficial and detrimental effects have been reported. Such effects of ethylene on disease development can be caused by a direct action of the gas to the pathogen or by an indirect action via possible modifications of the host's metabolism (Kader, 1985). For example, ethylene-induced resistance to decay was observed on oranges for green and blue molds (El-Kazzaz et al., 1983a; Porat et al., 1999) or on sweet potato against black rot (Stahmann et al., 1966). Promotion of phytoalexin and lignin biosynthesis or activation of antifungal hydrolases by ethylene are mechanisms that have been proposed as responsible for the induction of pathogen resistance in the fruit tissue (Boller, 1988; Brown and

Lee, 1993). In contrast, application of ethylene for degreening of oranges greatly enhanced the development of stem-end rot caused by *Diplodia natalensis* P. Evans (Brown and Lee, 1993; Porat et al., 1999). Ethylene was found to predispose the fruit to fungal invasion by enhancing the activity of certain abscission enzymes (Brown and Burns, 1998), but also to directly stimulate the growth of the fungus (Brown and Lee, 1993). Similarly, El-Kazzaz et al. (1983b) reported that strawberries wound-inoculated with *Botrytis cinerea* Pers.:Fr. and exposed to exogenous ethylene during cold storage developed more severe gray mold symptoms than nonexposed fruit.

Most of the research regarding the effects of ethylene on postharvest decay has been focused on diseases of nonclimacteric fruits such as citrus or strawberry, and very little work has been done with stone fruits or table grapes. Brown rot, caused by *Monilinia fructicola* (G. Wint.) Honey, and gray mold, caused by *B. cinerea*, are the most important postharvest diseases of stone fruits and table grapes, respectively, in California (Nelson, 1985; Ogawa and English, 1991). Unfortunately, there is also limited information on the effects of exogenous ethylene on the postharvest quality of stone fruits and table grapes (Brecht and Kader, 1982; Lill et al., 1989; Tonini et al., 1989). The objectives of the present work were to evaluate the influence of continuous exposure to exogenous ethylene during long-term storage at low temperature on the development of postharvest brown rot on selected cultivars of climacteric (peach, plum, nectarine, and apricot) and nonclimacteric (sweet cherry) stone fruits, and on the development of postharvest gray mold on table grapes (nonclimacteric). Effects of ethylene on external and internal fruit quality during cold storage were also assessed.

## 2. Materials and methods

The influence of exogenous ethylene exposure during cold storage on decay development and fruit quality was assessed in five peach cultivars, one plum cultivar, one nectarine cultivar, two apricot cultivars, three sweet cherry cultivars,

and three table grape cultivars. The experiments were performed during 1999, 2000 and 2001 seasons with fruit from the San Joaquin Valley (California, USA). Depending on the experiment, different ethylene concentrations and storage temperatures were tested. A summary of all experiments is presented (Table 1).

### 2.1. Fruit preparation

Peaches [*Prunus persica* (L.) Batsch.], plums (*P. salicina* Lindel), nectarines [*P. persica* (L.) Batsch. var. *nucipersica* (Suckow) Schneid], and apricots (*P. armeniaca* L.) for decay development assessment were purchased from an organic grower, selected, randomized, superficially disinfected by immersion for 1 min in diluted bleach (0.5% sodium hypochlorite), rinsed with fresh water, allowed to air dry at room temperature and used in the experiments before any postharvest treatments were applied. Fruit of these species for quality evaluations were commercially grown and handled in local packinghouses.

Mature sweet cherries (*P. avium* L.) were picked in commercial orchards, selected, randomized, and hydrocooled in chlorinated water ( $75 \mu\text{l l}^{-1}$  sodium hypochlorite) at 0.5–2 °C for about 30 min just after arrival at the laboratory. Cherries were used in the experiments immediately after hydrocooling.

Table grapes (*Vitis vinifera* L.) were picked in commercial orchards and brought to the laboratory before receiving any postharvest sulfur dioxide treatment. Individual berries were removed with the pedicels attached, selected, randomized, disinfected for 1 min in a bleach solution (0.5% sodium hypochlorite), rinsed with fresh water, and allowed to air dry at room temperature.

In all cases, initial fruit quality was determined the day of arrival as described below. Values for the most important initial maturity and quality attributes are presented (Table 2). In the case of climacteric fruit such as peaches, plums, nectarines, and apricots, fruit were harvested after physiological maturity. Thus, all fruit were able to ripen properly without the application of exogenous ethylene.

### 2.2. Fruit inoculation

Peaches, plums, nectarines, and apricots were inoculated once on the equator with 20  $\mu\text{l}$  of an aqueous suspension containing  $2 \times 10^4$  spores per ml of *M. fructicola* as described by Palou et al. (2002b). Cherries were wounded once along the suture with a needle 1 mm wide by 3 mm in length and inoculated following the same procedure. Inoculated fruit, placed on plastic cavity trays or grids in 7.8-l polypropylene containers with paper towels on the bottom, were held at room temperature in the laboratory for about 24 h before ethylene exposure. Each plastic container contained nine peaches or plums, 11 nectarines, 15 apricots or 28 cherries.

