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Postharvest Performance Evaluation of Plum (*Prunus salicina* Lindel., 'Casselman') Fruit Grown under Three Ozone Concentrations

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Abstract. We investigated the effects of three seasonal atmospheric ozone (O₃) concentrations on fruit quality, internal breakdown, weight loss, cuticle structure, and ripening characteristics of plum fruit from 3-year-old 'Casselman' trees in the 1991 season. Trees were exposed to 12-hour daily mean O₃ concentrations of 0.034 [charcoal-filtered air (CFA)], 0.050 [ambient air (AA)], or 0.094 [ambient plus O₃ (AA + O)] μl-liter⁻¹ from bloom to leaf-fall (1 Apr. to 31 Oct. 1991). Fruit quality and internal breakdown incidence measured at harvest and after 2, 4, and 6 weeks of storage at 0C were not affected by any of the O₃ treatments. Following an ethylene (C₂H₄) preconditioning treatment, the rate of fruit softening, C₂H₄ production, and CO₂ evolution was higher for plums harvested from the AA + O than from those grown in CFA. Weight loss of fruit from the AA + O exceeded that of fruit from CFA and AA. Anatomical studies of mature plums indicated differences in wax deposition and cuticle thickness between fruit grown in AA + O, AA, and CFA. Differences in gas permeability, therefore, may explain the difference in the ripening pattern of 'Casselman' plum fruit grown in high atmospheric O₃ partial pressures.

The San Joaquin Valley of California produces >2 million t-year of fruit and nut crops. This fruit production region is exposed to ambient ozone (O₃) concentrations that consistently exceed U.S. Environmental Protection Agency standards of 0.12 μl-liter⁻¹ at various times during the growing season (Cabrera et al., 1988; Olszyk, et al., 1988). High O₃ concentrations induce yield reduction in annual and perennial crops (Adaros et al., 1990; Brewer and Ashcroft, 1983; Mebrahtu et al., 1991; Musselman et al., 1978). Reduction in net photosynthesis due to high O₃ exposure has been given as an explanation for reduced plant growth and yield (Lehnherr et al., 1988; Reich and Amundson, 1985; Takemoto et al., 1988). A similar situation has been reported for 'Valencia' orange [*Citrus sinensis* (L.) Osbeck] grown under O₃ concentrations >0.020 μl-liter⁻¹ (Olszyk et al., 1990). Recent studies have demonstrated that net photosynthesis and tree growth of various fruit and nut tree species and even cultivars within the same species decreased with increasing O₃ concentration (Retzlaff et al., 1991; Retzlaff et al., 1992a). Retzlaff et al. (1992b) reported that increased atmospheric O₃ concentration decreased yield of 'Casselman' plum trees during the orchard establishment period. Plum tree yields in 1990 were 8.8, 6.3, and 5.5 kg/tree in 0.038, 0.050, and 0.090 μl-liter⁻¹ atmospheric O₃ concentrations, respectively.

As O₃ can cause foliar symptoms on pine needles that ultimately result in leaf necrosis (Lutz and Heinzmann, 1990; Percy et al.,

1990; Turunen and Huttunen, 1990), it may also cause similar injury to the epicuticular wax, cuticle, and epidermal cells of fruit and, therefore, lower fruit storage and market life potential. Although it is well documented that high O₃ concentrations decrease tree growth and productivity, O₃ air pollution effects on fruit quality and postharvest performance are unknown. For this reason, we decided to study fruit quality and storage and physiological characteristics of 'Casselman' plum exposed to several atmospheric O₃ concentrations during the 1991 growing season.

Materials and Methods

Plant material and ozone treatments. Three O₃ concentration treatments were imposed on 3-year-old 'Casselman' plum trees growing at the Univ. of California Kearney Agricultural Center, Parlier, Calif. The three O₃ levels were attained by enclosing trees in open-top fumigation chambers, each attached to an air circulation unit according to Retzlaff et al. (1992b). Air circulated through the chambers was either charcoal filtered (CFA; 12-h seasonal mean O₃ concentration 0.034 μl-liter⁻¹), ambient air (AA; 0.050 μl-liter⁻¹), or ambient air with O₃ added (AA + O; 0.094 μl-liter⁻¹). Ozone for the AA + O treatment chambers was generated from ambient air with a Griffin Model GTC-2A Ozone Generator (Lodi, N.J.), resulting in 12-h seasonal mean O₃ concentrations ≈1.9 times ambient treatment chamber levels. The O₃ treatments were initiated while the trees were in bloom (1 Apr. 1991) and continued until the beginning of leaf fall (31 Oct.).

