Effect of ozone on qualities of fruits and vegetables in cold storage

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Skog, L. J. and Chu, C. L. 2001. **Effect of ozone on qualities of fruits and vegetables in cold storage.** Can. J. Plant Sci. **81**: 773–778. Ozone at the concentration of 0.04 μL L^{-1} appears to have potential for extending the storage life of broccoli and seedless cucumbers stored at 3°C. Response to ozone was minimal for mushrooms stored at 4°C and cucumbers stored at 10°C. Ozone generators producing 0.04 μL L^{-1} ozone reduced the ethylene level in vegetable storage rooms from 1.5–2 μL L^{-1} (as produced by apples placed in the room) to a non-detectable level. At concentrations of 0.4 μL L^{-1}, ozone was effective in removing ethylene from the atmosphere in an apple and pear storage room. The ozonized and non-ozonized apples and pears showed no difference in fruit quality. This study explored a potential use of ozone application in wholesale storage rooms where ethylene-producing and ethylene-sensitive fruits and vegetables may be stored together.

Key words: Ozone, ethylene, fruit, vegetable, apple, pear, broccoli, cucumber, mushroom, storage

Skog, L. J. et Chu, C. L. 2001. **Incidence de l'ozone sur les propriétés des fruits et des légumes réfrigérés**. Can. J. Plant Sci. **81**: 773–778. À la concentration de 0,04 μL L^{-1}, l'ozone semble prolonger la durée d'entreposage du brocoli et des concombres sans graines conservés à 3 °C. Les champignons stockés à une température de 4 °C et les concombres gardés à la température de 10 °C y réagissent toutefois de façon minime. Les génératrices qui produisent 0,04 μL L^{-1} d'ozone diminuent la concentration d'éthylène dans les entrepôts de 1.5–2 μL L^{-1} (quantité libérée par les pommes placées dans l'entrepôt) à une quantité indécelable. À la concentration de 0,04 μL L^{-1}, l'ozone détruit l'éthylène présent dans l'air d'un entrepôt servant à stocker des pommes et des poires. Les pommes et les poires traitées ou non à l'ozone ne présentaient aucune variation sur le plan de la qualité. L'étude portait sur l'utilisation potentielle de l'ozone dans les entrepôts de gros où l'on stocke des fruits et des légumes produisant de l'ozone avec d'autres n'en libérant pas.

Mots clés: Ozone, éthylène, fruit, légume, pomme, poire, brocoli, concombre, champignon, stockage

Fruits and vegetables are often shipped or stored with ethylene-sensitive and ethylene-producing commodities together, possibly at less than ideal temperatures for the specific commodity. Ethylene damage and increased decay may result. By reducing the level of ethylene, we may expect to keep some ethylene-sensitive fruits and vegetables better. Ozone generators are increasingly being marketed in the industry as a solution to this problem with a lack of scientific evidence to support their claims.

Ozone is a powerful oxidizing agent with a pleasant odor in very dilute concentrations (< 2 μL L^{-1}), and has a harmless decomposition product, oxygen. It is widely used as a deodorizing agent because of its ability to oxidize many objectionable odors and gases into non-objectionable products. Ozone is unstable and decomposes rapidly. Ewell (1936) found that ozone concentrations in an apple storage room decomposed from 1.0 μL L^{-1} to 0.2 μL L^{-1} in 0.5 h. Ozone is irritating and injurious in higher concentrations. Ozone has been recommended in the horticulture industry for both ethylene removal and antimicrobial purposes.

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A number of investigators (Ewell 1936; Kessler 1936; Smock and Watson 1941; Rice et al. 1982; Liew and Prange 1994; Sarig et al. 1996) have recommended the use of ozone to reduce produce decay and extend the storage period. Others (Baker 1933; Schomer and McColloch 1948; Spalding 1968; Perez et al. 1999), however, have reported that ozone has little or no effect on fruit or vegetable decay. Ozone also has potential use as an insecticide, especially for stored-food products (Erdman 1980; Kiss and Law 1991). Ozone cannot control superficial scald in apples, although the development of scald can be reduced (Schomer and McColloch 1948).

