THE GROWTH AND YIELD EFFECTS OF AMBIENT AIR POLLUTION ON VALENCIA ORANGE TREES

Final Report
to the
California Air Resources Board
Contract No. A733-087
December 21, 1987 - September 30, 1989

David M. Olszyk, Principal Investigator

August 1989

Statewide Air Pollution Research Center

University of California

ARB-88(6):mechba

ABSTRACT

This study was conducted to provide detailed information concerning the effects of ambient oxidants [measured as ozone (O_3)] and added sulfur dioxide (SO_2) on young orange trees [Citrus sinensis (L.) Osbeck]. Valencia orange trees were used, as they are grown in areas subject to air pollution and have not been studied for air pollution effects. The exposures were initiated in May 1984 with four chamber treatments: filtered air, filtered air plus 0.09 ppm SO_2 (continuously), half-filtered and half-ambient air, and ambient air. Outside control trees were used to determine chamber effects. There were seven trees per treatment. The ambient air pollutant exposures were terminated in August 1988. The SO_2 treatment ended in November 1987. Tree response to air pollutants was documented in terms of fruit yield and quality, leaf physiology and biochemistry, and leaf biomass production per tree.

Ambient oxidants dramatically reduced orange fruit yields for the first two harvests. A linear equation described the relationship between O_3 concentration in the oxidant treatment and orange yields across both years according to the formula: total fruit weight per tree in kg = 53.7 - (261.1 x O_3 average). The O_3 average was for all hourly values between 0800 and 2000 from April through October during the summer two years before the harvest year. The reduced fruit weight with oxidant exposure was associated primarily with reduced number per tree. Oxidants had little effect on fruit quality except for a slightly less orange color. Orange yields for all treatments decreased in 1988, indicating an "off" productivity year in 1988 vs "on" years in 1986 and 1987. Oxidants had no effect on orange yields or fruit quality in 1988.

Ambient oxidants had no effect on overall tree growth, leaf production, immature fruit loss, or flower drop. Individual leaves weighed less with higher oxidant concentrations. Oxidants resulted in stomatal closure and more negative leaf water potentials, indicating increased moisture stress to leaves. Net photosynthetic rate was not affected by oxidants. Leaf starch prior to flowering was higher with increasing oxidant concentrations, indicating an effect on carbon allocation which may be affecting flowering or fruit set. No other biochemical indicators were affected by oxidants.

Sulfur dioxide (applied continuously to orange trees at approximately 0.09 ppm) reduced fruit yields significantly in 1986 and 1987. Yields for SO_2 exposed trees were 23 and 35% lower than for filtered air trees in 1986 and 1987, respectively. The reduced yield for the SO_2 trees was associated with both reduced numbers and size of fruit. Sulfur dioxide exposure resulted in more elliptical fruit, but no other quality effects were found. Individual leaves weighed less with SO_2 exposure. Sulfur dioxide resulted in a higher leaf transpiration rate than for filtered trees, but had no overall effect on stomatal conductance, net photosynthetic rate, or leaf water potential. Leaf total sulfur concentration was increased with SO_2 exposure, but no other biochemical changes were found.

The chambers themselves had many effects on the orange trees. Many fruit were produced on chamber trees in 1986, compared to virtually no fruit on outside trees. Weight of fruit was 39% and 106% higher for 1987 and 1988, respectively, for ambient chamber trees vs. outside trees. Chamber tree fruit were larger, heavier, and had less acidic juice than fruit on outside trees. Growth and leaf production were much greater for chamber trees than for outside trees. Immature fruit drop was lower, whereas flower production was greater for chamber trees than outside trees. Some physiological and biochemical responses indicated more stress to leaves on chamber trees than to leaves on outside trees, e.g., higher stomatal conductance, more negative leaf water potential, and greater leaf starch. Other responses indicated less stress to leaves on chamber trees, e.g., higher photosynthetic rates, higher chlorophyll concentrations, and less weight per unit area compared to leaves on outside trees.

Therefore, the results collected to date clearly document the effects of air pollutants on Valencia oranges. However, additional research is needed to determine the impact of the chambers themselves on tree responses and the mechanistic bases for the oxidant and SO_2 effects.

ACKNOWLEDGMENTS

The author wishes to thank the following research staff of the Statewide Air Pollution Research Center for their efforts on this project: for technical assistance, Dr. Brent Takemoto, Mr. Gerrit Kats, Mr. Phil Dawson, Mr. Lynn Morrison, Ms. Wendy Hutton, Ms. Joanne Wolf, Mr. Ian Gocka, and Dr. C. Ray Thompson; for word processing assistance, Ms. Barbara Crocker and Ms. Christy LaClaire. Also we would like to thank Dr. Homero Cabrera (Project Monitor, Air Resources Board) for his advice and encouragement.

DISCLAIMER

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES
(continued)

LIST OF FIGURES

SUMMARY AND CONCLUSIONS

California has long been a major United States producer of tree fruit crops. The most important of these crops, oranges, are grown on about 175,000 acres on some of the State's most productive land. Counties with major production have been Tulare, Fresno, Kern, Ventura, and San Diego, with lesser amounts in adjoining areas. Total annual production was in excess of 2.0 million tons valued at $423 million in 1987. Valencia oranges (used for juice) accounted for approximately 43% of the volume produced. Most areas of production presently have had photochemical oxidant (primarily O_3), and some areas have SO_2 pollution which may reduce yields. However, few studies have been available which indicate the amount of yield and/or economic losses.

During the late 1950's and early 1960's, Taylor, Thompson, and coworkers at the University of California, Riverside (UCR) studied the chronic (low level, long-term) effects of photochemical oxidants and/or fluoride on Navel oranges and lemons grown in the Los Angeles Basin. These studies showed reduced water use, reduced apparent photosynthesis, increased leaf drop, and very substantial reduction in yields of both crops due to photochemical oxidants. Losses of one-third to one-half total production were recorded in different years even though there were no easily observed leaf injury symptoms on the trees.