Gray mold nesting on grapes was evaluated according to our published protocol (Palou et al., 2002a). Ten microliters of a suspension of  $2 \times 10^6$  spores per ml of *B. cinerea* were injected 10 mm deep into the flesh of individual berries using a Hamilton syringe (needle of 1 mm external diameter) and incubated at 20 °C for 4 days until mycelium was visible. One of these previously inoculated berries was placed in the center of a Petri dish in contact with surrounding healthy berries. The number of berries surrounding the inoculated one was 6–8 for ‘Redglobe’ grapes and 9–11 for ‘Thompson Seedless’ and ‘Red Seedless’ grapes. Six or seven of these Petri dishes were placed in 7.8-l polypropylene containers with paper towels on the bottom.

### 2.3. Ethylene exposure

After initial fruit quality was determined, fruit for quality evaluations during storage were placed on plastic cavity trays (in cluster bags in the case of table grapes) in cardboard boxes in 338-l water-sealed aluminum tanks. After 24 h of incubation, the paper towels in the polypropylene containers containing inoculated fruit were moistened. The moist paper towels elevated RH inside the containers from 95 to 98% as monitored by chilled-mirror dew-point psychrometry (Model 1100DP, General Eastern Instruments, Woburn, MA, USA). Both aluminum tanks and plastic containers were connected to an ethylene flow-through system that

Table 1  
Summary of experiments and results

Specie	Cultivar	Season	EC <sup>a</sup> ( $\mu\text{l l}^{-1}$ )	ST <sup>b</sup> ( $^{\circ}\text{C}$ )	Evaluations <sup>c</sup>	Results <sup>d</sup>		
Peach	Elegant Lady	1999	0, 3	0, 5	BRI, BRS, SKC, FC, FF, SSC, TA, FBR, FBL FM	ns (dns) * (Fig. 4A)		
		2000	0, 1	5, 10	BRI, BRS	ns (Fig. 1A)		
			10, 100		FF, SSC, TA, FBR, FBL FM	ns (dns) ns (Fig. 4B)		
	2001	0, 0.1, 1	5	BRI, BRS, FF, SSC, TA, FBR, FBL FM	ns (dns) ns (Fig. 4C)			
	O'Henry	1999	0, 3	0, 5	BRI, BRS, SKC, FC, FF, SSC, TA, FM, FBR, FBL	ns (dns)		
		Fairtime	1999	0, 3	0, 5	SKC, FC, FF, FM, FBR, FBL, SSC, TA	ns (dns)	
			Autumn Flame	1999	0, 3	0, 5	SKC, FC, FF, SSC, TA, FM, FBR, FBL	ns (dns)
			Autumn Rose	1999	0, 3	0, 5	BRI, BRS	ns (dns)
		Fortune	2000	0, 1	5, 10	BRI, BRS	ns (Fig. 1B)	
2001			10, 100		5	FF, SSC, TA, FM, FBR, FBL	ns (dns)	
	Nectarine	August Red	2000	0, 1	5, 10	BRI, BRS, FF, SSC, TA, FM, FBR, FBL	ns (dns) ns (Fig. 1C)	
10, 100					FF, SSC, TA, FM, FBR, FBL	ns (dns)		
Apricot	Patterson	2000	0, 1, 10, 100	5	BRI, BRS, SSC, TA, FM, FBR, FBL FF	ns (dns) * (Fig. 3A)		
		2001	0, 1	5	BRI, BRS, SSC, TA, FM, FBR, FBL FF	ns (dns) * (Fig. 3C)		
	Castlebrite	2000	0, 1, 10, 100	5	BRI, BRS, SSC, TA, FM, FBR, FBL FF	ns (dns) * (Fig. 3B)		
					BRI, BRS, SKP, SB, FF, SSC, TA SKC	ns (dns) * (Fig. 2)		
	Cherry	Brooks	2001	0, 0.01, 0.1, 1	0, 5	BRI, BRS, SKP, SB, FF, SSC, TA SKC	ns (dns) * (Fig. 2)	
Tulare		2001	0, 0.01	0, 5	SKP, SB, FF, SSC, TA	ns (dns)		
			0.1, 1		SKC	ns (Fig. 2)		
Table grape	Red Seedless	1999	0, 0.125, 0.25, 0.5, 1	0	GMN	ns (dns)		
			0, 1	5	GMN, RB, FF, SSC, TA	ns (dns)		
	Thompson Seedless	2000	0, 1	5	GMN, RB, FF, SSC, TA	ns (dns)		
							0, 1	5

<sup>a</sup> Exogenous ethylene concentration.

<sup>b</sup> Storage temperature.

