Project No. 89-D1 - Screening Almond Cultivars for Ozone Susceptibility

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Objectives: Determine the effects of ozone on leaf photosynthesis and tree growth of five different almond cultivars.

Interpretive Summary: An experiment was set up at the Kearney Agricultural Center (-20 SE of Fresno) to determine the susceptibility of five different almond cultivars (Nonpareil, Mission, Butte, Carmel and Sonora) to ozone, the primary air pollutant in the San Joaquin Valley. One-year old nursery trees of each cultivar were planted in 12 open-topped chambers in April 1989. The chambers were divided into three exposure treatments (four chambers/treatment). A clean-air treatment in which incoming air was charcoal-filtered served as a control treatment. The two pollution treatments were an ambient air treatment and an added ozone treatment (ozone partial pressures 2x ambient). Treatments began on June 1 and continued until November 1.

Although we have not completed the analysis of the data, there appear to be substantial differences in susceptibility to ozone between the five cultivars tested. In the twice ambient treatment, Nonpareil trees grew 40 percent less than the control treatment trees whereas the growth of the Mission cultivars was unaffected by the treatments. The Nonpareil cultivar also showed earlier leaf symptoms, decreases in leaf photosynthesis and premature leaf drop in response to the high ozone treatments. According to these measures, Mission was the least affected by ozone and the other cultivars showed intermediate levels of response.

This study documents that differences in ozone susceptibility do exist between almond cultivars grown in California. If air pollution problems continue to increase in the central valley of California it will be important to take these differences in susceptibility into account when planning for the long term viability of the almond industry.

Current Work: The trees are being excavated, dry weights taken, and the information evaluated so that a detailed final report can be written.

Photosynthesis and growth response of almond (*Prunus dulcis* (Mill) D. A. Webb syn. *P. amygdalus* Batsch) to increased atmospheric ozone partial pressures.†

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Running Title: almond response to increased atmospheric ozone

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Abstract

Uniform nursery stock of five almond cultivars (Prunus dulcis (Mill) D. A. Webb syn. P. amygdalus Batsch, cv. Butte, Carmel, Mission, Nonpareil, and Sonora) propagated on peach (P. domestica L. Batsch.) rootstock were exposed to three different atmospheric ozone partial pressures. The trees were planted in open-top fumigation chambers on 19 April 1989 at the University of California Kearney Agricultural Center located in the San Joaquin Valley of California. Exposures of the trees to three atmospheric ozone partial pressures (charcoal filtered air, ambient air, or ambient air+ozone) lasted from 1 June to 2 November 1989. The mean 12-h (0800-2000 h PDT) ozone partial pressures measured in the open-top chambers during the experimental period were 0.038, 0.060, and 0.112 μPa Pa^{-1} ozone in the charcoal filtered, ambient, and ambient+ozone treatments, respectively. Leaf net CO_2 assimilation, trunk cross-sectional area growth, and root, trunk, foliage, and total dry weight of 'Nonpareil' were reduced by increased atmospheric ozone partial pressures. 'Mission' was unaffected by ozone and 'Butte', 'Carmel', and 'Sonora' were intermediate in their responses. Foliage of 'Nonpareil' also abscised prematurely in the ambient and ambient + ozone treatments. The results indicate that there are almond cultivars that are sensitive to ozone exposure.

KEYWORDS: OZONE, PHOTOSYNTHESIS, ALMOND, PRUNUS DULCIS, CROSS-SECTIONAL AREA

Introduction

Ozone (O_3) has been shown to decrease the rate of leaf photosynthesis whether plants are exposed to low partial pressures for an extended time (Reich, 1983; Reich and Amundson, 1985; Roper and Williams, 1990; Retzlaff et al., 1991) or to acute partial pressures of the pollutant for a short time (Hill and Littlefield, 1969; Roper and Williams, 1990). Impact of O_3 on the photosynthetic apparatus begins soon after initial exposure at low partial pressures, often with no sign of visible injury (Prinz, 1988).

Photosynthetic characteristics of wheat leaves exposed to elevated atmospheric O_3 are similar to those measured in senescing leaves, suggesting O_3 induced premature leaf senescence (Lehnherr et al. 1987). Prior to abscission of normally senescing leaves, the products of protein degradation usually move out of the leaves for storage in the permanent structures of perennial crops. Nitrogen content of the leaves prior to and just following leaf fall should indicate whether O_3 exposure affects this degradation in association with premature leaf senescence. Nitrogen content of attached Nonpareil almond foliage exposed to 0.030 μPa Pa^{-1} O_3 was previously found to be higher than the nitrogen content of attached foliage from trees in 0.051 or 0.117 μPa Pa^{-1} (Retzlaff, Williams, and DeJong, unpublished data). However, cottonwood leaves that abscised prematurely after five hours of 0.20 μPa Pa^{-1} O_3 exposure had a higher nitrogen content than leaves that either senesced at the normal time from the O_3 exposed trees or leaves that senesced from trees that were not exposed to O_3 (Findlay and Jones, 1989). The above information indicates that a distinction should be made with regard to premature leaf abscission in which there is no mobilization of nitrogen and premature leaf senescence involving the normal processes of senescence (Grigal et al., 1976; Oland, 1963; Sacher, 1973).

Tree growth reductions appear to be the result of the inhibitory effect of O_3 on photosynthesis (Houston, 1974; Reich and Amundson, 1985; Steiner and Davis, 1979; Townsend, 1974; Pye, 1988; Reich, 1983; Retzlaff et al., 1991). Cooley and Manning (1987) hypothesized that growth response of perennial plants to low atmospheric O_3 partial pressures may be the result of changes in assimilate partitioning. Further, these partitioning changes follow different patterns depending upon the plant species and status (vegetative or reproductive). In a recent field study, net photosynthesis and trunk circumference of selected cultivars of almond, plum, apricot, and pear decreased linearly with increasing O_3 partial pressure (Retzlaff et al., 1991). However, in the same study nectarine, peach, and cherry cultivars were unaffected by the O_3 treatments indicating species differences in response to atmospheric O_3 pollution.

In 1989 more than 165,000 ha of bearing almond trees were growing in California with 'Nonpareil' comprising 52 % of the total and 'Butte', 'Carmel', 'Mission', and 'Sonora' comprising 1, 12, 10, and <1 percent, respectively (Tippet et al., 1989). The San Joaquin Valley fruit production region is characterized by ambient O_3 partial pressures that consistently exceed U. S. Environmental Protection Agency standards of 0.12 μPa Pa^{-1} averaged over 1-hour (Cabrera et al., 1988). Previous research has indicated that 'Nonpareil' almond is one of California's most O_3 susceptible tree crops (Retzlaff et al., 1991; McCool and Musselman, 1990). Thus, almond is potentially at risk from O_3 air pollution in the San Joaquin Valley.

Therefore, the purpose of this study was to determine the physiological and growth responses of five commercial almond cultivars to three different atmospheric partial pressures of O_3. If cultivar differences in response to O_3 occurred, we were interested in determining their relationship to premature leaf senescence and changes in assimilate partitioning.