However, the sensitivity of the orange trees to ambient pollutants may have been different from that of outside trees, as the experiment was conducted in closed, plastic-covered greenhouses. A complicating factor was that there were only filtered and ambient air treatments, and little accurate air monitoring data from the different treatments. Thus, it was not possible to produce accurate dose-response models to describe the relationship between O_3 exposure (a surrogate for oxidants) and orange yield. Such models have become necessary for interpreting current O_3 and orange yield data for different counties, such as those used for the California Air Resources Board (ARB) Crop Loss Assessment Project. Besides the oxidant effects, the susceptibility of orange trees to long-term, low-level "chronic" exposure to SO_2 was not known.

Thus, to address the effects of air pollutants on citrus, the ARB in early 1983 funded a study to investigate physiological, growth, and yield responses of Valencia orange trees [Citrus sinensis (L.) Osbeck] to ambient oxidants or added SO_2.

Specific research questions to be addressed were:

(1) What are the yield effects of photochemical oxidants on orange trees based on current levels of oxidants found in the San Joaquin Valley and southern California?

(2) How susceptible are oranges to chronic SO_2 exposures which could occur if additional emissions of this gas occurred in citrus-producing areas of California?

(3) What parameters are most useful in indicating the effects of air pollutants on growth of orange trees?

(4) What parameters are the physiological and biochemical bases for air pollutant effects on orange trees?

(5) Are Valencia orange trees as sensitive to ambient oxidants as Navel orange trees were in the previous work?

From May 1984 to August 1988, chambers were used for three treatments: charcoal-filtered air to represent a "clean air" situation; half-ambient and half-filtered air to represent ozone concentrations in parts of the San Joaquin Valley; and ambient air to represent conditions in southern California. From May 1984 to October 1987, there was a fourth chamber treatment with filtered air plus approximately 0.10 ppm continuous SO_2 to represent potential conditions in the vicinity of industrial point sources. In addition, from May 1984 to August 1988, outside "control" trees were used to determine the effect of the chamber itself on responses to air pollutants. There were seven trees per treatment. Tree responses measured included three years of fruit yield, four years of tree canopy growth, three years of fruit and flower part drop, monthly leaf and fruit drop, weekly to monthly stomatal conductance, biweekly to monthly photosynthetic and water potential, and occasional measurements of biochemical constituents of orange tree leaves.

The treatments had dramatic effects on many tree response parameters. Increasing levels of ambient oxidants were associated with:

- Reduced orange yields, both total weight and number of fruit in two "on" production years. The large reduction in yield for

Valencia oranges exposed to ambient oxidants was similar to the response observed previously for Navel orange trees.

- No effect on yield for one "off" production year.
- Fewer orange fruit per tree.
- Lower individual leaf weights.
- Decreased stomatal conductance, increased transpiration, more negative leaf water potential, but unchanged net photosynthetic rate.
- Increased leaf starch prior to flowering.

Sulfur dioxide exposure was associated with:

- Reduced orange yields, both total weight and numbers of fruit in two "on" years.
- No effect on fruit number for one "off" year.
- More elliptical fruit (reduced height, greater width).
- Lower individual leaf weights.
- Increased transpiration in the summer.
- Increased leaf total sulfur concentration.

Trees in open top chambers differed in many respects from corresponding outside trees:

- Orange yields, both in terms of total weight and number of fruit across all years, were higher for chamber trees.
- Less acidic fruit juice for chamber trees.
- Larger fruit for chamber trees.
- Increased tree size in chambers.
- Increased leaf production for chamber trees.
- Larger, but thinner leaves for chamber trees.
- Less immature fruit drop, but greater flower drop for chamber trees.
- Decreased stomatal conductance and transpiration, more negative leaf water potential, and increased leaf photosynthetic rate, especially in summer months for chamber trees.
- Increased leaf starch concentration for chamber trees.

As a whole, these results indicated that oxidants at current levels or added SO_2 had dramatic adverse effects on the yield of orange trees. The effects occurred without visible leaf injury symptoms. The losses to Valencia oranges were of the same large order of magnitude as reported previously for Navel oranges. However, no direct comparison of results can be made because of the different exposure systems used to study these two types of oranges. There were no specific physiological or biochemical parameters that specifically indicated pollutant effects on trees on a single event basis. Measurements had to be averaged over several growing seasons to indicate pollutant stress. However, leaf starch prior to flowering may be an indicator of stress associated with yield reductions.

Conclusions

(1) Ambient oxidants resulted in a reduction in yield which can be defined by a linear O_3 concentration-yield loss equation during "on" years with normal fruit production.

(2) Ambient oxidants had no effect on fruit production during "off" harvest years of oranges.

(3) High concentrations of added SO_2 resulted in a reduction in yield.

(4) The reductions in yield with air pollutants were associated primarily with reduced numbers of fruit; reduced fruit size played only a minor role.

(5) There appeared to be some alterations in fruit quality with pollutant treatments which are likely related to the reduced number of fruit.

(6) The growth, physiological, and biochemical parameters did not indicate any definitive mechanism by which oxidants affected yield. Retention of starch in leaves instead of allocation to support fruit production may be a key to the mechanism as neither photosynthetic rates nor leaf loss were affected by oxidants in this study. Oxidant stress to leaves was shown by lower stomatal conductances and more negative water potentials, even though no visible injury from oxidants was observed.

(7) The open-top field chambers themselves had many dramatic effects on the orange trees; however, there was no consistent pattern of responses which would indicate that the chamber trees were more or less susceptible to air pollutants than outside trees such as in commercial fields.

RECOMMENDATIONS

(1) Obtain additional information on the general mechanism by which O_3 affects orange tree yield. The focus should be on the effects of oxidants on fruit production. If possible, this could include specific measurements to determine whether oxidants result in less flower production or actual abscission of young fruit.