<sup>c</sup> Nomenclature for decay evaluation: BRI, brown rot incidence; BRS, brown rot severity; and GMN, gray mold nesting; nomenclature for external quality evaluations: SKC, skin color; SKP, skin pitting; SB, stem browning; and RB, rachis browning; nomenclature for internal quality evaluations: FC, flesh color; FF, flesh firmness; SSC, soluble solids content; and TA, titratable acidity; nomenclature for internal breakdown evaluation: FM, flesh mealiness; FBR, flesh browning; and FBL, flesh bleeding.

<sup>d</sup> \*, Significant differences between ethylene concentrations at least at one of the storage temperatures according to Fisher's Protected LSD test ( $P = 0.05$ ); ns, no significant differences; dns, data not shown.

had been set up inside standard cold storage rooms at each desired temperature.

Flow rates and mixtures of compressed air and ethylene were established using a main mixing board and secondary flowboards with micrometering needle valves. Total flow rates were adjusted

to get an adequate gas exchange rate that prevented either respiratory gas accumulation or excessive gas flow in tanks or containers. Flow rates were measured with a digital flowmeter (Model ADM-1000, J&W Scientific, Folsom, CA, USA). Temperatures (0, 5, or 10  $^{\circ}\text{C}$ ) and

ethylene concentrations (ranging from 0.01 to 100  $\mu\text{l l}^{-1}$ ) tested in each experiment depended particularly on the physiological type of fruit (Table 1). In general, to simulate a broad range of possible commercial storage conditions, climacteric fruits (peach, plum, nectarine, and apricot) were exposed to 0.1, 1, 3, 10 or 100  $\mu\text{l l}^{-1}$  ethylene depending on the experiment, whereas nonclimacteric fruits (cherry, grape), which produce only small amounts of endogenous ethylene, were tested at concentrations up to 1  $\mu\text{l l}^{-1}$  ethylene. Ethylene-free air for control fruit (exogenous ethylene concentration  $\approx 0$ ) was ethylene-filtered by circulating compressed air through two jars containing fresh potassium permanganate pellets. The atmosphere of tanks and containers containing control fruit was kept free of endogenous ethylene by adjusting the flow of air that circulated through. Storage periods depended on storage temperature and commodity

and ranged from 14 to 28 days for stone fruits and from 28 to 60 days for table grapes. One aluminum tank and three polypropylene containers (replicates) were used for each ethylene treatment. Supply and exhaust ethylene concentrations were periodically monitored with a gas chromatograph equipped with a flame ionization detector (Carle AGC-211, EG&G Chandler Engineering, Tulsa, OK, USA).

#### 2.4. Decay development assessment

Brown rot incidence was determined weekly by counting the number of decayed fruit in each plastic container. Brown rot severity was assessed in each decayed fruit as lesion diameter. To evaluate gray mold nesting on grapes, the number of infected berries surrounding the central inoculated berry was counted weekly or monthly. A

Table 2  
Initial maturity and quality attributes

Specie	Cultivar	Season	Skin color			F (N) <sup>a</sup>	SSC (%) <sup>b</sup>	TA (%) <sup>c</sup>	
			L*	C*	h°				
Peach	Elegant Lady	1999	BC 33.7, GC 58.7 <sup>d</sup>	28.8, 42.8	25.1, 95.3	62.3	9.1	0.63	
		2000	– <sup>e</sup>	–	–	57.8	9.8	0.74	
		2001	–	–	–	54.3	11.9	0.72	
	O'Henry	1999	BC 37.5, GC 66.8	32.4, 48.9	29.1, 83.0	56.9	10.2	0.66	
		Fairtime	1999	BC 40.3, GC 75.2	24.8, 51.5	30.5, 90.5	41.4	9.4	0.57
		Autumn Flame	1999	BC 38.3, GC 66.6	21.4, 46.5	27.0, 82.6	49.8	12.4	0.59
Plum	Fortune	2000	–	–	–	35.1	12.9	0.62	
		2001	–	–	–	29.4	12.5	0.46	
Nectarine	August Red	2000	–	–	–	56.5	12.6	1.04	
Apricot	Patterson	2000	–	–	–	30.2	12.1	0.98	
		2001	–	–	–	21.8	12.4	1.06	
	Castlebrite	2000	–	–	–	32.9	11.0	1.56	
Cherry	Brooks	2001	30.3	32.7	24.9	1.01	17.4	1.03	
	Tulare	2001	30.4	34.8	25.3	1.76	19.4	0.82	
	Bing	2001	33.8	35.0	24.8	2.17	19.6	1.08	
Table grape	Thompson Seedless	2000	–	–	–	1.14	20.3	0.45	
	Redglobe	2000	–	–	–	2.65	18.3	0.33	

<sup>a</sup> Cheek firmness determined with a UC firmness tester with a 7.9-mm tip on peaches, plums, nectarines, and apricots, and a 3-mm tip on cherries and table grapes.

<sup>b</sup> Soluble solids concentration.

<sup>c</sup> Titratable acidity determined as percent of malic acid on stone fruits and percent of tartaric acid on table grapes.

<sup>d</sup> Blush color and ground color.