Materials and Methods

Plant Materials and O_3 Treatments

Nursery stock of five almond cultivars (Prunus dulcis (Mill) D. A. Webb syn. P. amygdalus Batsch, cv. Butte, Carmel, Mission, Nonpareil, and Sonora) were planted 19 April 1989 in twelve permanent open-top chambers at the University of California Kearney Agricultural Center near Fresno, California (30° 40' N 119° 40' W). Trees were on Nemaguard peach (P. domestica L. Batsch) rootstock and trunk diameters at planting were 1.27 cm for 'Butte', 'Carmel', 'Mission', and 'Nonpareil' and 0.95 cm for 'Sonora'. All trees were uniformly pruned at planting to a height of 70 cm and one tree of each cultivar was planted per chamber.

Cultural practices for these trees were the same as those used commercially to establish young orchards. Trees were flood irrigated approximately once a week throughout the growing season.

The open-top chambers used in this study were igloo shaped with a 3.7 x 3.7 m square base and a 3.1 m diameter circular open-top 2.7 m above the chamber floor. Chamber frames were constructed of metal conduit with the walls consisting of 12 mil clear polyvinyl plastic. Air ducts within the chambers were two 20 cm and two 15 cm diameter PVC pipes that extended along the chamber floor from one side wall to the other (3.7 m long) equidistant from one another. Holes (5 x 13 cm) were cut in the PVC pipe 31 cm apart to permit air flow upwards into the chambers. Plastic walls were put on the chambers 29-31 May 1989 and blowers were turned on at that time. Blowers provided approximately 67.1 m^3 min^{-1} air, enough flow to change the air volume in the chambers 2 times/minute. Chamber blowers were operated 24 hours per day. Plastic chamber walls were removed on 28 November 1989.

O_3 treatments imposed in this study were charcoal filtered air (C), ambient air (A), and ambient air+O_3 (T). Treatments were randomly assigned to a chamber and there were four replications of each treatment. O_3 treatments were initiated on 1 June and continued until 2 November 1989. O_3 partial pressures in the chambers were measured with a Dasibi (Glendale, CA) Model 1003 AH O_3 Analyzer. Calibration occurred weekly and involved cleaning and frequency count checks. An Apple IIe (Cupertino, CA) microcomputer interfaced with Cyborg's (Newton, MA) Integrated System for Automated Acquisition and Control (Model 91A) permitted sequential sampling of chamber O_3 partial pressure hourly from 0800 to 2000 hr Pacific Daylight Time (PDT) daily. Chambers were connected to the monitoring system via teflon tubing and solenoid valves. Inlets for air samples were suspended 1 meter above the soil in the center of each chamber (at the approximate mid-point of the plant canopy). Air from each chamber was passed through the monitoring system for 2 minutes prior to measuring O_3 partial pressures to permit residue purging from common sampling lines and the O_3 monitor.

O_3 for the ambient air+O_3 (T) treatment chambers was generated from dry ambient air with an OREC (Phoenix, AZ) Model 03B-AR/O O_3 Generator and delivered by teflon tubing to the delivery air stream of these chambers. The air was dried by a General Cable Corp. (Westminster, CO) Puregas Heatless Air Drier (Model F200A109-132). The O_3 generator was computer automated to operate at full potential from 0800 to 2000 hr PDT. This resulted in O_3 partial pressures approximately 2 times ambient.

Final O_3 partial pressure data analysis was conducted utilizing the means procedure (PROC MEANS) of the statistical analysis system (SAS Institute, 1985). O_3 12-h means (0800-2000 hr PDT) and the cumulative number of hours greater than 0.10 and 0.20 μPa Pa^{-1} (O_3 partial pressures of 0.10 and 0.20 μPa Pa^{-1} are

equivalent to O_3 concentrations of 0.10 and 0.20 μl l^{-1}) were calculated for each treatment. These O_3 partial pressures were used to assess effects of O_3 pollution on photosynthesis and growth of five almond cultivars.

Gas Exchange

One month after treatment initiation, leaf net CO_2 assimilation was measured on all cultivars (one cultivar per day over a five day period). This process was repeated at monthly intervals and at the end of the study each cultivar had been measured five times. On each measurement day, leaf net CO_2 assimilation was measured on four leaves from each tree of a particular cultivar in every treatment/chamber (16 leaves/treatment, 48 total leaves measured per sample day). Fully expanded leaves that had been in direct sunlight prior to data collection were selected for measurement. These leaves were from similar canopy positions and were above the last fully expanded leaf on 8 June 1989 near the point where the shoots were tagged for length measurements. Measurements were made between 1030 and 1130 h. Leaf temperatures ranged from 22 to 30 °C across all dates and Photon Fluence Rate was greater than 1000 μmol m^{-2} s^{-1} before 1 November and greater than 600 μmol m^{-2} s^{-1} on 1 November. Following each measurement, sample leaves were harvested and both leaf dry weight and Kjeldahl nitrogen were determined for each leaf.

Photosynthesis measurements were made using an Analytical Development Corporation (Hoddesdon, England) Portable Infrared Gas Analyzer (IRGA) (Model LCA-2), Air Supply Unit with Mass Flowmeter (Model ASUM), Data Processor for the LCA-2 (Model DL-2), and a broad-leaf Parkinson Leaf Chamber. The IRGA was used in the differential mode. Air for the leaf chamber was taken from inside of the open-top chamber in which the tree was growing. Data were recorded on the data processor until all measurements on that particular date had been taken.

Growth Measurements

From 1 June 1989 (treatment initiation) and at monthly intervals through 1 November 1989, circumference of each tree trunk was measured. Painted bands, fifteen centimeters above the soil-line, were used as reference points in order to minimize measurement errors. Increase in trunk cross-sectional area was calculated monthly from the circumference data.

Four growing shoots per tree were tagged on 8 June 1989 above the last fully expanded leaf on all cultivars so that any increases in branch length and leaf number above this point could be determined. In all five cultivars, the shoot length and leaf number determinations were made on 30 June, 2 and 31 August, 28 September, and 7 November 1989. Trees were inspected for foliar symptoms of O_3 injury when measurements were taken. Premature leaf fall was measured by collecting leaves from the ground below each tree on 2, 24, and 31 October 1989. On 4 December 1989, all leaves on the ground below the trees were collected and any remaining foliage on the trees was stripped off in order to determine final foliage biomass.

Trees were harvested on 13 February 1990 to determine root, trunk, branch, and total biomass. Roots were removed from the ground by a backhoe and sifted from the soil by hand following the procedure of Araujo and Williams (1988). The soil removed during this procedure was dependent upon the distribution of the trees' roots within the soil volume. Soil volume sifted was approximately 1.75 m^3 from a 1.2 x 1.2 x 1.2 m hole centered around each individual tree. All tree organs were dried in a forced air oven at 70 °C until there was no further weight change.