(2) Biochemical work to establish the metabolic basis for the reduction in yield due to oxidants exposure, looking at starch reserves in leaves and roots before flowering. The work should focus on controlled greenhouse research with plants grown in hydroponic systems.

(3) Perform additional biochemical research to establish the metabolic basis for changes in starch reserves due to oxidant exposure. This research should focus on translocation of reserves from leaves to roots, and from roots back to developing fruit. The enzymatic and/or hormonal control of translocation may be especially important.

(4) Obtain additional information on the mechanism by which the chambers reduce leaf drop. This is important as it would help to indicate whether orange trees are as susceptible to air pollutants in outside air as in the experimental chambers. This, in turn, has implications regarding the usefulness of the O_3 exposure-yield reduction equation generated from this study for estimating actual oxidant effects on orange yield in the field. If susceptibility to air pollutants in chamber trees is different from outside trees, then new methods must be developed to evaluate the effects of air pollutants on trees.

I. INTRODUCTION

A. Importance of Oranges to California and Past Air Pollution Research

California has long been a major United States producer of tree fruit crops. Oranges, the most important of these crops, are grown on about 173,000 acres of some of the State's most productive land (3). Counties with major production have been Tulare, Fresno, Kern, Ventura, and San Diego, with lesser amounts in adjoining areas. Total annual production was in excess of 2.0 million tons valued at $423 million in 1987. Valencia oranges (used for juice) accounted for approximately 43% of the volume produced. Most areas of production presently have had photochemical oxidant (primarily O_3), and some areas have SO_2 pollution which may reduce yields. However, very little data on yield losses, which could have significant economic effects, have been available.

During the late 1950's and early 1960's, Taylor, Thompson, and coworkers (34-36) studied the chronic (low-level, long-term) effects of photochemical oxidants and/or fluoride on Navel oranges and lemons, which occur in the Los Angeles Basin. These studies showed reduced water use, reduced apparent photosynthesis, increased leaf drop, and very substantial reductions in yields of both crops due to photochemical oxidants. Losses in total production were recorded in different years even though there were no easily observed leaf injury symptoms on the trees.

However, the experiment was conducted in closed, plastic-covered greenhouses and the sensitivity of the orange trees to ambient pollutants may have been different from that of outside trees. A complicating factor was that there were only filtered and ambient air treatments, and little accurate air monitoring data from the different treatments. Thus, it is not possible to produce accurate dose-response models to describe the relationship between O_3 dose (as a surrogate for oxidants) and orange yield. Such models have become necessary for interpreting current O_3 and orange yield data for different counties such as used for the ARB Crop Loss Assessment Project.

Furthermore, the susceptibility of orange trees to long-term, low-level "chronic" exposure to SO_2 has not been well documented. Thomas (33) cited results of O'Gara who did one-hour exposures in small greenhouses in Utah with SO_2 on 100 crop, ornamental, or forest species. He reported

citrus to be very resistant to acute foliar injury by SO_2 compared to the other species tested. Matsushima and Harada found that exposures of three species of one-year-old citrus with 1 and 5 ppm SO_2 for two hours/day for 40 days caused no foliar injury (20-21). Later work showed Satsuma orange (Citrus unshiu) to have accelerated leaf drop after exposure with 5 ppm SO_2 for two hours/day for 34 days. After spraying with a Bordeaux mixture, leaf drop was accelerated in 13 days of exposure with SO_2. These studies also were done in closed greenhouses (22).

Thus, to address the effects of air pollutants on citrus, in early 1983 the ARB funded a study to investigate physiological, growth, and yield responses of Valencia orange trees (Citrus sinensis) to ambient oxidants or added SO_2.

B. Statement of the Problem

Previous field research indicated that citrus trees suffered substantial yield losses and altered growth, leaf drop, and physiology with exposure to ambient oxidants, primarily O_3. However, the exposures were conducted in fiberglass greenhouses which may have altered the tree response compared to outside trees. Only Navel oranges were tested and not Valencia oranges. Furthermore, the studies used only the past very high oxidant exposure conditions of the Los Angeles Basin to determine tree response. There were no oxidant exposures representative of current levels in the important orange producing areas in the San Joaquin Valley. Thus, there was an important need to carefully investigate the effects of ambient oxidants on Valencia oranges using the best available current exposure and response measurement technology.

An investigation of the effects of oxidants began in early 1983. The initial two-year contract was not adequate for determination of important tree responses to air pollution because orange trees require two years after planting for the normal pattern of fruit set to occur and, thus, there were no yield data obtained over that time. Nearly four additional years of study were required not only to obtain yield data, but also additional growth, physiological, and biochemical data to investigate the mechanistic bases for the pollution effects.

C. Objectives

Specific research questions to be addressed in the study were:

(1) What are the yield effects of current levels of photochemical oxidants on orange trees, based on treatments representative of the San Joaquin Valley and southern California?

(2) How susceptible are oranges to chronic exposure to SO_2 such as would occur if additional emissions of this gas occurred in citrus-producing areas of California?

(3) Are Valencia oranges as sensitive to ambient oxidants as Navel oranges were in the previous work?

(4) What growth parameters are most useful in indicating the effects of air pollutants on orange trees?

(5) What parameters are the physiological bases and biochemical bases for effects to orange trees from air pollutants?

These objectives have been addressed over approximately six years of study under four separate contracts from the ARB. Three previous contracts, No. A2-130-33 (4/1/83-7/1/85), No. A4-134-33 (6/7/85-8/31/87), and No. A6-066-33 (11/17/86-5/16/88), ended with submission of progress reports and their acceptance by the ARB. The current contract has covered not only research during the last seven experimental months of the study, but also the re-analysis of all data and production of a final report summarizing results from the entire study. The report includes details regarding the most important data for all years of study, with summaries included in the text and important numerical data in appendices.