<sup>e</sup> –, No data.

berry was considered infected when mycelium of *B. cinerea* had contacted it.

### 2.5. Fruit quality evaluations

Skin (blush and ground) and flesh colors of peaches were measured weekly with a colorimeter (Model CR-200, Minolta USA Co., Ramsey, NJ, USA) in four replicates of ten fruit each per treatment and evaluation date. Cherry skin color was measured in five replicates of ten fruit each using a spectrophotometer (Model CM-2002, Minolta USA Co.) attached to a personal computer. Color was assessed according to the Commission Internationale de l'Eclairage (CIE) and described as the three independent attributes of hue angle ( $h^\circ$ ), lightness ( $L^*$ ), and chroma ( $C^*$ , saturation). Cherry skin pitting was determined as number of pits on the fruit surface. Cherry stem browning was evaluated as percentage of the stem not showing green color. Five replicates of ten fruit each were used. Rachis and stem condition on table grapes was assessed as a qualitative score in which (1) healthy; (2) slight browning of capstems; (3) browning of capstems and lateral stems, and (4) severe browning of capstems, lateral stems, and main rachis. Three clusters per treatment and evaluation date were examined.

Flesh firmness, soluble solids concentration, and titratable acidity were determined weekly on all fruit according to Crisosto et al. (1993). A 7.9-mm tip in the U.C. Firmness tester was used for peaches, plums, nectarines, and apricots. A 3-mm tip was used for cherries and table grapes. Acidity of stone fruits and table grapes was reported as percent malic and tartaric acid, respectively. For each ethylene treatment and evaluation date, three replicates of five fruit each were used for peaches, plums, and nectarines, three replicates of ten fruit each were used for apricots and table grapes, and five replicates of ten fruit each were used for cherries.

### 2.6. Internal breakdown

Symptoms of internal breakdown (chilling injury) on peaches, plums, and nectarines were evaluated weekly as flesh mealiness (woolliness),

browning, and bleeding following the protocol described by Crisosto et al. (1999). For each species, treatment, storage temperature, and evaluation date three replicates of five fruit each were used.

### 2.7. Statistical analyses

Data were subjected to analysis of variance using SAS software (SAS Institute Inc., Cary, NC, USA). Data on incidence of decay and internal breakdown were arcsine transformed. When appropriate, means were separated by Fisher's Protected LSD test ( $P = 0.05$ ).

## 3. Results

Results of all experiments are summarized in Table 1. Specification of shown and not shown data is included.

### 3.1. Decay development

Brown rot incidence and severity were not significantly different on either 'Elegant Lady' peaches, 'Fortune' plums or 'August Red' nectarines continuously exposed to 0 (control), 1, 10, or 100  $\mu\text{l l}^{-1}$  exogenous ethylene at 5 or 10 °C for 7, 14, 21, or 28 days of storage (Fig. 1). Likewise, for 'Elegant Lady', 'O'Henry', and 'Autumn Rose' peaches, there were no significant differences in either disease incidence or severity between control fruit and fruit exposed to 3  $\mu\text{l l}^{-1}$  ethylene at 5 °C for 7, 14, 21, or 28 days (data not shown). Moreover, differences were not significant when inoculated 'Elegant Lady' peaches and 'Fortune' plums were exposed to 0 (control), 0.1, or 1  $\mu\text{l l}^{-1}$  ethylene at 5 °C for 7, 14, 21, or 28 days (data not shown).

Similar results regarding brown rot incidence and severity were obtained with artificially inoculated 'Patterson' and 'Castlebrite' apricots exposed to 0 (control), 1, 10, or 100  $\mu\text{l l}^{-1}$  exogenous ethylene for 7 or 14 days of storage at 5 °C (data not shown).

Continuous exposure to exogenous ethylene at low concentrations (0.01, 0.1, or 1  $\mu\text{l l}^{-1}$ ) during a

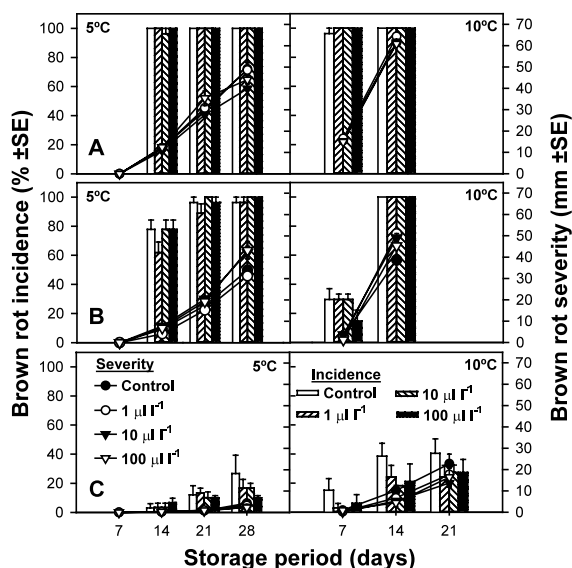


Fig. 1. Brown rot incidence (bars) and severity (lines) on 'Elegant Lady' peaches (A), 'Fortune' plums (B), and 'August Red' nectarines (C) wounded inoculated with *M. fructicola* and continuously exposed to 0 (control), 1, 10, or 100  $\mu\text{l l}^{-1}$  exogenous ethylene during storage at 5 or 10 °C.