Carbohydrate Analysis

Nonstructural carbohydrates in the roots, trunk, and branches were analyzed by high pressure liquid chromatography (HPLC) as described by Roper and Williams (1989). The trees in this study also contained measurable amounts of sorbitol and therefore it's concentration is included.

Statistical Analysis

A factorial arrangement of three O_3 partial pressures and five cultivars in a split-plot experimental design was replicated four times, with O_3 partial pressure as the main plot. The original analysis was set up in order that repeated measures of photosynthesis and growth through the time-course of the trial could be analyzed by cultivar in a split block design for O_3 partial pressure and date effects. However since there were date effects, no pooling of data across dates was included in the final analysis. So therefore, data collected on each individual date and/or only once during the study were analyzed by a two-way ANOVA. Linear contrasts with the 12-h mean O_3 partial pressures were used for a priori comparisons among treatment means ($\alpha<0.05$).

Results

O_3 Treatments

Cumulative monthly 12-h mean O_3 partial pressures (0800-2000 h PDT) peaked in July and declined to November. By November, 12-h mean O_3 partial pressures of the charcoal-filtered treatment were 63% of the ambient treatment, whereas the high O_3 treatment was 1.9 times that of ambient (Table 1). Monthly 12-h mean O_3 partial pressures also peaked in July and declined until November. The number of hours each treatment O_3 partial pressure exceeded 0.10 and 0.20 μPa Pa^{-1} also indicated large treatment differences (Table 1).

Gas Exchange

Increased atmospheric O_3 partial pressure reduced leaf net CO_2 assimilation rate in four of the five almond cultivars during the 1989 growing season (Figure 1). Leaf net CO_2 assimilation rate of 'Nonpareil' was reduced within two months after treatments were initiated (August). There were further reductions in 'Nonpareil' leaf net CO_2 assimilation on the three remaining measurement dates (September, October, and November) in the ambient and 1.9 times ambient O_3 partial pressure treatments. On the last measurement date (November), leaf assimilation rates of 'Butte', 'Carmel', and 'Sonora' also were reduced by increased atmospheric O_3 partial pressures. Increased atmospheric O_3 did not reduce leaf assimilation rate of 'Mission' during this study. It should be noted that leaf assimilation of 'Mission' in the C treatment was less than those of the other two treatments early in the study. Stomatal conductances of the five almond cultivars responded similarly to changing atmospheric O_3 partial pressures as the leaf assimilation response (data not shown).

Leaf Nitrogen

Seasonal average leaf nitrogen concentration (% dry weight) of 'Butte', 'Nonpareil' and 'Mission' was determined from leaf samples removed from the tree on each photosynthetic measurement date. Average leaf nitrogen concentration of 'Nonpareil' was reduced by increased atmospheric O_3 partial pressure (Table 2). Leaf nitrogen of 'Butte' was reduced in the T treatment. 'Mission' leaf nitrogen was unchanged by increased atmospheric O_3 partial pressure.

Tree Growth

Trunk cross-sectional area growth (through 1 November) of 'Butte' and 'Nonpareil' was significantly reduced in the T treatment compared to the C

treatment (Figure 2, Table 3). Cross-sectional area growth of 'Carmel', 'Mission', and 'Sonora' was not affected by increasing O_3 partial pressures.

Although shoot growth continued throughout the treatment period, branch length and leaf number of all five cultivars were unaffected by increasing O_3 partial pressure (data not shown). Shoot extension growth was highly variable and there were no significant response trends.

Above-ground biomass (leaf, branch, and trunk weight) of 'Butte' and 'Nonpareil' decreased with increasing atmospheric O_3 partial pressure (Figure 3; Table 3). Above-ground biomass of 'Carmel', 'Mission', and 'Sonora' was not affected by increased atmospheric O_3 partial pressure. Below-ground biomass (root dry weight) of 'Butte', 'Nonpareil', and 'Sonora' decreased with increasing atmospheric O_3 partial pressure (Figure 3; Table 3). Root dry weight of 'Carmel' and 'Mission' was not affected by increased atmospheric O_3 partial pressure. Overall, total tree biomass (total dry weight) of 'Butte' and 'Nonpareil' decreased with increasing atmospheric O_3 partial pressure (Figure 4; Table 3). Total tree biomass of the remaining cultivars was not affected by increased atmospheric O_3 partial pressure. The shoot/root ratio of 'Butte' and 'Nonpareil' decreased in the A treatment compared to the C treatment, but increased in the T treatment (Figure 4; Table 3). Shoot/root ratios of 'Carmel', 'Mission', and 'Sonora' increased with increasing atmospheric O_3 partial pressure.

Carbohydrates

The concentration of nonstructural carbohydrates (sugars, starch, and total (sugar + starch)) on a dry weight basis for the permanent organs of all five cultivars was unaffected by increasing atmospheric O_3 partial pressures (data not shown, can be calculated using data in Table 4 and Figure 3). Total nonstructural root carbohydrates of 'Butte' and 'Nonpareil' and the trunk

carbohydrates of 'Nonpareil' were reduced in the T treatment when compared to the C treatment on a per tree basis (Table 4). Total nonstructural carbohydrates in the above mentioned organs of the remaining three cultivars were unaffected by increasing atmospheric O_3 partial pressure.

Foliar Injury

Visual injury was observed on the foliage of 'Nonpareil' growing in the T treatment just prior to the first photosynthesis measurements (1 July 1989). Initially, visible injury consisted of chlorotic spots and yellow flecking on the leaf surfaces of older foliage. As time progressed, these chlorotic areas became larger and turned brown due to tissue necrosis. As the trees aged, foliar O_3 injury appeared on more and more of the older foliage. Signs of foliar injury were less pronounced on 'Butte', 'Carmel', 'Sonora', and 'Mission'. In these cultivars, injury occurred on the older foliage, but only appeared at the very end of the exposure period.

Soon after visible injury became evident in the T treatment, some of the lower/older leaves abscised. Leaves that were collected under the trees on 2 and 31 October 1989 indicated that more foliage abscised from 'Carmel', 'Nonpareil', and 'Sonora' in the T treatment than in the C treatment during this time period (Table 5). Increased atmospheric O_3 partial pressures had no effect on leaf abscision of 'Butte' and 'Mission'. Nitrogen concentrations of attached and abscised leaves of the 'Mission' were relatively unaffected by O_3 treatment and abscised leaves had about two-thirds as much nitrogen as the attached leaves (Table 2). On the other hand, leaf nitrogen concentration of the attached 'Nonpareil' leaves in the T treatment were significantly lower than in the C treatment and similar to the abscised leaves of all the O_3 treatments. The response of 'Butte' leaves was similar to that of 'Nonpareil' leaves.