II. METHODS

A. Pollutant Exposure

The pollutant exposures used open-top field chambers of a unique design specifically developed for this study. The chamber design, development, and initial testing for performance were described by Kats et al. (14). The chamber had to meet a stringent set of criteria for use in this study, including: (a) an area large enough to contain a bearing orange tree while retaining adequate space for air mixing around the tree's canopy, (b) shape to conform to the tree canopy while eliminating "dead" spaces which provide air mixing problems, (c) durability to last over a number of years, (d) the plastic had to remain clear over time to allow natural light penetration, (e) the chamber had to be relatively easy to fabricate and move, and (f) the costs had to be kept at a minimum.

All of these criteria were met with the "dome-shaped" chamber designed in early 1983. The idea for using the dome actually came from a popular science magazine where the plastic dome was advertized for use over outside "hot tubs" during the winter in California. The chamber consisted of a plastic dome, reinforced with sheet metal piping, and attached to a sheet metal base covered with transparent fiberglass. Figure 1 shows the general dimensions of the chamber.

The pollutant exposure and monitoring system for SO_2 consisted of a temperature controlled tank of liquid SO_2, heatless air drier, flowmeters, sample lines, scanning valve, and a ThermoElectron Company Model 43 SO_2 analyzer. The monitoring system for O_3 (measured as a surrogate for all oxidants) consisted of sample lines, scanning valve, and Bendix or Dasibi Model 1003 AH analyzer. The oxidant treatments of filtered air, half-filtered air, and ambient air were achieved by totally, partially, and not filtering the air entering the chambers, respectively. Electronic signals from both SO_2 and O_3 analyzers were fed into an ISAAC Cyborg® data acquisition system, which converts analog signals to digital signals and then processes and stores the data in an Apple computer system. Figure 2 shows the location of the different treatments in the field chambers. Each treatment, including the outside control treatment, was replicated seven times.

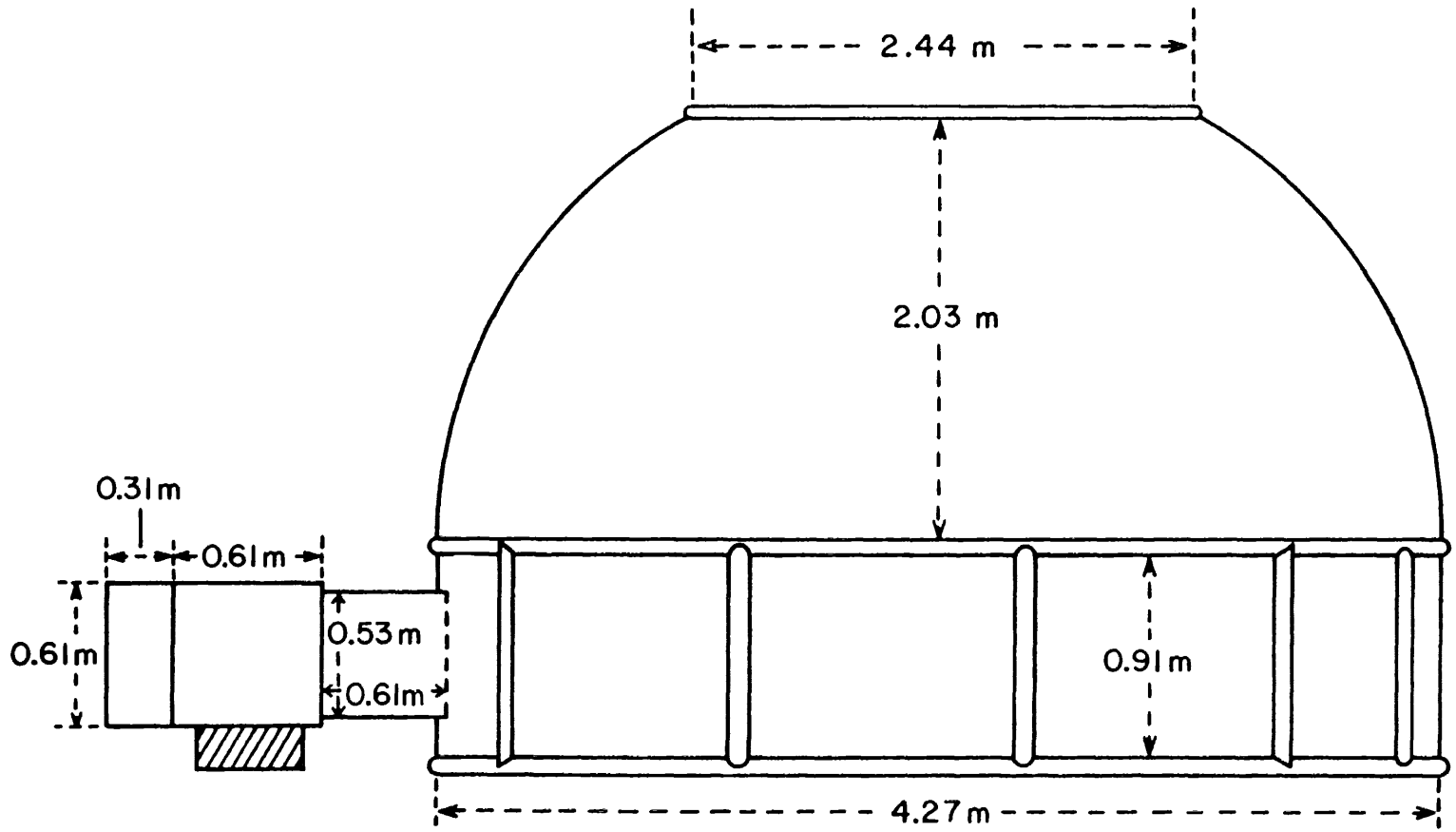

Figure 1. Diagram of orange tree exposure chamber.

