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

C.H. Crisosto¹, W.A. Retzlaff², L.E. William³, T.M. DeJong³, and J.P. Zoffoli⁴
Department of Pomology, University of California, Kearney Agricultural Center, 9240 South Riverbend Avenue, Parlier, CA 93648

Abstract. We investigated the effects of three seasonal atmospheric ozone (O₃) concentrations on fruit quality, internal breakdown, fruit weight loss, bruising, cuticle, ethylene production, respiration, and CO₂ evolution at 0°C. 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 Oᵢ₃ (AA+Oᵢ₃)] 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 0°C 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 + Oᵢ₃ than from those grown in CFA. Weight loss of fruit from the AA + Oᵢ₃ 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 + Oᵢ₃, 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.

Received for publication 24 July 1992. Accepted for publication 3 Nov. 1992. This study was funded in part by a grant from the California State Air Resources Board. The statements and conclusions of this report are those of the Univ. of California and not necessarily those of the California State Air Resources Board. The mention of commercial products, their source, or their use in connection with the research reported herein is not to be construed as either an actual or implied endorsement of said products. We thank Juvenal Luz and Vito S. Polito for their technical assistance. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

¹Assistant Professor.
²Postdoctoral Research Associate, Dept. of Viticulture and Enology.
³Associate Professor, Dept. of Viticulture and Enology.
⁴Professor, Dept. of Pomology, Univ. of California, Davis, CA 95616.
⁵Visiting Professor, Departamento de Fruticultura, Universidad Catolica de Chile, Santiago, Chile.

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; Mebrahuti 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 (Lehnheur 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 >0.200 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 treatments, 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 Huttenen, 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 Oᵢ₃ added (AA + 0; 0.094 μl·liter⁻¹). Ozone for the AA + Oᵢ₃ 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 Oᵢ₃ 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 (α < 0.05). The SAS program was used for ANOVA and regression analyses (SAS, 1988).

Abbreviations: AA, ambient air; AA + Oᵢ₃, ambient air plus ozone; CFA, charcoal-filtered air; LM, light microscopy; SEM, scanning electron microscopy; SSC, soluble solids concentration; TA, titratable acidity.
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).

Impact 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 of ethephon dip at 20C, with the end result being four treatments: AA + O, AA + O plus ethylene (C,H₄), CFA, and CFA plus C,H₄. 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 C,H₄ 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-200OR; Horiba Instruments, Irvine, Calif.), and C,H₄ 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 O,treatment-replication combination were collected at commercial maturity and prepared for fruit anatomical observations according to Luzá 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 pm 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 K,HPO , and 0.5% safranine 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%
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 O3 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 O3 treatments that were removed 2 weeks after storage and preconditioned with 100 µl ethephon/liter (data not shown).

**Impact and vibration bruising damage.** There were no differences in vibration and impact bruising damage for fruit among the O3 treatments (data not shown).

**Fruit weight loss.** Weight loss was high for fruit from all of the O3 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 O3 concentration during fruit growth and maturation

**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 O3 treatment levels. A lack of O3 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-O3 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 20°C, were not affected by any of the O3 treatment levels. During the 6-week storage period, fruit firmness decreased from 28.4 N to 23.4 N regardless of O3 treatment. SSC increased to a peak near 17% at week 4, but then decreased during the last 2 weeks. The $≈ 4.7\%$ 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.

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 µl ozone/liter; Weight loss = 0.5 + 1.1 (days), $r^2 = 0.99$ for 0.034 µl ozone/liter; Weight loss = 0.5 + 1.4 (days), $r^2 = 0.99$ for 0.094 µl 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 CO2, 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.

**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 CO2 and ethylene production during a 9-day postharvest period.
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 O₃ concentrations.

Ripening. Fruit from the CFA without C,H₄ preconditioning had the lowest CO₂ evolution rate, and fruit from the AA + O plus C,H₄ had the highest CO₂ evolution with a peak observed 3 days following the C,H₄ preconditioning (Fig. 2). Fruit from AA + O plus C,H₄ and CFA plus C,H₄ always evolved more CO₂ than fruit from O₃ treatments without C,H₄. Plums from AA + O and CFA without C,H₄ preconditioning produced very low levels of C,H₄ during the 9 days (data not shown). Fruit from the AA + O plus C,H₄ always produced more C,H₄ than fruit from the CFA plus C,H₄. One day after C,H₄ preconditioning, fruit from AA + O plus C,H₄ had a higher rate of C,H₄ evolution than fruit from the CFA plus C,H₄. Ethylene production of fruit from AA + O plus C,H₄ 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,H₄ 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,H₄, and CFA plus C,H₄ treatments were significantly different (P > 0.05) from those of the AA + O and CFA treatments without C,H₄ preconditioning. There was no significant relationship between fruit firmness (Y) and time (day) for the AA + O (Y = 28 - 0.4 day, r = 0.24) and CFA (Y = 31 - 0.9 day, r = 0.47) treatments without C,H₄ preconditioning. However, fruit firmness for AA + O plus C,H₄ (Y = 31 - 2.9 day, r = 0.89) and CFA plus C,H₄ (Y = 29 - 1.5 day, r = 0.77) decreased significantly over the 9-day experiment period. Fruit from the AA + O plus C,H₄ became soft (13.5 N) 6 days after preconditioning, while fruit from the CFA plus C,H₄ 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 O₃ 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 (

(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 = 0.24 for 0.094 µl-liter⁻¹ (ambient + ozone); flesh firmness = 31 - 2.9 (days), r = 0.89 for 0.094 µl-liter⁻¹ (ambient + ozone + C,H₄); flesh firmness = 31 - 0.9 (days), r = 0.47 for 0.034 µl-liter⁻¹ (charcoal); flesh firmness = 29 - 1.5 (days), r = 0.77 for 0.034 µl-liter⁻¹ (charcoal + C,H₄).
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.