Discussion

Leaf net CO_2 assimilation of 'Nonpareil' was lower in air containing 1.9 times ambient O_3 partial pressures compared to charcoal filtered air two months after treatments were initiated (Figure 1). Similar results for this cultivar were reported in a study conducted in 1988 (Retzlaff et al., 1991). In both studies, a decrease in leaf net CO_2 assimilation rate to increased atmospheric O_3 was detected soon after treatments were initiated and remained apparent on each subsequent measurement date. Decreases in leaf net CO_2 assimilation of 'Butte', 'Carmel', and 'Sonora' in the present study did not develop as rapidly as those of 'Nonpareil', but by the end of the study period decreases in leaf net CO_2 assimilation of these three cultivars were detected in the 1.9 times ambient O_3 partial pressure treatments compared to the trees grown in air containing less O_3. In contrast, increased O_3 partial pressure did not reduce leaf net CO_2 assimilation rate of 'Mission'. Leaf assimilation of 'Mission' in the charcoal filtered treatment was initially lower than that measured in the other two treatments. Later in the season, the Mission leaf assimilation rates in the charcoal filtered air were approximately the same as those in the other two treatments and similar to the other cultivars in the charcoal filtered treatment. The reason for the initial low leaf CO_2 assimilation rates of 'Mission' trees in the charcoal filtered chambers are unknown. Differences in leaf CO_2 assimilation response to increased O_3 partial pressures have been reported previously within the Prunus genus (Retzlaff et al., 1991). However, those differences were among individual species within the Prunus genus, not among different cultivars within a single species as reported here.

Plants with high rates of leaf CO_2 assimilation have high uptake rates of O_3 and as a consequence respond more negatively to increased O_3 partial pressures (Reich and Amundson, 1985). Leaf net CO_2 assimilation rates of 'Butte',

'Carmel', 'Mission', 'Nonpareil', and 'Sonora' grown in charcoal filtered air, averaged across all measurement dates, were 28.3± 1.4 (± SE), 24.6± 1.5, 22.0± 1.5, 25.8± 1.5, and 24.4± 1.2 μmol CO_2 m^{-2} s^{-1}, respectively. Thus, the photosynthetic potential was similar among all the cultivars and the potential uptake of O_3 by all cultivars should be similar. Therefore, the insensitivity to O_3 by 'Mission' and intermediate responses by 'Butte', 'Carmel' and 'Sonora', when compared to 'Nonpareil', apparently were not due to exclusion of O_3 by stomatal regulation (the response of stomatal conductance to O_3 was similar to that of the leaf CO_2 assimilation response to O_3, data not shown) as has been shown for Phaseolus vulgaris L. (Butler and Tibbitts, 1979).

Biochemical mechanisms that may impart resistance to a particular air pollutant would include the ability to detoxify the reactive species, an increased threshold for the pollutant, and the ability to repair pollutant damage quickly (Tingey and Taylor, 1982). 'Mission', and to a lesser extent 'Butte', 'Carmel', and 'Sonora', apparently have one or more of the above described mechanisms enabling them to reduce the adverse effects of O_3 on metabolism within the leaf.

Increased atmospheric O_3 partial pressure resulted in reduced cross-sectional area growth and biomass accumulation (Figures 2 and 3) of the almond cultivars in which photosynthesis was reduced and premature leaf abscission occurred (Table 5). Cross-sectional area relative growth rate of 'Nonpareil' almond previously was shown to decrease linearly with increased atmospheric O_3 partial pressure (Retzlaff et al., 1991). Further, trunk caliper of 'Nonpareil' almond was reduced following a two year exposure regime of 0.25 μPa Pa^{-1} O_3 (McCool and Musselman, 1990). The lack of a cross-sectional area growth response in 'Mission', 'Carmel', and 'Sonora' apparently is related to the lack of a reduction in photosynthesis to increased atmospheric O_3 partial pressures until

late in the season. Similarly, in red spruce (Picea rubens), neither photosynthesis nor growth were affected by a range of atmospheric O_3 partial pressures (Taylor et al., 1986; Laurence et al., 1989).

A conceptual model has been proposed to describe the effects of O_3 on the partitioning of assimilates in plants (Cooley and Manning, 1987). It states that under conditions of low partial pressures of O_3 (0.05 - 0.10 μPa Pa^{-1}) perennial plants divert assimilate to leaves at the expense of the roots, while at higher partial pressures (> 0.10 μPa Pa^{-1}) partitioning of carbon to all sinks is decreased (due to a reduction in the production of photosynthates) with a concomitant reduction in growth. Results obtained in this study allowed us to examine differences in the partitioning of assimilates of almond cultivars that were either sensitive or tolerant to O_3, and to relate this data to the model of Cooley and Manning.

The accumulation of total tree biomass by 'Carmel', 'Mission', and 'Sonora' was not significantly affected by increasing O_3 partial pressures (Figure 3). However, as the mean seasonal O_3 partial pressure increased the shoot/root ratio of these three cultivars increased, albeit small for 'Mission' (Figure 4). There was a linear reduction in biomass accumulation as a function of the mean seasonal O_3 partial pressure for 'Butte' and 'Nonpareil'. The shoot/root ratio for both of these cultivars decreased in going from 0.038 to 0.060 μPa Pa^{-1} O_3 and then increased at the highest seasonal mean O_3 partial pressure (0.112 μPa Pa^{-1}). The three less sensitive cultivars ('Carmel', 'Sonora', and 'Mission') responded as predicted by Cooley and Manning (1987). Less carbon was allocated to the roots and more to the above ground organs such that total tree biomass was unchanged in the three O_3 treatments (Figures 3 and 4). This was despite the fact that the actual amount of biomass allocated to the leaves was constant across the seasonal mean O_3 partial pressures for all three cultivars (Figure 3).

The amount of carbon allocated to the leaves and branches of the more O_3 sensitive ('Butte' and 'Nonpareil') cultivars decreased as the seasonal mean O_3 partial pressures increased. This does not agree with the model of Cooley and Manning (1987). The sensitive cultivars responded to O_3 partial pressures less than 0.10 μPa Pa^{-1} as would have been predicted by the model to O_3 partial pressures greater than 0.10 μPa Pa^{-1}. The exception was the fact that the shoot/root ratio decreased at the ambient O_3 partial pressure. The results presented here and elsewhere (Mortenson and Skyre, 1990) indicate that partitioning of dry matter will differ depending upon both the sensitivity of the plant to O_3 and the partial pressure to which the plants are exposed.

There was no effect of O_3 partial pressure on the concentration of nonstructural carbohydrates in the permanent structures of the trees. However, as biomass of an organ decreased due to increased partial pressures of O_3, the total amount (g $tree^{-1}$) of nonstructural carbohydrates found in that organ decreased (Table 4). This differs from the assumptions of Cooley and Manning (1987) and results of a study on Pinus ponderosa Laws. seedlings (Tingey et al., 1976). It is believed that air pollution may cause photosynthates to be retained in the leaves or stems either due to a reduction in their translocation or increased utilization due to a stimulation of respiration needed for repair processes. However for almond, while increasing O_3 partial pressures decreased the allocation of carbon to an organ, due to less available photosynthate, the relative partitioning of carbohydrates for structural and nonstructural purposes did not change. Data similar to this (reduced carbon allocation but no changes in carbon partitioning) have been found when field-grown grapevines (Vitis vinifera L.) are irrigated at rates resulting in different soil water contents, including deficits (L.E. Williams, unpublished data).