Statistical design and analysis. The design used was a randomized complete block with three O₃ concentration treatments and five replications. Postharvest data were analyzed using analysis of variance (ANOVA). Linear contrast with 12-h mean O₃ levels was used for a priori comparisons among treatment means ($\alpha < 0.05$). The SAS program was used for ANOVA and regression analyses (SAS, 1988).

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Abbreviations: AA, ambient air; AA + O, ambient air plus ozone; CFA, charcoal-filtered air; LM, light microscopy; SEM, scanning electron microscopy; SSC, soluble solids concentration; TA, titratable acidity.

Table 1. Effect of three atmospheric ozone concentration treatments during the growing season on 'Casselman' plum fruit quality at harvest and after different storage periods (0C) in addition to 5 days at 20C.

Evaluation time (wks after storage)	Treatments					
	Charcoal-filtered air		Ambient air		Ambient air + ozone	
	Firmness (N)	SSC (%)	Firmness (N)	SSC (%)	Firmness (N)	SSC (%)
0	30.0 ^z	15.8	32.0	15.6	31.0	15.3
2	29.0	16.2	30.0	16.2	28.0	16.2
4	24.0	17.3	23.0	16.8	23.0	16.9
6	25.0	16.4	23.0	14.8	23.0	15.2

^zThere were no significant differences among the different treatments within each evaluation time for fruit firmness and soluble solids concentration (SSC).

Postharvest evaluation. Fruit were picked at commercial maturity as determined by ground color (21 Aug. 1991), and the following fruit quality and physiological variables were measured: flesh firmness, surface color, soluble solids concentration (SSC), titratable acidity (TA), pH, bruising susceptibility to impact and rolling, weight loss, market life, and ripening. Twenty fruit per treatment from each of the five replications were collected for fruit quality determination at harvest. Flesh firmness was measured using a Univ. of California firmness tester with an 8-mm tip (Western Industrial Supply, San Francisco). Skin from opposite cheeks of each fruit was removed and flesh firmness calculated as an average of two measurements per fruit. A wedge from each fruit was removed and combined with wedges from each treatment within a replication to form a composite sample. From this composite sample, we extracted juice with a hand press, filtered it through cheesecloth, and determined SSC (by refractometer; Cambridge Instruments, Buffalo, N.Y.), pH, and TA at final pH 8.2.

Storage life. Sixty fruit per treatment per each of the five replications were stored in ethylene-free air at 0C and 90% relative humidity (RH) (vapor pressure deficit = 0.061 kPa). Twenty fruit per treatment from each of the five replications were removed following 2, 4, and 6 weeks of storage. After removal from storage, the samples were ripened at 20C for 7 to 10 days before evaluation. Flesh firmness, SSC, and TA were measured. Internal breakdown (chilling injury) symptoms were evaluated as flesh browning, texture (juiciness, mealiness), hardness, and bleeding according to Nanos and Mitchell (1991). These observations were made on the mesocarp around the pit after the fruit were cut transversely along the plane of the suture.

Bruising susceptibility. Fruit from each treatment were subjected to impact and vibration tests just after harvest. After bruising, fruit were placed in an open plastic bag and stored at room temperature (20C) for 48 h before evaluation. Bruising damage was measured as the percentage of fruit showing visible injury. In both experiments, visual bruising damage was evaluated externally and internally following the bruise-scoring system of Mitchell and Kader (1992).

Vibration bruising: Transit injury was simulated by allowing fruit to roll loosely in a container that was subjected to a vibration of 1.1×g acceleration at 550 cycles/min and a 6.4 mm stroke with flesh at 20C. Fifteen fruit per treatment from each of the five replications were evaluated.