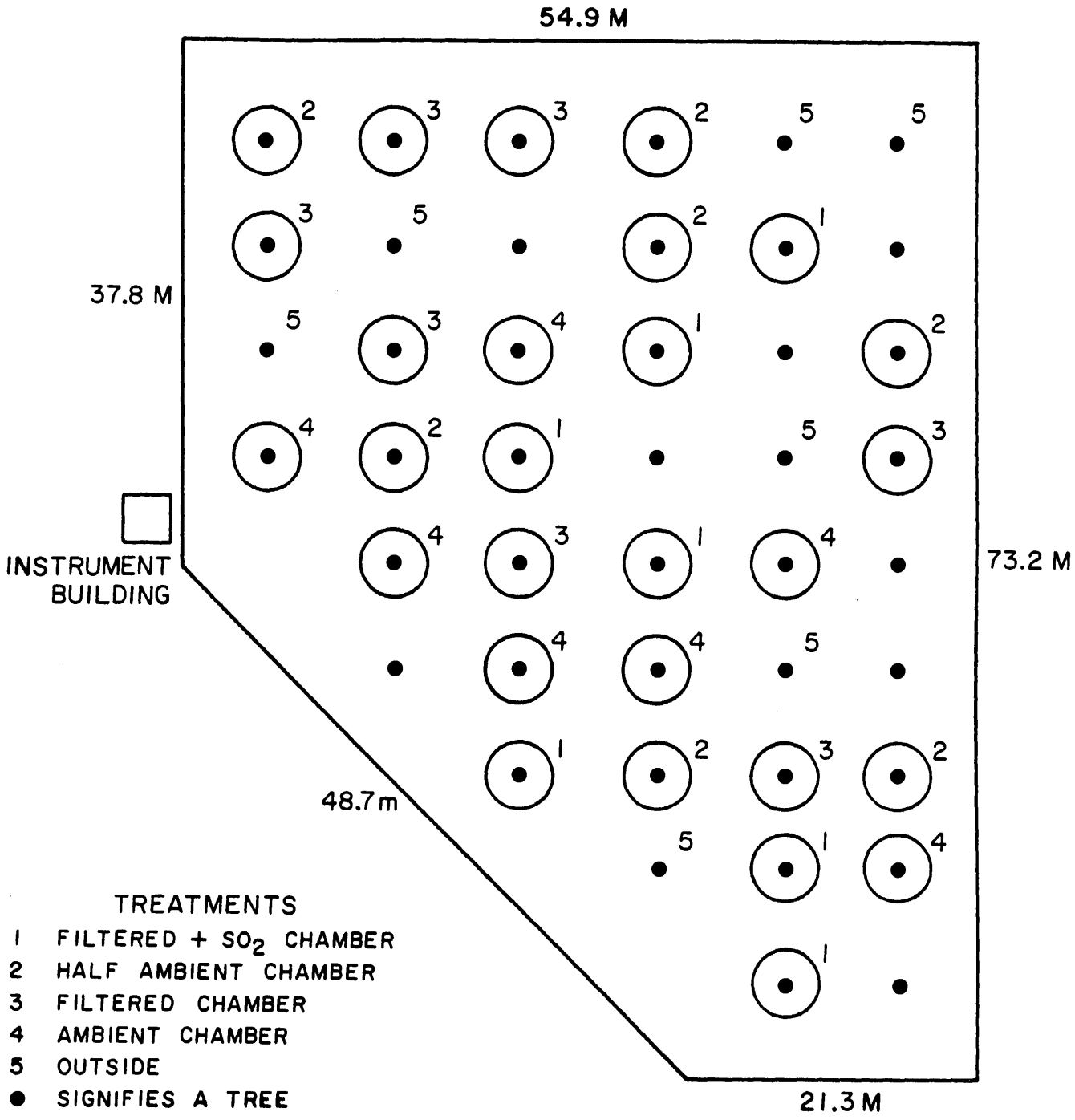

Figure 2. Diagram of orange tree exposure site.

The air samples from the treatments were taken with the assistance of a 24-channel scanning valve system. The scanning valve was necessary so that different tree environments could be sampled sequentially by the single O_3 and SO_2 analyzers. Since there were a total of 35 tree environments to be sampled and only 24 available channels, a scheme was developed at the start of the study to more intensively sample the air around those trees where the pollutant concentrations would be expected to be more variable. Thus, air was sampled from all seven half-ambient and seven SO_2 chambers. Air was sampled from four ambient and four filtered air chambers, as those treatments would be more critical for eventual O_3 exposure-tree response regression analysis. The air around only two outside trees was sampled, as the O_3 concentrations were expected to vary least around those trees.

Each channel was sampled for five minutes, with only the signal from the last minute saved by the computer. The data from the first four minutes was considered to be unreliable due to flushing of the lines of the previous sample. Since there were 24 channels each sampled for five minutes per cycle, each tree was sampled only once every two hours. The channels were allocated, however, so that approximately half of the channels per treatment were sampled each hour. This resulted in the following numbers of samples per hour per treatment: sulfur dioxide (3-4), half-ambient (3-4), ambient (2), filtered (2), and outside (1). All of the raw data were recorded on disk and then transferred to tape for final quality checks and analysis for the entire study period.

Because of the differing number of samples per hour per treatment, the raw data were reduced for final analysis. Only data from two channels per hour were used from each of the sulfur dioxide and half-ambient treatments. This resulted in the same number of values per hour as for the ambient and filtered chamber treatments. Occasionally, only one value per hour was available from the chamber treatments due to instrument malfunctions, line clogging, and other factors. Only the one value per hour was available for the outside treatments for the entire period of time. Thus, the final data from the chamber treatments were all on a similar averaging time and number per hour basis, but the outside tree data were only taken from half as many hours. These differences in sampling frequency were not

considered to be important, as the growing season averages over 12 hours per day were considered to be the most important for plant responses.