It is interesting to note that the dry weight of the 'Carmel', 'Mission' and 'Sonora' trees actually increased from the charcoal filtered air treatment (0.038 μPa Pa^{-1}) to the ambient (0.060 μPa Pa^{-1}) O_3 treatment. All trees in this study were pruned to the same approximate fresh weight at planting (229 g $tree^{-1}$). Even though 'Butte' and 'Nonpareil' responded negatively to increased O_3 partial pressures, compared to the other three cultivars, final tree dry weight of the two was the greatest in the ambient treatment and 'Butte' was the greatest in the twice ambient treatment (Figure 4). In the charcoal filtered treatment the average dry weight of 'Butte' and 'Nonpareil' (2400 g $tree^{-1}$) was approximately 70% greater than the average weight (1400 g $tree^{-1}$) of the other three cultivars. The rapid growth of these two cultivars in the charcoal filtered air chambers may have reduced the availability of solar radiation, mineral nutrients, or water to the other three cultivars, reducing their potential growth. Thus, when 'Butte' and 'Nonpareil' experienced stress (ambient O_3 partial pressures) and their growth was reduced, 'Carmel', 'Mission', and 'Sonora' were able to compete more effectively inside the ambient O_3 chambers and their growth was greater than that in the charcoal filtered treatment.

The data compiled in Figure 4 is similar to that found with tomato (Lycopersicon esculentum Mill.). Temple (1990) found that the most productive tomato cultivar was the most susceptible to O_3 and the least productive the most resistant. In this study the fastest growing almond cultivars were the most sensitive to increased O_3 partial pressures. However, unlike the tomato study there appears to be a direct relationship between reduction in leaf CO_2 exchange (Figure 1) and vegetative tree growth.

Foliage discoloration followed by leaf abscission often occurs following extended periods of chronic O_3 exposure (Prinz, 1988). Keller (1988) found that the life span of leaves of O_3-sensitive aspen clones was shortened by increased

atmospheric O_3 partial pressures. The above and additional data of Lehnherr et al. (1987) and Reich and Amundson (1985) indicates that ambient partial pressures of O_3 may cause premature leaf senescence. The processes of leaf senescence involve the recovery of mineral nutrients by the permanent structures of perennial plants (Grigal et al., 1976; Oland, 1963; Sacher, 1973). If O_3 causes premature leaf senescence followed by abscission (as opposed to just premature leaf abscission), then the concentrations of mineral elements such as nitrogen in leaves that have abscised should be similar to leaves that have not been exposed to O_3 but have aged naturally. There were no differences among O_3 treatments in the concentration of nitrogen in fallen leaves regardless of cultivar sensitivity to O_3 or when during the experimental period the leaves abscised (Table 2). This supports the contention that ambient O_3 partial pressures do cause premature leaf senescence. It would also indicate that chronic O_3 stress does not interfere with the recovery of mineral nutrients from leaves of perennial plants during senescence.

The lowered nitrogen concentrations of attached leaves exposed to increasing O_3 partial pressures of the sensitive cultivars (Table 2, leaves used to measure net CO_2 assimilation rate) during the growing season also indicates the remobilization of nitrogen long before leaf abscission. This differs from the results of Tingey et al. (1976). They found that total nitrogen in the tops (needles and stems) of P. ponderosa seedlings exposed to elevated partial pressures of O_3 were similar to the controls. The reduction in nitrogen in the leaves prior to abscission would also lead to a reduction in the capacity of those leaves to fix CO_2 since there is a linear relationship between leaf nitrogen and net CO_2 assimilation rate for several perennial fruit crop species (DeJong, 1982; Williams and Smith, 1985). However, the remobilization of nitrogen out of the sensitive almond cultivar leaves was not the sole reason for

a reduction of net CO_2 assimilation rate in this study. When leaf net CO_2 assimilation was expressed as a function of leaf nitrogen (either nitrogen concentration or nitrogen per unit leaf area) the rate was always lower than would have been predicted if there had been a linear relationship between leaf net CO_2 assimilation and nitrogen in this study. For example, net CO_2 assimilation rate expressed per unit leaf nitrogen of 'Nonpareil' on the September measurement date for the C, A and T treatments were 0.11, 0.11 and 0.05 μmol CO_2 mmol N^{-1} s^{-1}, respectively. As outlined by Heath (1980), there are numerous other possible metabolic causes for the additional reduction in leaf CO_2 assimilation of the sensitive almond cultivars.

The O_3 response differences in the five almond cultivars appears to be related to their different genetic backgrounds. 'Nonpareil' and 'Mission', which responded to O_3 at opposite ends of the spectrum, are either scions or seedlings of distinctly different European cultivars (Hauagge et al., 1987). 'Butte', which had an O_3 response similar to 'Nonpareil', resulted from a cross of 'Mission' (female) and 'Nonpareil' (male) (Hauagge et al., 1987). 'Carmel', with no or slight O_3 responses, similar to 'Mission', is a cross of 'Nonpareil' (female) and 'Mission' (male) (Hauagge et al., 1987). Similarly, 'Sonora' is a cross of 'Nonpareil' (female) and 'Eureka' (male), with 'Eureka' having 'Mission' as a parent (Kester et al., 1984). The lack of effects of chronic O_3 exposure on some of the almond cultivars indicates that it may be possible to breed or select for O_3 tolerance in almond.

To date, no correlation between decreases in photosynthesis and/or growth and productivity in response to O_3 has been established for deciduous fruit and nut trees. Yield losses of 10% or greater have been documented in citrus and annual agricultural crops at current ambient atmospheric O_3 partial pressures experienced in California (Olszyk et al., 1988; Olszyk et al., 1990). A ten

percent loss in 'Nonpareil' almond yield could reduce the total production value of almonds in California by approximately 26 million dollars. Preliminary yield data from a study currently being conducted by the authors with another Prunus species at the Kearney Agricultural Center indicates that losses due to ambient O_3 are much greater than ten percent.

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Table 1. Cumulative 12-hour (0800-2000 h PDT) mean O_3 partial pressures and the cumulative number of hours greater than 0.1 and 0.2 μPa Pa^{-1} for the experimental period from 1 June to 2 November 1989.

Treatment†	From 6/1/89 through 11/2/89	
	(μPa Pa^{-1})	
C	0.038‡	Cumulative Mean
A	0.060	Cumulative Mean
T	0.112	Cumulative Mean
	(# hours treated)	
C	1785	Total
	0	Cumulative Hours >0.1
	0	Cumulative Hours >0.2
A	1785	Total
	99	Cumulative Hours >0.1
	0	Cumulative Hours >0.2
T	1785	Total
	1017	Cumulative Hours >0.1
	96	Cumulative Hours >0.2

†C, A, and T refer to the charcoal filtered, ambient, and ambient + O_3 treatments, respectively.