Impact bruising: Fifty fruit per treatment from five replications were individually impacted twice (once on each cheek) from a fixed height (30.5 cm) using a free-falling steel ball (2.54 cm diameter and 66.7 g) dropped through a vertical column at 20C. Following impact, the treated areas were marked and the fruit kept at 20C for 48 h before evaluation. Fruit were peeled before bruising evaluation.

Fruit weight loss. Groups of five fruit per treatment from each of the five replications were carefully weighed and placed in a temperature-controlled room at 30C and 30% RH (vapor pressure deficit = 2.97 MPa). Fruit were reweighed daily for 12 days. Weight loss was calculated as the percent reduction from the original weight. Because visible symptoms of weight loss (fruit shriveling) and decay were observed in fruit from the AA + O by day 7, only weight loss measurements up to and including day 5 are reported.

Ripening pattern. Fruit from the AA + O and CFA were stored for 2 weeks at 0C. Fruit ripening was preconditioned on half of the cohorts by immersion for 12 h in 100 µl·liter⁻¹ ethephon dip at 20C, with the end result being four treatments: AA + O, AA + O plus ethylene (C₂H₄), CFA, and CFA plus C₂H₄. Then, fruit from these four treatments were allowed to ripen in ventilated jars at 20C. Flesh firmness was measured every other day on 30 fruit per treatment from each of four replications during the ripening period until the average firmness was <13.5 N. Firmness measurements were terminated after 9 days on fruit that did not ripen.

Carbon dioxide evolution and C₂H₄ production were also measured for all treatments. Fruit from each treatment were placed in glass respiration jars attached to a flow board and then kept in a 20C room. Air flow through the sample jars was adjusted using a flow board so that the internal atmosphere contained no more than 0.3% CO₂ (Nanos and Mitchell, 1991). Air samples were taken from the outlets of the jars every other day during ripening. Carbon dioxide concentration in the gas samples was measured with a Horiba infrared CO₂ gas analyzer (Model SX-2, PIR-2000R; Horiba Instruments, Irvine, Calif.), and C₂H₄ concentration was measured with a Carle gas chromatograph equipped with a flame ionization detector (Model 411, Carle Instruments, Tulsa, Okla.).

Anatomical studies. Three plums from each O₃ treatment-replication combination were collected at commercial maturity and prepared for fruit anatomical observations according to Luza et al. (1992). Samples were prepared by cutting 4-mm³ pieces from the midcheek area of the fruit. Samples were placed into the fixative immediately and subjected to a mild vacuum for 30 min.

Procedures for light microscopy (LM) observations. Samples were fixed in a 4% glutaraldehyde solution containing 0.2 M dipotassium phosphate and 0.1 M citric acid monohydrate at pH 7.0. Samples were then washed in the buffer at room temperature, dehydrated through an ethanol series, and infiltrated with glycol methacrylate resin (DuPont-Sorvall; Wilmington, Del.). Sections were cut at 5 µm using glass knives on a Sorvall JB4 microtome (Polaron Instruments, Hatfield, Pa.). To analyze general cellular structure, sections were stained with 0.5% toluidine blue in 0.15 M K₂HPO₄ and 0.5% safranin in 0.2 M Tris-HCl, and counterstained with calcofluor white MR2 (American Cyanamid Co.). For cuticle observations, slides were stained with Sudan black in 100%