Ozone treatment may affect fruit quality. Perez et al. (1999) reported that at the end of 3 d of cold storage, the vitamin C content of ozonized (0.35 μL L^{-1}) strawberries was three times that of control fruit. A detrimental effect of ozone treatment on strawberry aroma was observed, with a 40% reduction in emissions of volatile esters in ozonized fruits. Kute et al. (1995) reported that 0.3 or 0.7 μL L^{-1} of ozone did not affect the ascorbic acid levels in strawberry fruit after 1 wk of treatment and storage. The total soluble solids levels steadily increased in ozonized fruit, reaching significantly higher levels than in controls by the end of the week.

The objective of this study was to determine the effectiveness of ozone in preventing ethylene-mediated deterio-

ration and postharvest decay in both ethylene-sensitive and ethylene-producing commodities, when stored at close to ideal and less than ideal temperatures. As mushrooms have no known site of ethylene activity (Abeles 1984), effects from ozone would be antimicrobial only.

MATERIALS AND METHODS

General

In all vegetable trials, treatments were duplicated by using two ozonized rooms and two non-ozonized rooms. Three subsamples were conducted at different locations within each room. All storage rooms were 1.86 m in width, 2.05 m in length, and 2.76 m in height. All vegetables, except mushrooms, were imported off-season by a produce distributor. The age of the produce and cultivar were therefore unknown. All produce except for mushrooms, were stored in vented plastic bins. The relative humidity was 95–98% in all storage trials.

For the vegetable trials, ozone was generated with a prototype ozone generator equipped with a feedback sensor (Holistech, Toronto, ON, Canada). Ozone concentration was confirmed with an external ozone analyzer (Dasibi Model 1008, Glendale, CA, USA). In the vegetable trials, approximately 50 kg of fully ripe McIntosh apples were added to the storage as a source of ethylene. Apples were added or removed in order to maintain the ethylene concentration at various temperatures. The ethylene concentration was allowed to stabilize prior to ozone treatment to ensure the initial treatment and control concentrations were similar. In all trials, the ethylene concentration was measured every weekday. The air sample in each storage room was obtained by sampling from a 3.175 mm o.d. × 100 cm stainless steel tube with one end located at the center of the room and the other end outside the door. Air samples for ozone and ethylene measurements were taken without opening the room. In the apple and pear rooms, the ozone level in each room was measured using an ozone detector tube (0.05–0.7 ppm, No. 6733181, Drager Canada Ltd., Mississauga, ON, Canada). A gas chromatograph (model GC-8A, Shimadzu Corporation, Kyoto, Japan) with dual FID detectors and 3.175 mm o.d. × 120 cm stainless steel column packed with Porapak Q (80/100 mesh) was used for measuring ethylene in the air of storage rooms and also for measuring the internal ethylene concentration of apples during storage. A microcomputer (Vectra 486/33VL, Hewlett-Packard Company, USA) with ChemStation software was used to record, calculate and report the ethylene concentration.

Hunter Color 'L', 'a' and 'b' readings were taken for all the vegetable trials both initially and after storage (1.25 cm port, Hunter Labscan II, Hunter Associates, Virginia, USA) Hue angle was calculated from arctan b/a (°) and chroma was calculated from $(a^2 + b^2)^{0.5}$. For the mushrooms only, the browning index was calculated from $\Delta a/L \times 100$.

Statistical analyses were performed using Proc GLM of the Statistical Analysis System (SAS Institute, Cary, NC). Means were separated by Tukey's Honest Significant Difference.

Broccoli

Two trials were performed on broccoli (*Brassica oleracea* L. Italica Group) with varying experimental conditions. In Trial 1, the broccoli was stored at 3 ± 1°C for 21 d in the presence and absence of 0.04 μL L^{-1} ozone. In Trial 2, the broccoli was stored at 10 ± 1°C for 7 d in the presence and absence of 0.04 μL L^{-1} ozone.

Each subsample consisted of three heads of broccoli. After storage, each head of broccoli was rated separately by three individuals for floret opening (rated 1 to 10: 1 = florets tight, 2 = florets beginning to swell, 3 = some florets beginning to open, 4 = all florets beginning to open, 5 = some florets showing trace yellow, 6 = all florets showing trace yellow, 7 = florets 25% open, 8 = florets 50% open, 9 = florets 75% open, 10 = florets full open), yellowing (rated 1 to 10: 1 = dark green, 2 = 0–25% of area yellow, 3 = 25–50% yellow, 4 = 50–75% yellow, 5 = more than 75% yellow, 6 = 100% yellow with some trace areas turned brown, 7 = more than 25% brown, 8 = more than 50% brown, 9 = more than 75% brown, 10 = 100% brown) and base browning (rated 1 to 10: 1 = no browning, 2 = less than 25% of cut area pale brown, 3 = 25–50% pale brown, 4 = 50–75% pale brown, 5 = 75% to entire base pale brown, 6 = entire base pale brown with some areas dark brown, 7 = 25–50% base dark brown, 8 = 50–100% dark brown, 9 = 75% to entire base dark brown, 10 = entire base dark brown). Three color readings were taken on each broccoli head, one in the center and the others 4 cm from the center, 180° apart.