‡Standard errors of all values are less than 1% of the mean.

Table 2. Leaf nitrogen concentration (% dry weight) of attached and abscised foliage from three almond cultivars exposed to season-long atmospheric O_3 partial pressures.

		Nitrogen†	
		Attached	Abscised
		---------- (% dry weight) ----------	
	C‡	2.0 (0.1)§	1.7 (0.1)
Butte	A	2.1 (0.2)	1.8 (0.1)
	T	1.7 (0.1)	1.8 (0.1)
	P>F¶	*	NS
	C	3.0 (0.1)	2.0 (0.1)
Mission	A	3.0 (0.1)	2.0 (0.2)
	T	2.8 (0.1)	1.8 (0.2)
	P>F	NS	NS
	C	3.0 (0.1)	2.0 (0.1)
Nonpareil	A	2.6 (0.1)	1.9 (0.2)
	T	2.3 (0.1)	2.2 (0.1)
	P>F	*	NS

† Nitrogen from attached and abscised refer to leaves removed from trees following photosynthesis measurements (averaged across all dates) and leaves collected after they had fallen from the tree.

‡ C, A, and T refer to the charcoal filtered, ambient, and ambient + O_3 treatments, respectively.

§ Values in parenthesis represent one standard error.

¶ A significant linear treatment effect (*) indicates that, within a cultivar, a decline in response associated with a linear increase in O_3 partial pressure is significant at the 5% level. n = 20 (attached) n = 4 (abscised).

Table 3. Probabilities of statistically significant linear treatment effects of O_3 on dry weights (Figures 3 and 4) and cross-sectional area growth (Figure 2) of five almond cultivars exposed to season-long atmospheric O_3 partial pressures.

	Root	Trunk	Branch	Foliage	Total	S/R	Cross-sectional Area Growth
Butte	*†	NS	*	NS	*	NS	*
Carmel	NS	NS	NS	NS	NS	NS	NS
Mission	NS	NS	NS	NS	NS	NS	NS
Nonpariel	*	*	NS	*	*	NS	*
Sonora	NS	NS	NS	NS	NS	*	NS

† A significant linear treatment effect (*) indicates that, within a cultivar, a decline in response associated with a linear increase in O_3 partial pressure is significant at the 5% level.

Table 4. Total nonstructural carbohydrates in the permanent organs of five almond cultivars exposed to season-long atmospheric O_3 partial pressures.†

		Root‡	Trunk‡	Branch‡
		---------- (g tree^{-1}) ----------		
Butte	C	139 (18)	25 (3)	85 (7)
	A	126 (10)	29 (1)	63 (8)
	T	91 (18)	29 (3)	59 (3)
	P>F	*	NS	NS
Carmel	C	107 (33)	20 (5)	40 (16)
	A	84 (11)	20 (3)	43 (14)
	T	74 (20)	19 (2)	42 (3)
	P>F	NS	NS	NS
Mission	C	87 (10)	23 (3)	42 (4)
	A	98 (18)	23 (5)	61 (22)
	T	92 (18)	25 (4)	61 (5)
	P>F	NS	NS	NS
Nonpareil	C	113 (22)	25 (3)	68 (10)
	A	121 (23)	28 (5)	61 (19)
	T	59 (16)	14 (4)	33 (9)
	P>F	*	*	NS
Sonora	C	70 (14)	13 (3)	29 (12)
	A	82 (14)	17 (2)	31 (7)
	T	48 (7)	15 (2)	27 (3)
	P>F	NS	NS	NS

† Other information as found in Table 2. n = 4.

‡ Total Nonstructural Carbohydrate = Percent Total Nonstructural Carbohydrate X Dry Weight.

Table 5. Cumulative leaf dry weight that had fallen prior to 2 and 31 October and 4 December from five almond cultivars exposed to season-long atmospheric O_3 partial pressures.†

		10/2/89	10/31/89	12/4/89
		---------- (g tree^{-1}) ----------		
	C	18 (4)	48 (10)	514 (56)
Butte	A	13 (3)	37 (7)	429 (38)
	T	19 (3)	58 (5)	371 (47)
	P>F	NS	NS	NS
	C	9 (2)	23 (5)	232 (55)
Carmel	A	9 (2)	28 (9)	311 (83)
	T	29 (7)	67 (7)	292 (33)
	P>F	*	*	NS
	C	6 (2)	18 (4)	294 (15)
Mission	A	8 (2)	27 (6)	303 (44)
	T	9 (3)	31 (7)	312 (27)
	P>F	NS	NS	NS
	C	18 (2)	36 (2)	540 (106)
Nonpareil	A	20 (5)	40 (10)	443 (44)
	T	50 (14)	97 (22)	206 (43)
	P>F	NS	*	*
	C	16 (6)	30 (10)	293 (45)
Sonora	A	12 (3)	29 (8)	289 (52)
	T	20 (3)	57 (5)	281 (45)
	P>F	NS	*	NS

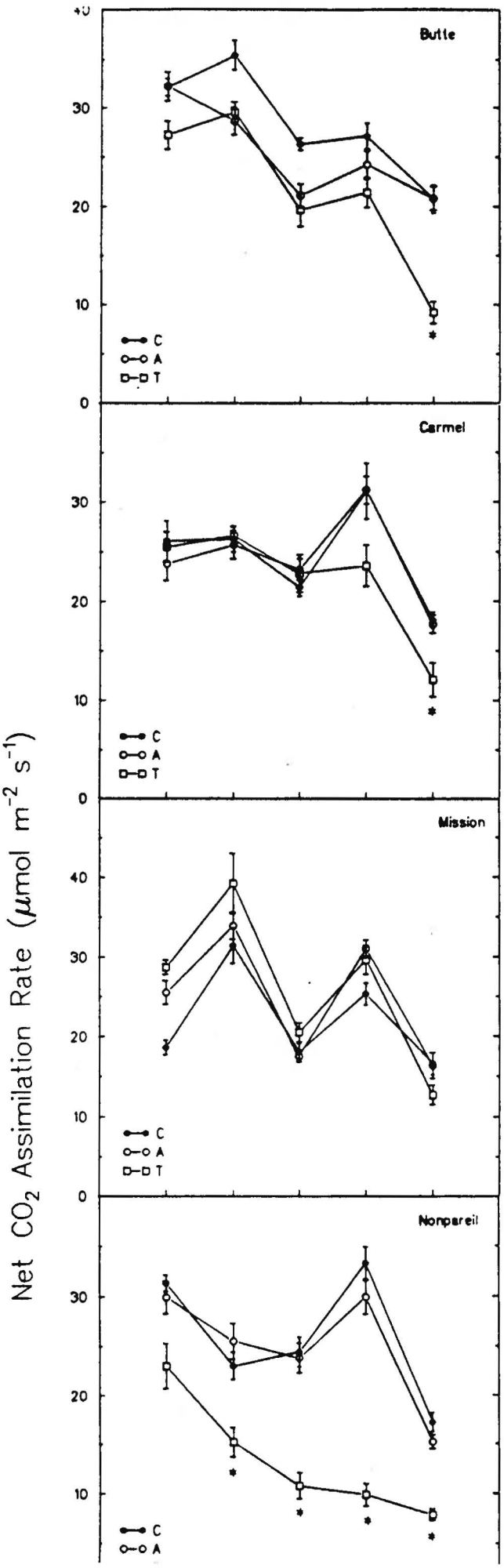
† Other information as found in Table 2. n = 4.