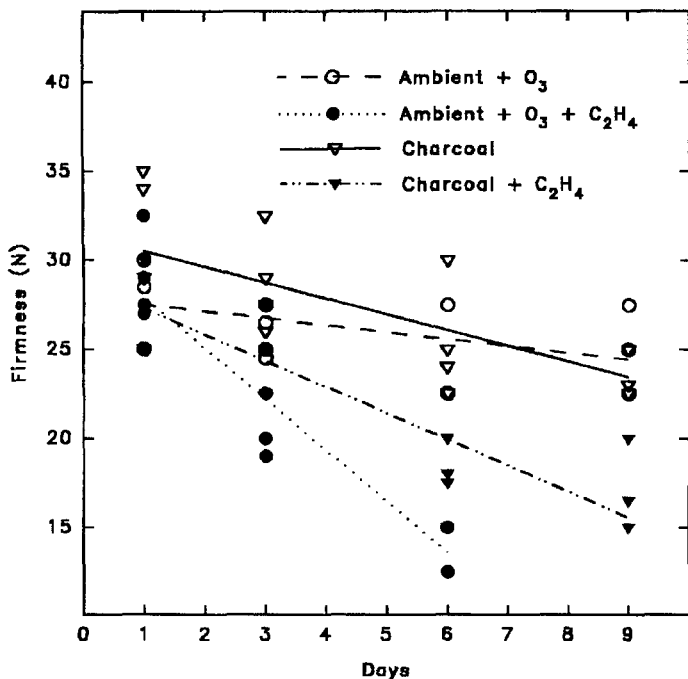


Fig. 3. Relationship between flesh firmness (N) and time (days) during ripening for 'Casselman' plum grown under two atmospheric ozone concentration treatments. Regression models: Flesh firmness = $28 - 0.4$ (days), $r_2 = 0.24$ for $0.094 \mu\text{l}\cdot\text{liter}^{-1}$ (ambient + ozone); flesh firmness = $31 - 2.9$ (days), $r_2 = 0.89$ for $0.094 \mu\text{l}\cdot\text{liter}^{-1}$ (ambient + ozone + C_2H_4); flesh firmness = $31 - 0.9$ (days), $r_2 = 0.47$ for $0.034 \mu\text{l}\cdot\text{liter}^{-1}$ (charcoal); flesh firmness = $29 - 1.5$ (days), $r_2 = 0.77$ for $0.034 \mu\text{l}\cdot\text{liter}^{-1}$ (charcoal + C_2H_4).

increased plum fruit weight loss. Since epicuticular waxes and the cuticle act as a partial barrier to water vapor movement from inside the cuticle to the environment (Gaffney, 1978), the above data for plum may indicate cuticle or epicuticular wax differences in response to increased O_3 concentrations.

Ripening. Fruit from the CFA without C_2H_4 preconditioning had the lowest CO_2 evolution rate, and fruit from the AA + O plus C_2H_4 had the highest CO_2 evolution with a peak observed 3 days following the C_2H_4 preconditioning (Fig. 2). Fruit from AA + O plus C_2H_4 and CFA plus C_2H_4 always evolved more CO_2 than fruit from O_3 treatments without C_2H_4 . Plums from AA + O and CFA without C_2H_4 preconditioning produced very low levels of C_2H_4 during the 9 days (data not shown). Fruit from the AA + O plus C_2H_4 always produced more C_2H_4 than fruit from the CFA plus C_2H_4 . One day after C_2H_4 preconditioning, fruit from AA + O plus C_2H_4 had a higher rate of C_2H_4 evolution than fruit from the CFA plus C_2H_4 . Ethylene production of fruit from AA + O plus C_2H_4 decreased for 3 days, then increased the next 3 days, reaching a maximum at 9 days, the end of the test.

Fruit flesh softening was increased by C_2H_4 preconditioning (Fig. 3). Regression analysis using time (days) as a predictor of fruit firmness (N) showed that the slope for the AA + O plus C_2H_4 and CFA plus C_2H_4 treatments were significantly different ($P > 0.05$) from those of the AA + O and CFA treatments without C_2H_4 preconditioning. There was no significant relationship between fruit firmness (Y) and time (day) for the AA + O ($Y = 28 - 0.4$ day, $r_2 = 0.24$) and CFA ($Y = 31 - 0.9$ day, $r_2 = 0.47$) treatments without C_2H_4 preconditioning. However, fruit firmness for AA + O plus C_2H_4 ($Y = 31 - 2.9$ day, $r_2 = 0.89$) and CFA plus C_2H_4 ($Y = 29 - 1.5$ day, $r_2 = 0.77$) decreased significantly over the 9-day experiment period. Fruit from the AA + O plus C_2H_4 became soft (13.5 N) 6 days after preconditioning, while fruit from the CFA plus

C_2H_4 treatment never reached 13.5 N, reaching only 16.0 N after 9 days (Fig. 3).