Cucumber

Two trials were performed on seedless cucumbers (*Cucumis sativus* L.). In Trial 1, the cucumbers were stored for 17 d at 3 ± 1°C in the presence and absence of 0.04 μL L^{-1} ozone. In Trial 2, the cucumbers were stored for 12 d at 10 ± 1°C in the presence and absence of 0.04 μL L^{-1} ozone. Each subsample consisted of four cucumbers.

After storage, the cucumbers were rated separately by three individuals for desiccation (rated 1 to 5: 1 = none, 2 = trace shriveling, 3 = apparent shriveling, 4 = pronounced shriveling, 5 = severe shriveling) and overall appearance (rated: 1 to 5: 5 = excellent, no visible decay or chilling injury, 4 = trace of visible decay or chilling injury, 3 = trace to 10% of surface decayed or chilling injured, 2 = 10–25% of surface decayed or chilling injured, 1 = more than 25% of surface decayed or chilling injured). Microbial plate counts were performed on cross sections of the cucumbers using BBL Standard Methods Agar (Becton Dickinson, Cockeysville, MD). Hunter color readings were performed 5 cm from the blossom end with two readings taken for each cucumber. Initially and after storage, cucumber texture was measured 5 cm from the blossom end, without the skin, using an Effegi type pressure tester with an 8-mm probe. Two readings were taken per cucumber.

Mushroom

'Button' mushrooms [*Agaricus bisporus*, (Lange) Sing.] were received fresh from a local grower and immediately placed in cold storage. Mushrooms were treated in commercial plastic trays without plastic overwraps. Each subsample

Table 1. Effect of 0.04 μL L^{-1} ozone on broccoli stored 21 d at 3°C (Trial 1) or 7 d at 10°C (Trial 2) in the presence of 1.5 – 2 μL L^{-1} ethylene

Treatment	Hunter 'L' color	Hue angle	Chroma	Floret opening	Yellowing	Base browning
			Stored 21 days at 3°C			
Initial	33.77*c*	–56.03*c*	8.58*b*	NA	NA	NA
Control	39.16*a*	–70.53*a*	11.40*a*	2.6*a*	3.0*a*	1.4*b*
Ozone	34.76*b*	–65.91*b*	8.36*b*	2.1*b*	1.8*b*	3.6*a*
			Stored 7 days at 10°C			
Initial	32.22*c*	–63.26*c*	6.81*c*	NA	NA	NA
Control	43.80*a*	–89.81*a*	15.00*a*	1*a*	6.1*a*	2.0*a*
Ozone	41.80*b*	–86.01*b*	13.55*b*	1*a*	2.4*b*	2.3*a*

a–c Means separation in each column within a storage period is by Tukey's HSD. Means followed by the same letter are not significantly different ($P \leq 0.05$). NA = not assessed as the initial samples were free of defects.

consisted of two trays. Within the trays, samples were divided into an upper and lower level for evaluation (5–7 mushrooms/level). Storage was for 14 d at 4 ± 1°C in the presence and absence of 0.04 μL L^{-1} ozone. After storage, the mushrooms were rated separately by three individuals for blotch (rated 1 to 5: 1 = none, 2 = trace to 10% of surface covered, 3 = 10–25% of surface covered, 4 = 25–50% of surface covered, 5 = more than 50% of surface covered) and surface browning (rated 1 to 5: 1 = white, 2 = off-white, 3 = light tan colored, 4 = tan colored, 5 = dark tan colored). One color reading was measured in the center of each mushroom.

Apple and Pear

Two refrigerated rooms at 0 ± 1°C were used for the storage of approximately 160 kg of each of the Empire and Delicious apples (*Malus* × *domestica* Borkh) and approximately 140 kg of each of the Anjou and Bosc pears (*Pyrus communis* L.). Both storage rooms were same size, 1.86 m in width, 2.05 m in length, and 2.76 m in height. The ozone level in the treated room was maintained at approximately 0.4 μL L^{-1} throughout the storage period of 107 d by placing two ozone generators (Model S200, A.H. Simpson Industries Ltd., Toronto, ON) in the center of the room about 120 cm above the floor. Ozone was measured by using Drager ozone detecting tubes (0.05–0.4 μL L^{-1} range, Dragerwerk AG, Lubeck, Germany).