**Literature Cited**

Adaros, G.H., J. Weigal, and H.J. Jager. 1990. Effects of incremental ozone concentrations on the yield of bush beans (Phaseolus vulgaris var. nanus [L.] Aschers.) Gartenbauwissenschaft 4:162-167.

Brewer, R.F. and R. Ashcroft. 1983. The effects of ambient air pollution on Thompson Seedless grapes. Final Report on Air Resources Board Contract AI-132-33. The effect of present and potential air pollution on important San Joaquin Valley crops: Grapes.

Cabrera, H.S., S.V. Dawson, and C. Stromberg. 1988. A California air standard to protect vegetation from ozone. Environ. Pollution 53:397-408.

Gaffney, J.J. 1978. Humidity: Basic principles and measurement techniques. HortScience 13:551-555.

Juniper, B.E. and C.E. Jeffree, 1983. The plant surfaces in defense and attack, p. 37-45. In: B.E. Juniper and C.E. Jeffree (eds.). Plant surfaces, Edward Arnold Company.

Lehnerr, B., F. Machler, A. Grandjean, and J. Fuhrer. 1988. The regulation of photosynthesis in leaves of field-grown spring wheat (Triticum aestivum L. cv. Albis) at different levels of ozone in ambient air. Plant Physiol. 88:1115-1119.

Lutz, C. and U. Heinzmann. 1990. Surface structural and epicuticular wax composition of spruce needles after long term treatment with ozone and acid mist. Environ. Pollution 64:313-322.
Luza, J.G., R. Van Gorsel, V.S. Polito, and A.A. Kader. 1992. Chilling injury in peaches: A cytochemical and ultra-structural cell wall study. J. Amer. Soc. Hort. Sci. 117:114-118.

Mebrahtu, T., W. Mersie, and M. Rangappa. 1991. Path coefficient analysis of ozone effects on seed yield and seed yield components of bean (Phaseolus vulgaris L.) J. Hort. Sci. 66:59-46.

Mitchell, G.F. and K.K. Kader. 1992. Factors affecting deterioration rates, p. 165-178. In: J.H. LaRue and R.S. Johnson (eds.). Peaches, plums, and nectarines-growing and handling fresh market. Public. 3311, Univ. of Calif., Div. Agr. and Natural Resources, Oakland.

Musselman, R.C., W.J. Kender, and D.E. Crowe. 1978. Determining air pollution effects on the growth and productivity of ‘Concord’ grape-vines using open-top chambers. J. Amer. Soc. Hort. Sci. 103:645-648.

Nanos, G.D. and F. Gordon Mitchell. 1991. Carbon dioxide injury and flesh softening following high temperature conditioning in peaches. HortScience 26:562-563.

Olszyk, D., CR. Thompson, and M.P. Poe. 1988. Crop loss assessment for California: Modeling losses with different ozone standard scenarios. Environ. Pollution 53:303-311.

Olszyk, D.M., G. Kats, C.L. Morrison, P.J. Dawson, I. Gocka, J. Wolf, and C.R. Thompson. 1990. Valencia orange fruit yield with ambient oxidant or sulfur dioxide exposures. J. Amer. Soc. Hort. Sci. 115:878-883.

Ojanpera, K., S. Sutinen, H. Pleinjel, and G. Sellden. 1992. Exposure of spring wheat, Triticum aestivum L., cv. Drabant, to different concentrations of ozone in open-top chambers: Effects on the ultrastructure of flag leaf cells. New Phytol. 120: 39-48.

Percy, K.E., C.R. Krause, and K.F. Jensen. 1990. Effect of ozone and acidic fog on red spruce needle epicuticular wax ultrastructure. Can. J. For. Res. 20: 117-120.

Reich, P.B. and R.G. Amundson. 1985. Ambient levels of ozone reduce net photosynthesis in tree and crop species. Science 230:566-570.

Retzlaff, W.A., L.E. Williams, and T.M. DeJong. 1991. The effect of different atmospheric ozone partial pressures on photosynthesis and growth of nine fruit and nut tree species. Tree Physiol. 8:93-105.

Retzlaff, W.A., T.M. DeJong, and L.E. Williams. 1992a. Photosynthesis and growth response of almond to increased atmospheric ozone partial pressures. J. Environ. Quality 21:208-216.

Retzlaff, W.A., L.E. Williams, and T.M. DeJong. 1992b. Photosynthesis, growth, and yield response of ‘Casselman’ plum to various ozone partial pressures during orchard establishment. J. Amer. Soc. Hort. Sci. 117:703-710.

SAS. 1988. SAS/STAT User’s Guide: Release 6.03 ed. SAS Institute, Cary, NC.

Takemoto, B.K., A. Bytnerowicz, and D.M. Olszyk. 1988. Depression of photosynthesis, growth, and yield in field-grown green pepper (Capsicum annuum L.) exposed to acidic fog and ambient ozone. Plant Physiol. 88:477-482.

Temple, P.J. 1990. Growth and yield responses of processing tomato (Lycopersicon esculentum Mill.) cultivars to ozone. Environ. and Expt. Bot. 30:283-291.

Tenga, A.Z., A.M. Beverley, and D.P. Ormrod. 1990. Recovery of tomato plants from ozone injury. HortScience 25:1230-1232.

Turunen, M. and S. Huttunen. 1990. A review of the response of epicuticular wax of conifer needles to air pollution. J. Environ. Quality 19:35-45.