Cuticle structure. SEM and LM in combination with several staining techniques showed cuticle structural differences among fruit from the three O_3 treatments (Fig. 4). SEM photomicrographs indicated differences in the arrangement of epicuticular wax among treatments. In the AA + O, wax was deposited in a reticulate net with large pores (Fig. 4 G and H). In contrast, fruit from the CFA showed a much tighter reticulated pattern of wax deposition (Fig. 4 A and B). An intermediate wax deposition was observed on fruit from the AA (Fig. 4 D and E). In this case, portions of the fruit surface exhibited a loose wax net deposition type (similar to that for AA + O fruit), and the other portion of the fruit exhibited a tight wax net (similar to that for CFA fruit). Alteration of epicuticular waxes has been reported in conifer needles and spring wheat (*Triticum aestivum* L.) as a result of O_3 air pollution (Ojanpera et al., 1992; Percy et al., 1990; Turunen and Huttunen, 1990). Epicuticular wax structure modification consisting of crystal formation near or over stomata, fissures, clogging, and even fusion of wax tubes has been reported on Norway spruce [*Picea abies* (L.) Karst.] needles after long-term exposure to O_3 and acid mist (Lutz and Heinzmann, 1990).

LM indicated differences in cuticle thickness in response to increased O_3 concentration. Fruit from CFA (Fig. 4C) presented a more continuous and much thicker cuticle than fruit from AA + O (Fig. 4I). Fruit epidermis samples from AA had a combination of thick and thin cuticles (Fig. 4F). Leaf, stem, and fruit cuticle thickness has been related to plant pathogen defense mechanisms and resistance to moisture loss (Juniper and Jeffree, 1983).

There were no visible differences in fruit tissue anatomy among the three O_3 treatments. In general, plum fruit exhibited one row of epidermal cells without trichomes, four to six rows of hypodermal cells, and beneath the hypodermis many large mesocarp cells (Fig. 4 C, F, and I).

'Casselman' plum fruit is a very very low-ripening cultivar that is not capable of generating C_2H_4 , and it must be exposed to exogenous C_2H_4 to initiate ripening and softening (Mitchell and Kader, 1992), which normally occurs during commercial postharvest handling operations. Our fruit did not soften during postharvest storage and ripening tests, because C_2H_4 -free air was used. Differences in softening were observed between fruit from AA + O and CFA only when fruit was preconditioned with C_2H_4 before ripening. The differences in softening between O_3 treatments may be due to increased uptake of C_2H_4 (increased permeability) or more C_2H_4 sites in the cell membranes. The increased C_2H_4 peak obtained just after ethephon dipping with the AA + O-grown fruit, the higher weight loss rate, and the thinner cuticle and epidermal waxes in AA + O-exposed fruit suggests that fruit from the AA + O take up more ethephon, thus more C_2H_4 is produced within their tissue, in contrast to fruit grown in CFA. Increased water loss due to cuticle and epidermal wax modification by increased O_3 concentrations indicate that fruit grown in the presence of $0.094 \mu\text{l}/\text{liter}$ (12-h seasonal mean) are more susceptible to fruit shriveling symptoms development than plums grown in lower O_3 concentrations. A susceptibility to water loss due to O_3 injury could be even more important for nectarine and peach, because plum fruit have lower rates of water loss (Mitchell and Kader, 1992). Cuticle and wax deposition modification by increased atmospheric O_3 exposure has potentially detrimental effects on very low, medium, and readily ripening plum fruit postharvest life, such as increased susceptibility to water loss, decreased resistance to pathogen infection, and increased sensitivity to C_2H_4 . The influence of increased atmospheric O_3 concentra-