Apples and pears were harvested and stored in these two rooms starting on 5 October 1998. Additional Empire apples (approximately 160 kg, harvested on the same date) were also placed in both rooms in order to generate ethylene. Eight plastic containers of apple or pear samples were placed randomly in various locations inside each cold room. Internal ethylene concentrations of apples (Chu 1988) were determined after 63 and 107 d of storage. Fruit quality was determined at harvest and after 107 d of storage. At both evaluations, tests were also performed after 7 d at room temperature (20 ± 1°C) for the apples and after 3 d at room temperature for the pears. Apple firmness was measured by using an electronic pressure tester (model EPT–1, Lake City Technical Products Inc., Kelowna, BC) with an 11-mm-diameter probe. Pear firmness was measured using an Effegi pressure tester with a 9-mm-diameter probe. Total soluble solids were measured using an Abbe refractometer (Model 10450, American Optical Corporation, Buffalo, NY). Titratable acidity was measured using an automatic Titrameter (model 385, Fisher Scientific Company, USA) to the end point of 8.1 pH.

RESULTS AND DISCUSSION

For the vegetable rooms, the ethylene level in the non-ozonized rooms (and in the ozonized rooms prior to treatment) gradually increased during the first 24 h after venting and then remained relatively stable between 1.5 and 2 μL L^{-1} with an average of 1.7 μL L^{-1}. Ozone treatment reduced the ethylene to undetectable levels for vegetable trials.

Broccoli

When stored at 3°C in the presence of 1.5–2 μL L^{-1} ethylene (Trial 1), Hunter 'L' and hue angle values indicated a color shift from green to yellow for both the control and ozone-treated samples (Table 1). The color change was significantly less pronounced for the ozone-treated samples. Floret opening and visually assessed yellowing were also significantly lower for the ozone-treated samples. Increased base (cut surface) browning occurred in the ozone-treated samples, however, trimming of the stalks could easily remove the brown area. Laisk et al. (1989) suggested that ozone decomposes at the cell wall and plasma membrane rather than penetrating intercellularly. One of the primary responses to ozone treatment is increased membrane permeability and electrolyte leakage (Beckerson and Hofstra 1980; Liew and Prange 1994). The observed browning was likely a result of increased membrane permeability and subsequent loss of cellular compartmentation with increased activity of oxidative enzymes.

When stored at 10°C in the presence of 1.5–2 μL L^{-1} ethylene (Trial 2), both the control and ozone-treated samples were significantly more yellow than the initial; however, again, the control broccoli was significantly more yellow than the ozone-treated samples (Table 1).

Under the conditions used in these trials, ozone appears promising for extending the storage life of broccoli. Although ozone maintained the quality of stored broccoli, it was not equal to the fresh harvested quality.

Cucumber

The Hunter 'L' reading and the hue angle value indicated yellowing of both the ozone-treated and control samples (Table 2) although no differences were detected visually. Firmness for the ozone-treated fruit was significantly high-

Table 2. Effect of 0.04 μL L^{-1} ozone on cucumbers stored 17 d at 3°C in the presence of 1.5 - 2 μL L^{-1} ethylene

Treatment	Hunter 'L' color	Hue angle	Chroma	Firmness (N)	Desiccation	Appearance	Plate count (log^{10}cfu g^{-1})
Initial	25.23*b*	–38.86*a*	8.86*a*	34*a*	NA	NA	NA
Control	28.44*a*	–53.59*b*	8.71*a*	22*b*	0.9*b*	2.2*b*	8.5*a*
Ozone	28.13*a*	–52.94*b*	8.66*a*	36*a*	2.7*a*	3.5*a*	7.4*b*

a–c Means separation in each column is by Tukey's HSD. Means followed by the same letter are not significantly different ($P \leq 0.05$). NA = not assessed as the initial samples were free of defects.