21-day cold storage period at 5 °C did not significantly influence incidence or severity of brown rot on wound inoculated 'Brooks' cherries (data not shown).

Gray mold development on 'Thompson Seedless' and 'Redglobe' table grapes, as indicated by the number of infected berries surrounding an inoculated berry, was not significantly different when the fruit were continuously exposed to 0 (control) or 1  $\mu\text{l l}^{-1}$  ethylene during storage at 5 °C for up to 35 days (data not shown). Furthermore, no significant differences between ethylene concentrations of 0 (control), 0.125, 0.25, 0.5, and 1  $\mu\text{l l}^{-1}$  were found for gray mold nesting ability on 'Red Seedless' table grapes after 60 days of storage at 0 °C (data not shown).

### 3.2. External quality

Skin color (red and ground colors) of 'Elegant Lady', 'O'Henry', 'Fairtime', and 'Autumn Flame'

peaches was not significantly influenced by continuous exposure to 3  $\mu\text{l l}^{-1}$  ethylene during storage at 0 or 5 °C for 7, 14, 21, and 28 days (data not shown).

After 21 days of storage at 5 °C, skin color attributes ( $h^\circ$ ,  $L^*$ ,  $C$ ) of cherry cultivars 'Brooks' and 'Bing', but not 'Tulare', were significantly lower (the color was darker and less red mahogany saturated) on fruit continuously exposed to 0.01, 0.1, or 1  $\mu\text{l l}^{-1}$  ethylene than on control fruit. In contrast, no significant differences were found after storage at 0 °C (Fig. 2). Exposure to these ethylene concentrations did not affect skin pitting and stem browning on any of the tested cultivars during storage at 0 or 5 °C (data not shown).

Continuous exposure to 1  $\mu\text{l l}^{-1}$  ethylene during a 35-day cold storage period at 5 °C did not significantly influence the incidence of rachis and stem browning on 'Thompson Seedless' and 'Redglobe' table grapes (data not shown).

### 3.3. Internal quality

Continuous exposure to exogenous ethylene at 0.1, 1, 3, 10, or 100  $\mu\text{l l}^{-1}$  during storage at 0, 5, or 10 °C for 7, 14, 21, or 28 days did not affect flesh firmness, SSC and TA on 'Elegant Lady', 'O'Henry', 'Fairtime', and 'Autumn Flame' peaches, 'Fortune' plums, and 'August Red' nectarines. Similarly, flesh color of 'Elegant Lady', 'O'Henry', 'Fairtime', and 'Autumn Flame' peaches was not influenced by exposure to 3  $\mu\text{l l}^{-1}$  ethylene during up to 28 days of storage at 0 or 5 °C (data not shown).

However, 'Patterson' (Fig. 3A) and 'Castlebrite' (Fig. 3B) apricots exposed to 1, 10, or 100  $\mu\text{l l}^{-1}$  exogenous ethylene were significantly softer than control apricots after 7 and 14 days of storage at 5 °C. Data on cheek firmness are presented but significant differences were also found for firmness on fruit tip, suture, and shoulder. Likewise, in 2001 trials, 'Patterson' apricots exposed to 1  $\mu\text{l l}^{-1}$  ethylene during a 14-day storage period at 5 °C became softer than control fruit (Fig. 3C). No significant differences were observed for SSC and TA between control and ethylene-treated apricots (data not shown).

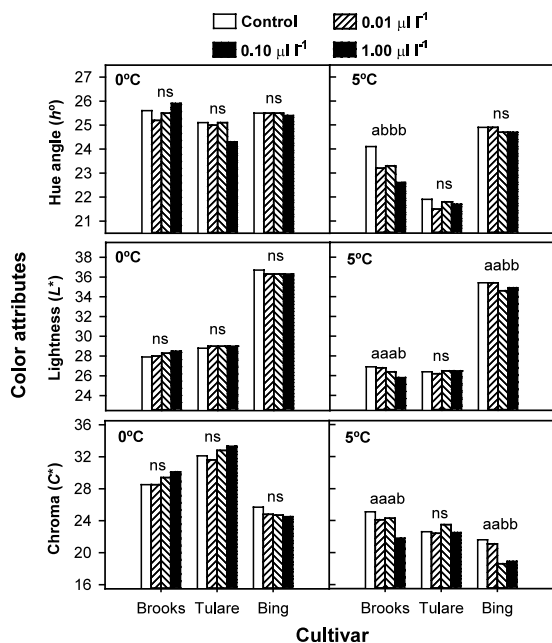


Fig. 2. Skin color attributes on 'Brooks', 'Tulare', and 'Bing' cherries continuously exposed to 0 (control), 0.01, 0.1, or 1 μl l<sup>-1</sup> exogenous ethylene during 21 days of storage at 0 or 5 °C. Different letters and 'ns' indicate significant and no significant differences between treatments, respectively, according to Fisher's Protected LSD test ( $P = 0.05$ ).