The SO_2 treatments were initiated on May 15, 1984 and terminated on October 22, 1987, following consultation with ARB staff. The filtered, half-ambient, and ambient treatments began in April 1984 (chambers came on line over a period of several weeks) and continued until August 10, 1988. However, O_3 air monitoring ended on July 22, 1988 as the O_3 instrument began to malfunction. At that time the blowers were turned off and then pulled back from the chamber to provide one opening. The doors were left permanently open to provide a second opening for air flow through the chamber. It was hoped that this ventilation would help to at least partially alleviate overheating in the chamber so that the oranges would remain on the trees until picked in June 1989.

B. Tree Culture

Valencia orange trees [Citrus sinensis (L.) Osbeck] growing on the experimental site at UC Riverside were used for this study. The trees were planted during July 1983, and have been exposed to experimental treatments since May 1984. The trees, grafted on Troyer Citrange rootstocks, were selected by the grower based on similar stem diameters of approximately 0.032 m. Figure 3 shows a time line for significant events during the life of these orange trees. The trees were approximately two years old at the time of planting in July, and had set fruit that spring. These fruit were picked from the trees on November 15, 1983 so that the variable number of fruit picked per tree would not affect flowering in the spring of 1984. The trees flowered in the spring of 1984; however, no fruit were set. Possible causes for lack of set included injury to root systems at transplanting the previous summer, the sudden change in tree microclimate with installation of chambers at the end of the flowering period in April 1984, or changes in tree metabolism after removal of the fruit in 1983.

Originally, a grove of Valencia orange trees planted in 1978 was to have been used for this study. However, in June of 1983 it was determined that these trees could not be used due to differences in Tristeza disease inoculation and rootstock type between trees. Thus, the new grove was planted.

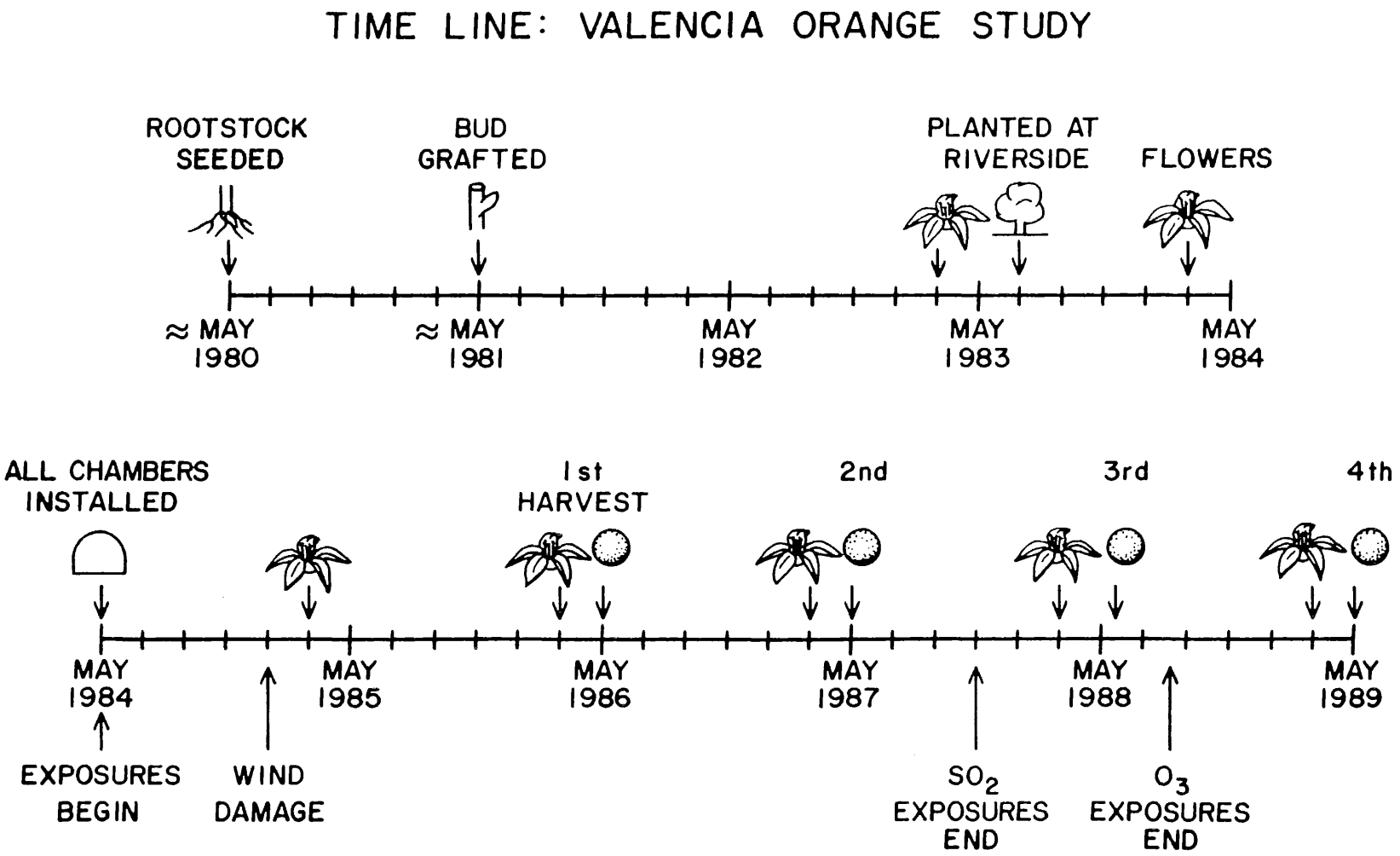

Figure 3. Time line for culture of Valencia orange trees 1981-1989.