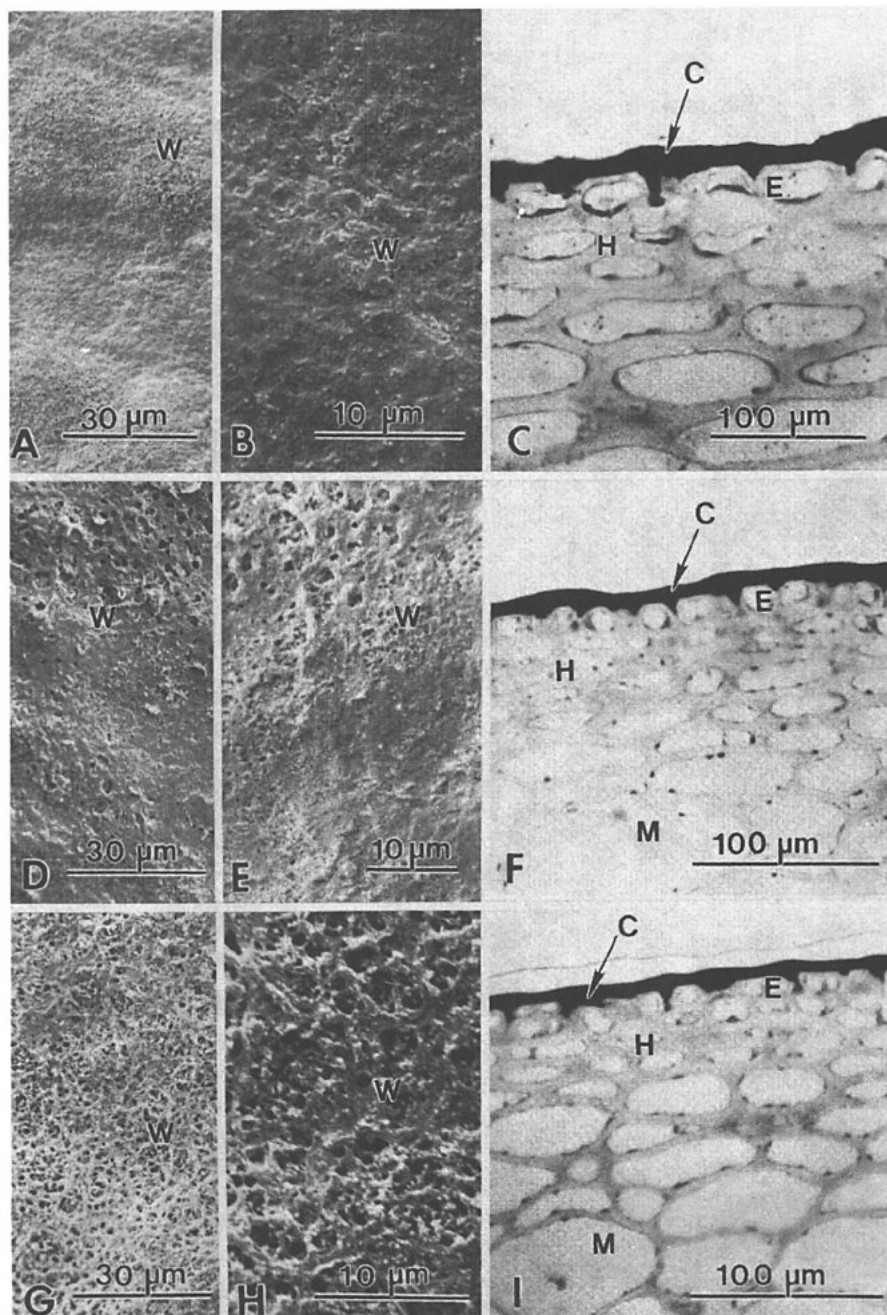


Fig 4. SEM and LM observations following harvest of 'Casselman' plum fruit grown under three atmospheric ozone concentration treatments. (A) 0.034 $\mu\text{l}\cdot\text{liter}^{-1}$ ozone, SEM; (B) 0.034 $\mu\text{l}\cdot\text{liter}^{-1}$ ozone, SEM; (C) 0.034 $\mu\text{l}\cdot\text{liter}^{-1}$ ozone, LM, Black Sudan; (D) 0.050 $\mu\text{l}\cdot\text{liter}^{-1}$ ozone, SEM; (E) 0.050 $\mu\text{l}\cdot\text{liter}^{-1}$ ozone, SEM; (F) 0.050 $\mu\text{l}\cdot\text{liter}^{-1}$ ozone, LM, Black Sudan; (G) 0.094 $\mu\text{l}\cdot\text{liter}^{-1}$ ozone, SEM; (H) 0.094 $\mu\text{l}\cdot\text{liter}^{-1}$ ozone, SEM; (I) 0.094 $\mu\text{l}\cdot\text{liter}^{-1}$ ozone, LM, Black Sudan. W = wax; E = epidermis; H = hypodermis; M = mesocarp; C = cuticle.

tions during fruit maturation and ripening needs to be studied in more detail on climacteric and nonclimacteric fruit to further understand the influence of O_3 on fruit postharvest life potential.