No significant differences in firmness, SSC, and TA between ethylene concentrations of 0 (control), 0.01, 0.1, and 1 μl l<sup>-1</sup> were found for 'Brooks', 'Tulare', and 'Bing' cherries stored at 0 or 5 °C for 21 days (data not shown).

Similarly, storage of 'Thompson Seedless' and 'Redglobe' table grapes at 5 °C for up to 35 days under a continuous flow of 1 μl l<sup>-1</sup> ethylene did not affect flesh firmness, SSC, and TA (data not shown).

### 3.4. Internal breakdown

In the 1999 season, development of visual mealiness symptoms was significantly delayed on 'Elegant Lady' peaches stored with 3 μl l<sup>-1</sup> exogenous ethylene compared with control fruit. After 14 days of storage, while mealiness incidence for ethylene-exposed fruit was 13%, it was 60% for control fruit. After 21 days of storage, however, the incidence was 76 and 87%, respectively, and

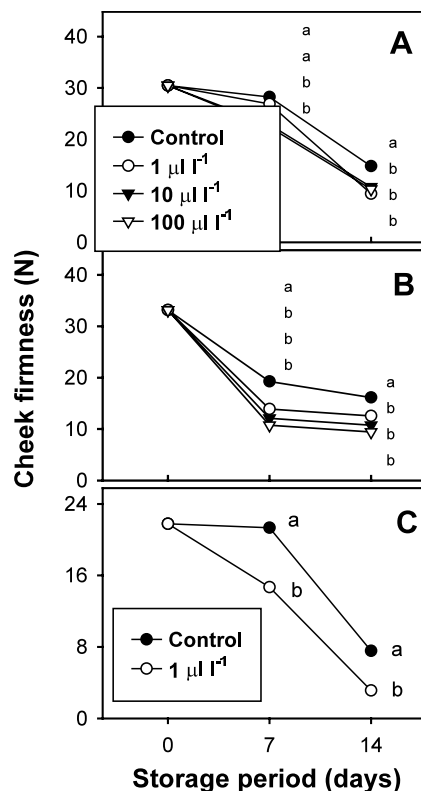


Fig. 3. Flesh softening on 'Patterson' (A and C) and 'Castlebrite' (B) apricots continuously exposed to 0 (control), 1, 10, or 100 μl l<sup>-1</sup> (A and B), or 0 (control) or 1 μl l<sup>-1</sup> (C) exogenous ethylene during storage at 5 °C. Different letters indicate significant differences between treatments according to Fisher's Protected LSD test ( $P = 0.05$ ).

the difference was not statistically significant (Fig. 4A). In contrast, continuous ethylene exposure at 3 μl l<sup>-1</sup> did not affect the proportion of mealy fruit for 'O'Henry', 'Fairtime', and 'Autumn Flame' peaches stored either at 0 or 5 °C (data not shown).

In 2000 trials, no significant differences in flesh mealiness between ethylene concentrations of 0 (control), 1, 10, or 100 μl l<sup>-1</sup> were found during cold storage at 5 °C for 'Elegant Lady' peaches (Fig. 4B), 'August Red' nectarines, 'Fortune' plums, and 'Patterson' and 'Castlebrite' apricots (data not shown). For 'Elegant Lady' peach, the



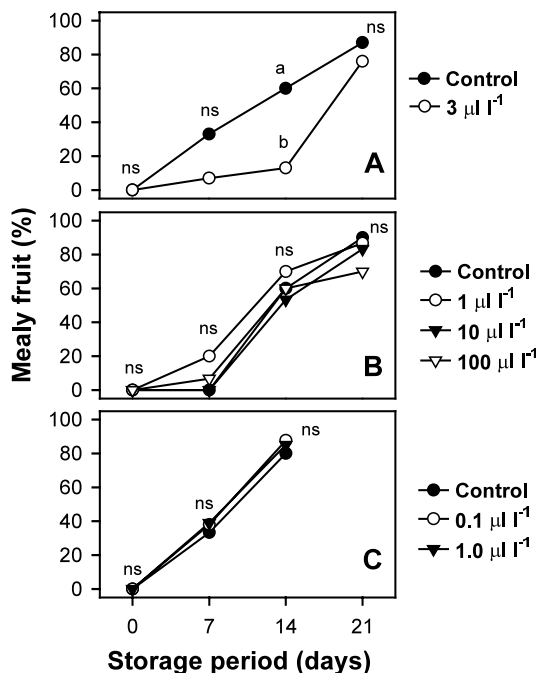


Fig. 4. Incidence of internal breakdown on 'Elegant Lady' peaches continuously exposed to different concentrations of exogenous ethylene during storage at 0 °C (A) or 5 °C (B and C). Different letters and 'ns' indicate significant and no significant differences between treatments, respectively, according to Fisher's Protected LSD test ( $P = 0.05$ ).