Soil samples were taken on June 22, 1983 to determine if there were any obvious nutrient deficiencies. The measured elements were within adequate ranges for citrus (i.e., 8-28 ppm for phosphorus, 104-186 ppm for potassium, 8-61 ppm for zinc, and an electrical conductivity of 1.2-2.0). The soil was sampled on September 1, 1983 for sulfate, which ranged from 60-215 mg/liter.

Soil samples were taken on June 24, 1988 for citrus nematodes. These pathogens can cause considerable damage to citrus tree roots if present in high enough quantities. The soil counts for nematodes were very low at 0-94 per 50 cc of soil. However, fumigation with methyl bromide was still carried out for the experimental plot in order to ensure control of any nematodes that were present.

The trees received foliar zinc sprays after major flushes of growth had just expanded, usually twice a year. The trees received irrigation with a nutrient solution (57 g nitrogen as urea per tree per irrigation), as necessary, applied over periodic irrigations since planting. The trees were watered at regular intervals via furrow irrigation. The irrigation system consisted of an irrigation water supply, liquid feed proportioner for fertilizer addition, polyvinylchloride main lines, separate lines to each tree, individual valves for each tree, and drip irrigation tubing to an irrigation furrow underneath the drip line of each tree. Two Irrometers® (one 0.31 m and one 0.61 m long) were placed together under the drip line of the tree, and checked periodically. The trees were irrigated if both Irrometers® read over 50 centibars to avoid overwatering in these rather compact soils. The irrigation rate was approximately 11.4 liters of water m^{-2} at 7031 kg m^{-2} pressure per tree. All other management practices were as normally prescribed for Valencia oranges based on specific cultural practices (e.g., pesticide sprays, pruning, etc.).

The most common pests were red spider mites, cottony cushiony scale, and aphids. The mite and scale problems especially persisted during the study, primarily for the chamber trees. The insect persistence in the chambers may have been related to the confining nature of the chamber environment, general tree stress due to the chamber, or actual increased sensitivity to pests due to the pollutant treatments. However, only casual observations of the pests were made and no actual studies were undertaken to determine the cause for the insect outbreaks.

A number of chamber trees also had an apparent abiotic stress problem. Symptoms consisted of branch dieback associated with defoliation, and a secretion of sap from the dead branch or twig, often near its insertion into a larger branch. Experts in citrus diseases were contacted, but no cause was found for the problem, which seemed to be worse for SO_2 treated trees as noted on January 5, 1986. Four of the seven SO_2 treated trees had dieback, whereas only two other trees, both with charcoal filtered air, exhibited the dieback.

There was a fence around two sides of the site to provide extra security for the chambers. The fencing contained redwood slats to provide a barrier to wind and, thus, decrease the direct force of the wind against the domes.

C. Environmental Measurements

Important environmental parameters [i.e., light (quantum) intensity, leaf temperature, air temperature, and relative humidity] were monitored continuously during most of the study. Measurements of light and humidity began on January 4, 1986 and lasted through July 22, 1988. Measurements of leaf and air temperatures were made from June 29, 1985 through July 22, 1988. These measurements were used to determine (a) the occurrence of any variability in the environment between chambers and outside trees which could be associated with differences in tree responses to air pollutants, and (b) the environmental basis for any seasonal changes in plant response.

Quantum Intensity. Quantum intensity was measured continuously with Lambda Instrument Company LI 190SB quantum sensors (400-700 nm wavelengths). The sensors had a millivolt output for use with the Apple®-ISAAC® data acquisition system. Measurements were made from one sensor located just above the canopy of an outside tree and one just above the canopy of a chamber tree at a height of approximately 2 m. Data from the chamber for the latter part of the study were of questionable reliability due to shading of the light sensor. Shading of the sensor would have occurred anywhere in the chamber due to the large size of the tree canopy. Thus, it became impossible to get a true reading of the effect solely of the chamber on quantum intensity. Hourly data were summarized

across the daylight hours (0800-2000) and averaged for this period from Saturday through Friday of each week.

Leaf Temperature. Leaf temperature was measured continuously using fine wire thermocouples attached to the undersides of shaded leaves with surgical tape. The leaves were inside the canopy at a height of approximately one meter. The thermocouple was moved to a new leaf whenever the old one became senescent. The thermocouples were read by the Apple®-ISAAC® data acquisition system. Data were reported as the mean of one or two outside and one ambient chamber measurement(s). Data were reported as hourly averages for 0800-2000 Saturday through Friday.

Air Temperature. Air temperature was measured continuously at a height of about one meter using fine wire thermocouples. The thermocouples were shielded from the sun within tubes which were equipped with fans to draw air over the thermocouple junction. Air temperature was measured by the Apple®-ISAAC® data acquisition system. Data were reported as the mean of one or two outside and one or two ambient chamber measurement(s). Data were reported as hourly averages for 0800-2000 Saturday through Friday.

Relative Humidity. Relative humidity was measured continuously using dewpoint sensors located in the air stream going to the air pollutant analyzers from the chambers or outside air. Thus, dewpoint was determined for each sampling point approximately once per hour. The dewpoint sensors for each chamber or outside sampling point were read by the Apple®-ISAAC® data acquisition system. Relative humidity itself was calculated from the air temperature and dewpoint temperature. Data were reported as the mean of one or two outside and one or two ambient chamber measurements. Data were reported as hourly averages for 0800-2000 Saturday through Friday.

D. Yield and Quality Measurements

Yield and quality parameters were approximately the same as those used in earlier citrus studies, focusing on economic yield (36).

Yield. Yield was determined as weight and number of ripe fruit per tree. The fruit were picked by hand over one-day harvests on May 8, 1986, May 20, 1987, and May 23, 1988. An additional harvest was made in June, 1989 after the experiment was over in order to see if there was any carry-

over effect of the treatments on tree responses to pollutants. Only total number and weight of fruit/tree were recorded.