Figure 1. Leaf net CO_2 assimilation (measured at monthly intervals) of five almond cultivars exposed to season-long atmospheric O_3 partial pressures. Vertical bars represent ± one standard error. Asterisks (*) represent dates on which there was a significant linear treatment effect ($\alpha<0.05$). n = 16.

Figure 2. Trunk cross-sectional area growth from 1 June to 1 November 1989 of five almond cultivars exposed to season-long atmospheric O_3 partial pressures. Vertical bars represent ± one standard error. Probabilities of statistically significant linear O_3 treatment effects are shown in Table 3. n = 4.

Figure 3. Leaf, branch, trunk, and root dry weight of five almond cultivars exposed to season-long atmospheric O_3 partial pressures. Probabilities of statistically significant linear O_3 treatment effects are shown in Table 3. n = 4.

Figure 4. Total dry weight and shoot/root ratio of five almond cultivars exposed to season-long atmospheric O_3 partial pressures. Probabilities of statistically significant linear O_3 treatment effects are shown in Table 3. n = 4.


Butte
Carmel
Mission
Nonpareil
C
A
T
Net CO_2 Assimilation Rate (μmol m^{-2} s^{-1})

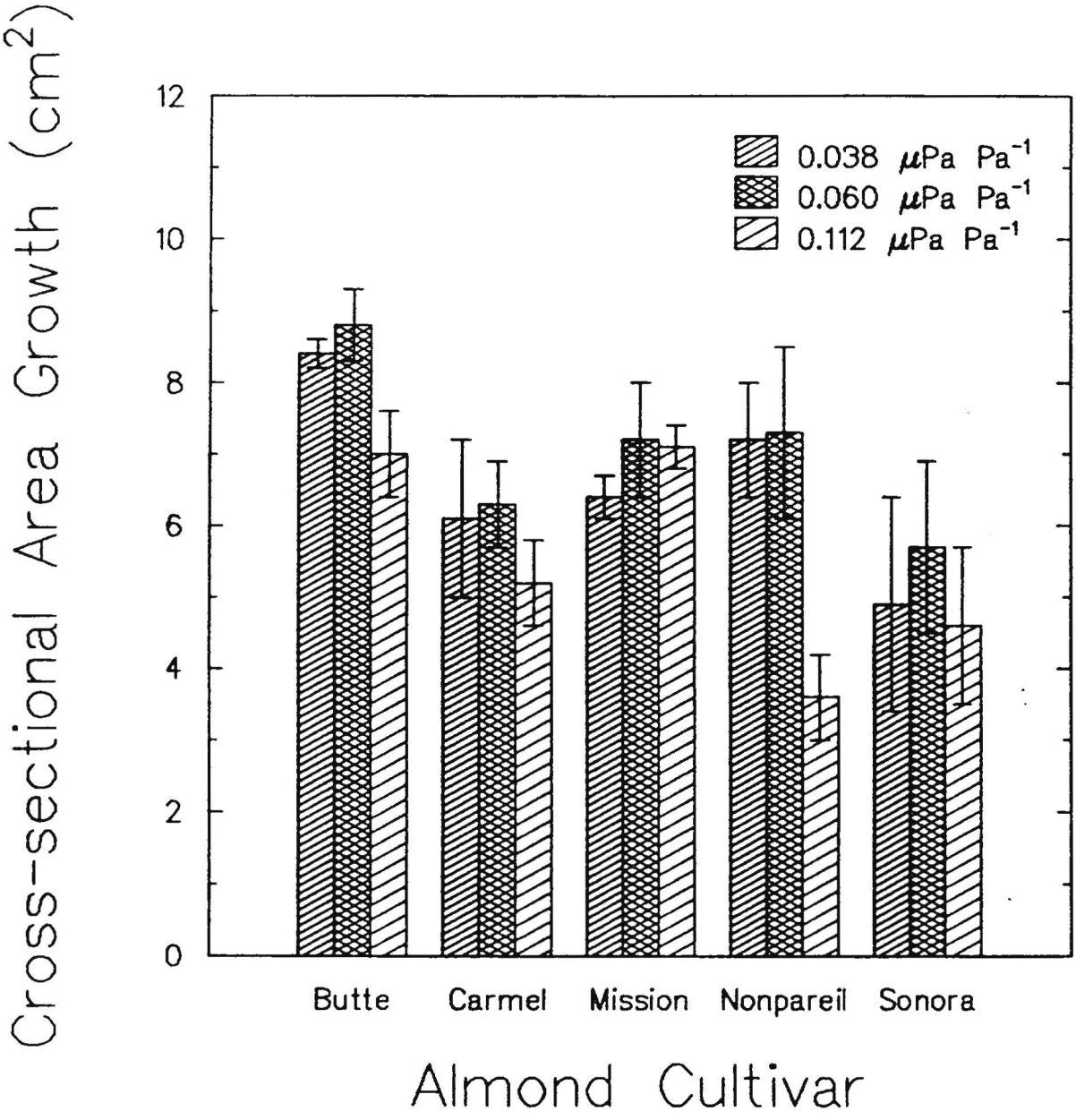
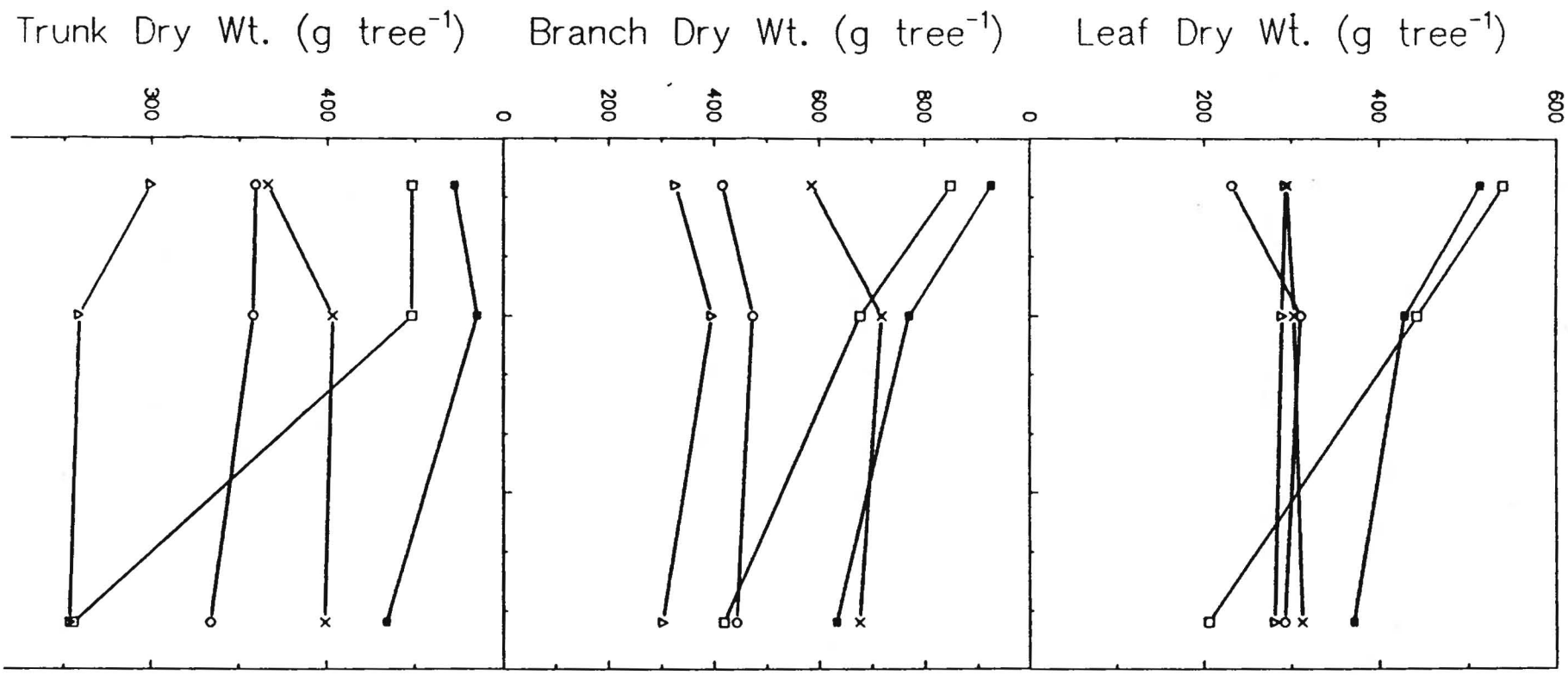