proportion of mealy fruit was 50–75% and 60–90% after 21 and 28 days of storage at 5 °C, respectively (Fig. 4B). Furthermore, in the 2001 season, mealiness incidence on 'Elegant Lady' peaches was not different on fruit exposed to 0 (control), 0.1, or 1 µl l<sup>-1</sup> ethylene. In this experiment, the percentage of mealy fruit ranged from 80 to 88% after 14 days of storage at 5 °C (Fig. 4C). Continuous exposure to 1 µl l<sup>-1</sup> ethylene during a 14-day cold storage period at 5 °C did not significantly affect mealiness incidence on 'Patterson' apricots (data not shown).

In every test, the presence or absence of exogenous ethylene did not affect the incidence of flesh browning and bleeding symptoms (data not shown).

#### 4. Discussion

The repeated lack of influence of continuously applied exogenous ethylene on both incidence and severity of brown rot during cold storage observed in our trials suggests that the development of latent or recent infections of *M. fructicola* on stone fruits would not be affected by the presence of ethylene, even at high concentrations. Under our experimental conditions (continuous ethylene exposure at 0, 5, or 10 °C), the gas apparently neither directly stimulated growth in vivo of *M. fructicola* nor induced in the fruit significant mechanisms of resistance against the pathogen. In tests in vitro, while germination and germ tube elongation of spores of *M. fructicola* were slightly stimulated by exposure to exogenous ethylene (1 or 10 µl l<sup>-1</sup>), the treatments had no influence on growth rate of the fungus on PDA at 20 °C (El-Kazzaz et al., 1983c).

It is known that, as one form of stress-induced ethylene production, diseased plant tissues evolve more ethylene than healthy ones (Archer and Hislop, 1975). Different postharvest pathogens, for instance *P. digitatum* (Chalutz et al., 1977) or *B. cinerea* (Qadir et al., 1997), produce ethylene when they are grown in vitro in the presence of the ethylene precursor methionine. Since methionine is the common precursor of ethylene in higher plants, it is difficult to determine if the ethylene produced in an infected fruit region is of fungal or plant origin. This is even more difficult in the case of wound pathogens because wounded fruit tissue (not infected) produces considerably more ethylene than healthy tissue (Archer and Hislop, 1975). However, considerable efforts have been made to elucidate the origin and the role of ethylene in fruits or vegetables infected by postharvest pathogens such as *P. digitatum* (Chalutz, 1979; Achilea et al., 1985), *B. cinerea* (Niklis et al., 1997; Qadir et al., 1997), or *C. fimbriata* (Stahmann et al., 1966; Okumura et al., 1999). Although we did not study the biosynthesis of ethylene in stone fruits infected by *M. fructicola*, and further specific research should be pursued to elucidate the origin and role of endogenous ethylene on the development of postharvest brown rot on climacteric and non-climacteric stone fruits, our results suggest that

ethylene plays no role in the pathogenicity of *M. fructicola*. Measurements of outlet concentrations of ethylene from the containers with control fruit (not exposed to exogenous ethylene) indicated that peaches, plums, nectarines, apricots, and cherries infected by *M. fructicola* produced variable amounts of ethylene, and our goal was to maintain an ethylene-free atmosphere surrounding the fruit. This was accomplished by adjusting the air flow through the containers. Since no significant differences in brown rot incidence and severity were found between control and ethylene-exposed fruit, ethylene, independent of its origin, may not affect the pathogenicity of the fungus. In addition, the amounts of exogenous ethylene applied to either climacteric or nonclimacteric stone fruits were, in each case, high enough to assume that disease development would not also be affected by a peak of naturally produced endogenous ethylene. Similarly, Chalutz (1979) concluded that, although ethylene is normally produced at high rates in citrus fruit infected by *P. digitatum*, the gas has no role in the pathogenicity of the fungus because disease severity and appearance do not differ when ethylene is present or absent in and around the infected fruit.

In contrast to *M. fructicola*, some information is available concerning production of ethylene by *B. cinerea* or *B. cinerea*-infected plant tissue and the effects of the gas on growth of *B. cinerea* or development of gray mold. *B. cinerea* has the capacity to produce ethylene in vitro (Qadir et al., 1997) and diseased fruit evolve considerably more ethylene than healthy or wounded fruit (Niklis et al., 1997). Ethylene not only might be required for development of the fungus but also may stimulate fungal growth directly (El-Kazzaz et al., 1983b,c). In our tests, regardless, gray mold nesting on table grapes was not significantly affected by continuous exposure to ethylene levels of  $1 \mu\text{l l}^{-1}$  or lower. However, we did not measure gray mold development in the fruit (disease severity) but aerial mycelial growth as percentage of infected berries surrounding a central inoculated berry. Moreover, ethylene concentrations and storage temperature were lower in our tests than in those of other workers.