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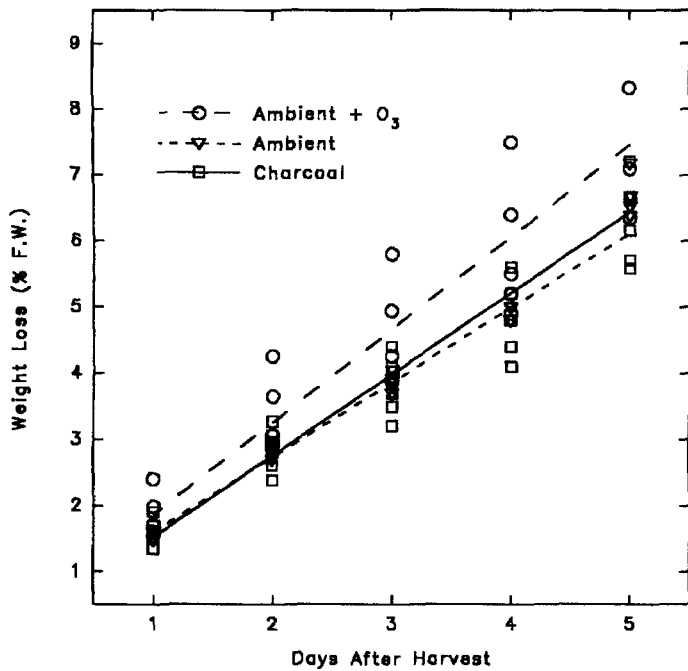


Fig. 1. The relationship between weight loss (percent of initial fresh weight) and time after harvest (days) for 'Casselman' plum grown under three atmospheric ozone concentration treatments. Regression models: Weight loss = $0.3 + 1.2$ (days), $r^2 = 0.98$ for $0.050 \mu\text{l}$ ozone/liter; Weight loss = $0.5 + 1.1$ (days), $r^2 = 0.99$ for $0.034 \mu\text{l}$ ozone/liter; Weight loss = $0.5 + 1.4$ (days), $r^2 = 0.99$ for $0.094 \mu\text{l}$ ozone/liter.

acetone and mounted in glycerol. Sudan black turned fatty substances black, providing information about the general appearance and thickness of the cuticle. Photomicrographs were taken using Kodak Pan-X film for bright field and Kodak Tri-X for fluorescent images.

Procedures for scanning electron microscopy observations. Fixed samples for scanning electron microscopy (SEM) were dehydrated with ethanol as described above, except that the 100% ethanol was replaced with amylacetate. The samples were critical point dried with CO_2 , mounted on stubs with silver paint, and then sputter-coated with 40 to 50 nm of Au. Photographs and observations were made on an ISI DS-130 scanning electron microscope operated at 10 kV.

Results and Discussion

Fruit quality. Following the 1991 growing season, flesh firmness and SSC (Table 1), and the percent surface that was red, pH, and TA (data not shown) were not affected significantly by any of the O_3 treatment levels. A lack of O_3 effect on fruit SSC and TA has been reported for tomatoes (Temple, 1990; Tenga et al., 1990) and oranges (Olszyk et al., 1990) grown under high- O_3 concentrations.

Storage life. Fruit firmness, SSC (Table 1), and internal breakdown (data not shown), measured after 2, 4, and 6 weeks of storage followed by 7 to 10 days at 20C, were not affected by any of the O_3 treatment levels. During the 6-week storage period, fruit firmness decreased from 28.4 N to 23.4 N regardless of O_3 treatment. SSC increased to a peak near 17% at week 4, but then decreased during the last 2 weeks. The $\approx 4.7\%$ greater increase in SSC measured at 4 weeks can be explained by increased SSC due to water loss. Cumulative weight loss occurring throughout the fruit harvesting, postharvest handling, and storage periods may reach 6% without showing any visible symptoms on 'Casselman' plum (Mitchell

and Kader, 1992). The reduction in SSC by 6 weeks might have been due to respiration losses.