Table 3. Effect of 0.04 μL L^{-1} ozone on mushrooms stored 14 d at 4°C

Treatment	Location	Blotch	Surface browning	Hunter 'L' color	Hue angle	Chroma	Browning index
Initial		NA	NA	88.11*a*	83.07*a*	12.02*a*	NC
Control	Top	2.4*a*	2.8*a*	75.10*ab*	80.21*ab*	14.20*b*	1.46*b*
	Lower	2.7*a*	2.6*a*	73.64*ab*	79.64*ab*	14.19*b*	1.76*b*
Ozone	Top	1.7*a*	2.6*a*	70.65*b*	76.78*b*	14.80*b*	2.82*a*
	Lower	2.5*a*	1.7*a*	72.13*b*	80.21*ab*	12.74*a*	0.97*c*

a–c Means separation in each column is by Tukey's HSD. Means followed by the same letter are not significantly different ($P \leq 0.05$). NA = not assessed as the initial samples were free of defects. NC= not calculated as index is based on initial reading.

er than the control treatment and was equal to initial levels. Appearance (based on chilling injury and amount of decay) and microbial counts were also superior for the ozone-treated samples. Appearance deterioration was primarily due to chilling injury in the ozone-treated samples and due to decay in the control samples. Severe decay prevented chilling injury assessment of the control samples. The ozone samples appeared more desiccated than the control. Rao et al. (2000) have indicated that ozone reacts with plants in both solid phase (e.g., cuticular components) and liquid phase (including the dissolution of ozone followed by reaction with lipids and proteins). It is likely that a combination of these effects resulted in the observed desiccated appearance. If the desiccated appearance could be minimized, ozone could be a promising treatment for extending the storage life of cucumbers when they are stored under chilling conditions (3°C). There was no chilling injury or effect of ozone when the cucumbers were stored at 10°C (data not presented).

Mushroom

After 14 d at 4°C, ozone-treated mushrooms exhibited lower Hunter 'L' readings than the initial reading (Table 3). The hue angle of the top layer of the ozone-treated mushrooms was significantly lower than the fresh cut mushrooms, indicative of phytotoxicity due to the ozone. The browning index for the ozone-treated mushrooms was higher than the control mushrooms for the upper level, but lower for the lower level. Chroma of the lower level of the ozone-treated samples was equal to the initial reading. Visually, the upper level of the ozone-treated mushrooms appeared to have less blotch after storage than the controls; however, due to high sample variation, differences were not statistically significant. The ozone treatment did not affect the blotch or surface browning rating of the mushrooms compared with the control samples. The low browning index and lack of blotch control in the lower level of the ozone-treated mushrooms could indicate that the ozone was not penetrating to the lower level.

Apple and Pear

Ozone treatment reduced the ethylene level in the apple and pear storage room considerably (Fig. 1). The ethylene level in the ozonized storage room was < 1.0 μL L^{-1} during the first 20 d, and < 2.0 μL L^{-1} for the rest of the storage period. The ethylene level in the non-ozonized room gradually increased to about 25 μL L^{-1} by the end of the storage period.

The ozonized and non-ozonized apples and pears showed no difference in quality deterioration. After 63 and 107 d of cold storage, there was no difference in internal ethylene concentration between ozonized and non-ozonized apples. After 107 d of storage at 0°C and also after 1 additional week at 20°C, there was no significant difference in apple firmness, total soluble solids, titratable acidity or scald index between ozonized and non-ozonized apples. Li et al. (1989) observed a pronounced positive effect of ozone and negative ions (with a lesser effect of negative ions alone) on quality of stored apples; however, the ozone concentration used in their study was significantly higher (1–5 μL L^{-1}). After 107 d of storage at 0°C and also after an additional 3 d at 20°C, there was no significant difference in pear firmness and total soluble solids. There were no symptoms of ozone injury found on the apples or pears.

CONCLUSIONS

Ozonizing the air in a cold storage room can reduce the level of ethylene in the air. Li et al. (1989) found that production of 1-aminocyclopropane–1-carboxylic acid (ACC) decreased in tomatoes stored in the presence of ozone and negative ions. It is not known if the decrease in ethylene in the storage rooms was a result of the decrease in the ACC