With the exception of skin color of ‘Brooks’ and ‘Bing’ cherries stored at  $5^\circ\text{C}$ , exogenous ethylene did not influence any of the parameters of fruit external quality that were tested. Nevertheless, visual differences in cherry skin color, although statistically significant, were not important enough to have a commercial impact. While chlorophyll degradation is clearly regulated by ethylene, it appears that synthesis of pigments, in both climacteric and nonclimacteric fruit, can be either ethylene-dependent or independent depending on the type of pigments involved and the fruit species and cultivars (Lelièvre et al., 1997).

In general, internal quality attributes of grapes and climacteric and nonclimacteric stone fruits were not affected by exogenous ethylene. Only in apricots was fruit softening significantly enhanced by ethylene exposure. This was observed in 2000 trials with both ‘Patterson’ and ‘Castlebrite’ apricots, and confirmed in 2001 trials with ‘Patterson’ apricots that were initially softer than those used in the 2000 season (Table 2, Fig. 3). In agreement with our results, Brecht et al. (1982) reported that a treatment with  $100 \mu\text{l l}^{-1}$  ethylene for 48 h at  $20^\circ\text{C}$  accelerated softening of apricots. In contrast, softening and skin color of nectarines were not affected by exposure to  $100 \mu\text{l l}^{-1}$  exogenous ethylene during 4 weeks of storage at 0 or  $10^\circ\text{C}$  (Brecht and Kader, 1982). Likewise, storage of nectarines at  $0^\circ\text{C}$  for 4 weeks with  $15 \mu\text{l l}^{-1}$  ethylene did not modify fruit firmness significantly (Dong et al., 2001). Moreover, Tonini et al. (1989) observed no beneficial effects from ethylene removal on quality of nectarines stored in controlled atmosphere. In general, climacteric stone fruits are considered relatively insensitive to exogenous ethylene application (Lill et al., 1989). Regarding nonclimacteric commodities, our results with cherries and grapes are similar to those of Siriphanich (1980) with strawberries, who found no effect of ethylene at 1, 10 or  $100 \mu\text{l l}^{-1}$  on fruit quality. Porat et al. (1999) reported that weight loss and internal quality of oranges during cold storage at  $2^\circ\text{C}$  for 8 weeks were not affected by a previous 60 h ethylene treatment with  $10 \mu\text{l l}^{-1}$ . Further, El-Kazzaz et al. (1983a) concluded that SSC, TA, pH, and total phenolics content of oranges were not modified by exposure to an ethylene concen-

tration as high as  $1000 \mu\text{l l}^{-1}$ , for 2–6 days at  $20^\circ\text{C}$ . In contrast, across 23 kinds of nonclimacteric produce, including oranges, strawberries, green beans, lettuce, Chinese cabbage, and other vegetables, Wills et al. (1999) found about a 60% extension in postharvest life when produce were stored in less than  $0.005 \mu\text{l l}^{-1}$  ethylene compared with  $0.1 \mu\text{l l}^{-1}$ . The presence of ethylene during cold storage at either low (Wills and Kim, 1995) or high levels (El-Kazzaz et al., 1983b) enhanced softening of strawberries, but did not affect juice SSC and TA.

In most cases, application of exogenous ethylene at different concentrations did not influence the incidence of internal breakdown on tested cultivars of peach, nectarine, plum, and apricot. However, in one experiment (1999) the percentage of mealy fruit was significantly delayed on ‘Elegant Lady’ peaches stored at  $0^\circ\text{C}$  with  $3 \mu\text{l l}^{-1}$  ethylene (Fig. 4A). The lower initial maturity of these fruit compared with that of ‘Elegant Lady’ peaches used in 2000 and 2001 trials, could explain this result (Table 2). Furthermore, we previously observed (Crisosto et al., 2001) that when flesh mealiness of ‘Elegant Lady’ peaches stored at 0 or  $5^\circ\text{C}$  was assessed as percentage of free juice according to the method described by Crisosto and Labavitch (2002), it was significantly lower (higher free juice content) in fruit continuously exposed to  $3 \mu\text{l l}^{-1}$  ethylene than in control fruit. A beneficial effect from ethylene application during cold storage has also been reported for nectarines (Dong et al., 2001). They indicated that exposure to exogenous ethylene during storage increased ethylene production after storage, thereby promoting the sequence of cell hydrolysis necessary for normal ripening. Detailed work in this subject is currently conducted by our group.

In conclusion, the lack of response to exogenous ethylene at ranges from  $0.1$  to  $100 \mu\text{l l}^{-1}$  for peach, plum, and nectarine, and from  $0.01$  to  $1.00 \mu\text{l l}^{-1}$  for cherry and table grape suggests that no significant reduction of decay losses nor extension of postharvest life would be accomplished by actively removing ethylene from stone fruit and table grape cold storage rooms. Nevertheless, since ethylene enhanced apricot softening, evaluation of commercial techniques devoted to create an ethyl-

ene-free environment during apricot postharvest storage or transportation should be pursued.

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