The number of fruit displaying internal breakdown symptoms increased linearly during storage, but it never reached commercially important values. At week 2, only 2.7% (average of all three O_3 treatments) of the fruit was affected by internal breakdown, but it increased to 8.7% by week 4 and to 17% by the end of the 6-week storage period. A similar situation occurred on forced ripened fruit from the three O_3 treatments that were removed 2 weeks after storage and preconditioned with $100 \mu\text{l}$ ethephon/liter (data not shown).

Impact and vibration bruising damage. There were no differences in vibration and impact bruising damage for fruit among the O_3 treatments (data not shown).

Fruit weight loss. Weight loss was high for fruit from all of the O_3 treatments. Visible shriveling symptoms first began to appear after 5 days when weight losses exceeded 6% of the initial fresh weight. Linear regression analysis indicated a strong relationship ($r^2 = 0.99$ to 1.0) between weight loss as a percentage of initial fruit weight and time (days) for all of the treatments (Fig. 1). A comparative analysis (t test) of the regression equation slopes indicated a significantly ($P < 0.001$) greater weight loss for fruit from the AA + O than for fruit from the AA or CFA. Thus, increased O_3 concentration during fruit growth and maturation

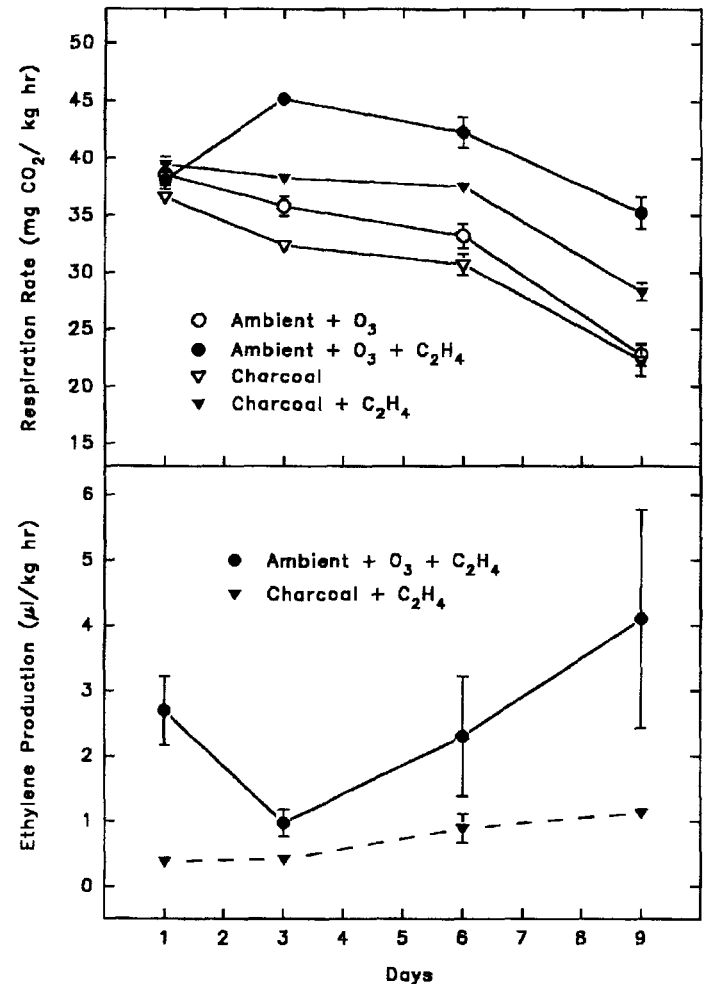


Fig. 2. Effect of 0.034 (charcoal filtered) and 0.94 (ambient + ozone) μl ozone/liter concentration on 'Casselman' plum during the growing season with and without ethylene preconditioning treatment on CO_2 and ethylene production during a 9-day postharvest period.

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