Cross-sectional Area Growth (cm^2)
12
10
8
6
4
2
0
0.038 μPa Pa^{-1}
0.060 μPa Pa^{-1}
0.112 μPa Pa^{-1}
Butte
Carmel
Mission
Nonpareil
Sonora
Almond Cultivar

Figure 2.


Trunk Dry Wt. (g tree^{-1})
Branch Dry Wt. (g tree^{-1})
Leaf Dry Wt. (g tree^{-1})
300
400
0
200
400
600
800
0
200
400
600

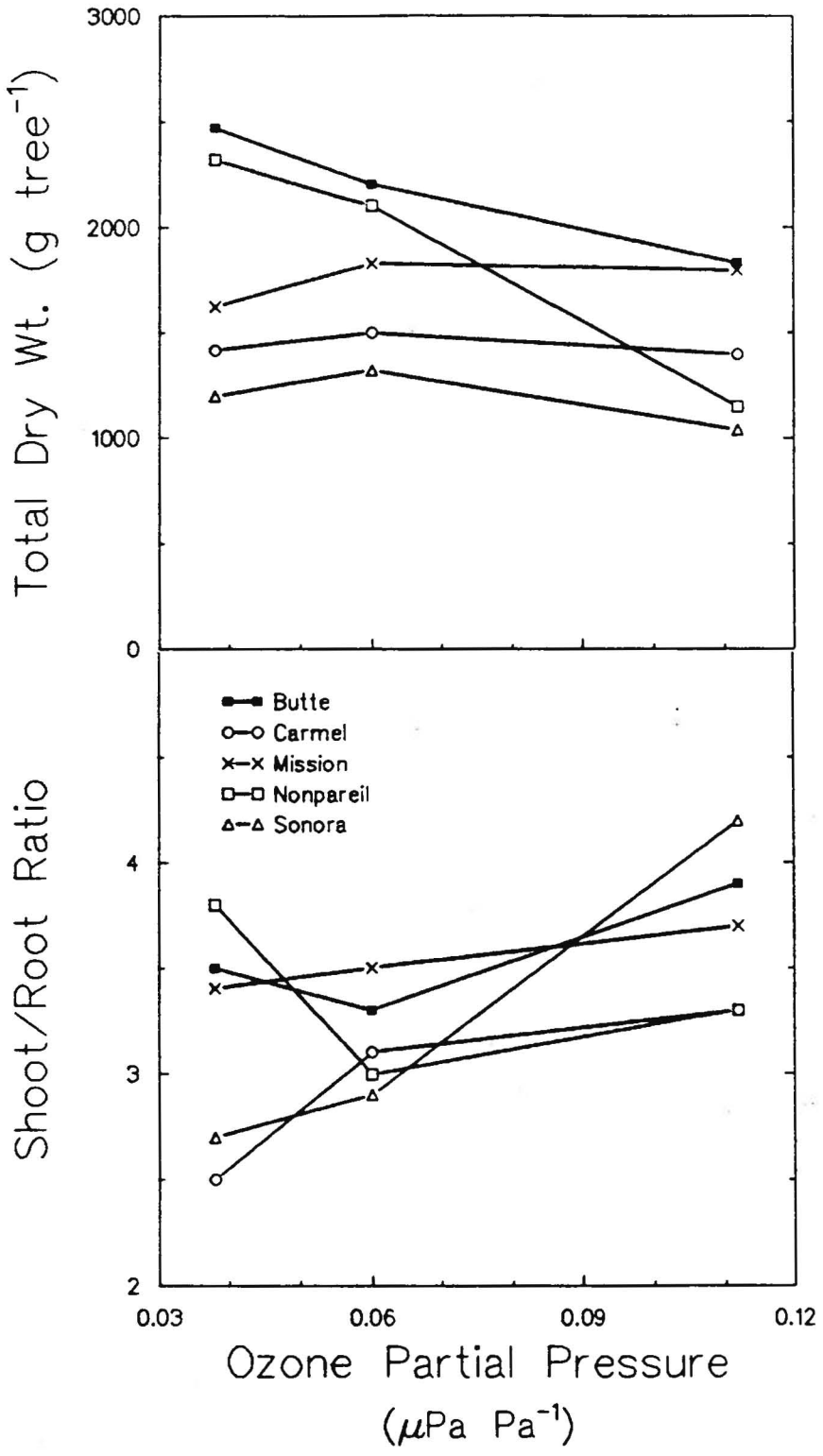

3000
2000
1000
0
Total Dry Wt. (g tree^{-1})
Butte
Carmel
Mission
Nonpareil
Sonora
4
3
2
Shoot/Root Ratio
0.03
0.06
0.09
0.12
Ozone Partial Pressure
(μPa Pa^{-1})

Figure 4.