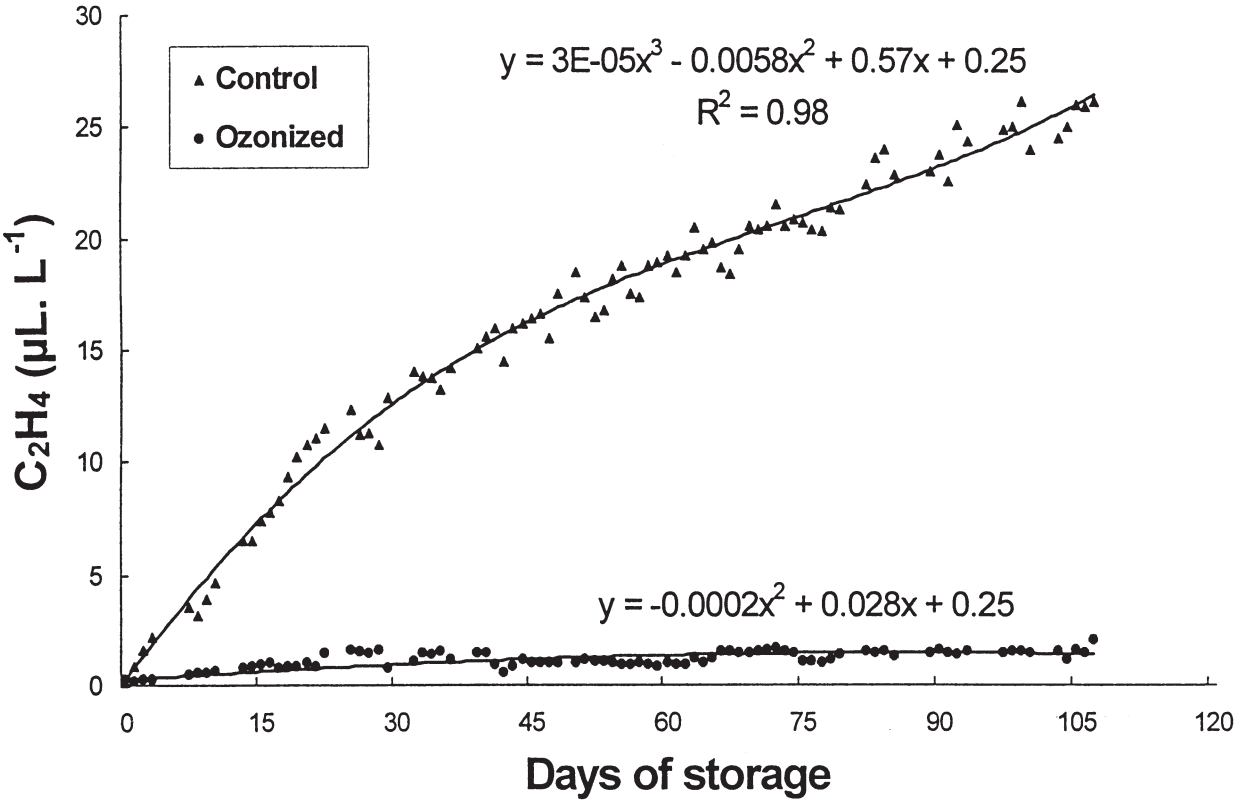

Fig. 1. Ethylene levels in ozonized (0.4 μL L^{-1}) and non-ozonized apple and pear cold storage rooms.

ethylene precursor or due to oxidation of the atmospheric ethylene. The lack of any difference in internal ethylene concentrations or fruit quality resulting from ozone treatment of the apples would tend to indicate that ethylene was being oxidized rather than a decrease in ethylene production. Ozone generators may be of most use in places where ethylene-producing (e.g., apples and pears) and ethylene-sensitive fruits and vegetables (e.g., broccoli) may be stored in the same room. It may not change the quality of apples and pears because they produce ethylene and, therefore, have a higher level of ethylene inside the fruit. However, the quality of the ethylene-sensitive crops may be improved.

It is likely that the observed antimicrobial effects (primarily on the cucumbers) are a result of the microbial resistance of the plant being maintained by the ozone treatment rather than a direct effect of the ozone on the plant pathogen. Other researchers (Liew and Prange 1994; Bradford and Suslow 2000) have indicated that concentrations significantly higher than the 0.04–0.4 μL L^{-1} ozone used in these trials are required for fungicidal effects.

Phytotoxicity of ozone-sensitive produce is of concern. All three of the vegetable crops tested exhibited adverse effects due to ozone treatment, although for two of the crops the effects were minor and overall quality was better than the control treatment. Other researchers have also observed phytotoxicity to *Agaricus* mushrooms and broccoli (Bradford and Suslow 2000). While some fruits and vegetables may be sensitive to ozone, apples and pears can tolerate 0.4 μL L^{-1} of ozone in the air for 107 d without having any symptom of ozone injury.

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