Quality. Fruit quality was measured in terms of size, rind thickness, rind color, individual weight, sugar concentration (% by weight), and acidity (% by weight) for 10 fruit per tree in 1986 and 30 fruit per tree in 1987 and 1988. Size was measured as two separate diameters (height and width) per individual fruit. Fruit circumference was determined according to the formula, circumference = π x d, where d was the average of height and width. Rind thickness was measured with a ruler to the nearest mm. Rind color was rated on a 3 (green) to 13 (orange) scale according to a commercial color chart. To ensure uniformity in color evaluations, all ratings were done by the same individual in all three years and under similar light conditions.

Juice quality was measured as percent sugar and percent acid. The subsamples of 10 (1986) or 30 (1987 and 1988) fruit per tree were juiced using a home juicer. The juice from all subsamples per tree was pooled and strained through cheesecloth. The percent sugar was determined using a refractometer and tables, relating index of refraction to percent sugar. The percent acid was determined by titration with a pH meter of the strained juice mixture which contained 10 ml juice, 50 ml H_2O and two drops of phenolphthalein indicator. Titration was with 0.1562 N NaOH. The formula for calculating percent citric acid was:

$$\frac{\text{ml of NaOH used x N NaOH}}{\text{ml of juice x specific gravity of juice}}$$

E. Growth Measurements

Growth was determined by monthly measurements of leaf drop, flower and small fruit drop, and tree size.

Leaf Drop. Leaf drop, a sensitive indicator of tree stress, was measured monthly. The leaves falling to the ground beneath each tree were picked up continuously over the year and placed in covered buckets alongside each tree. On about the tenth of each month the leaves were transferred to paper bags, dried in fiberglass greenhouses for one to two weeks, and weighed. Both total dry weight per tree and weight of a subsample of 10 or 30 leaves were determined. The individual leaf weight was

an indicator of the relative size of individual leaves per treatment. Leaf drop was pooled for April through October 1984. Samples were collected approximately monthly on the 10th of each month for most months from December 10, 1984 through August 10, 1988.

Fruit and Flower Drop. All flower parts and young, small fruit which had dropped from the trees during the spring blooming period were retained and measured in 1985, 1987, and 1988. Data were not collected for 1986. The fruit and flower parts were swept into paper bags, cleaned, and weighed. Two to four separate collections were made each year. The data were used as an indicator of the effects of the different treatments on flower production, fruit set, and very premature fruit drop.

Larger fruit which dropped from the trees were collected periodically. Collections were made from one to four months, depending on whether fruit dropped or not. The measurements indicated unseasonable loss of fruit which could have contributed to the final yield. The fruit loss was averaged from May of the "set" year through April of the following "harvest" year to represent extra fruit drop relevant to the harvest.

Tree Size. Tree size was determined as stem diameter, tree height, canopy diameter in north-south and east-west directions, and trunk height from soil. Measurements were made during December 1984, May 1986, and March 1988. Crown height was determined as tree height minus trunk height except for March 1988 when only total tree height was used because the lower limbs of nearly all trees now trailed on the ground. The north-south crown diameter, east-west crown diameter, and crown height were averaged to determine average tree crown diameter. The average crown diameter (d) was used to calculate crown volume (assuming the canopy was a sphere) according to the formula: volume = $4/3\ \pi(1/2d)^3$.

F. Physiological Measurements

Physiological measurements were made to (a) assess the responses of the tree which may be the metabolic basis for any observed growth or yield effects, (b) assess any differences in tree metabolism which may occur between outside and chamber trees, and (c) identify physiological parameters for tree response which may be useful under field conditions.

Physiological measurements were made on fully expanded leaves from the most recent flush of growth prior to the day of measurement. These

leaves generally were a more glossy yellow-green color than the older leaves on the tree. Measurements were made every two to four weeks, with data collected from late morning to early afternoon between approximately 1030 and 1400.

Stomatal conductance and transpiration were measured with a Lambda Instruments Co. LI-1600 steady state porometer beginning May 11, 1984. Measurements were made approximately weekly through September 25, 1986. The porometer was calibrated at the factory approximately every six months. A check was made before each use to determine if the silica gel was dry, thermocouples were operating properly, air flows were correct, and the system was without leaks. Stomatal conductance and transpiration were measured every two to four weeks from April 18, 1986 through August 5, 1988, using a Lambda Instruments Co. LI-6000 portable gas exchange unit. This is a closed system which detects water vapor in the air with a capacitance sensor. There was some overlapping between the two instruments in the middle of 1986.

Net photosynthesis was measured with a ^{14}C radioisotope porometer (1), approximately monthly from February 4, 1985 through April 4, 1986. Net photosynthesis was also measured with the LI-6000 porometer, using its portable infrared analyzer to detect CO_2 in the air. The radioisotope and LI-6000 porometers gave similar net photosynthetic measurements. The system is equipped with a microcomputer to store and process the data. The LI-6000 was used every two to four weeks between April 18, 1986 and August 5, 1988.

Leaf water pressure potential was measured with a pressure bomb (28). Measurements were made every two to four weeks from February 3, 1985 through August 5, 1988.

G. Biochemical Measurements

In this study, in the absence of obvious visible leaf injury from ozone, it was important to determine if changes in leaf biochemistry preceded or were concomitant with plant development in polluted environments. In addition, little was known with respect to whether enclosing trees in chambers with open tops altered responses to gaseous pollutants. Thus, the objective of the biochemistry measurements was to

determine levels of selected metabolites in Valencia orange leaves exposed to the different treatments.

A number of biochemical parameters were measured to evaluate their potential as indicators of air pollutant stress in citrus. The selected parameters had been used to evaluate plant responses to pollutants in previously published reviews and papers.

Leaf chlorophyll and carotenoid concentrations have been measured in many studies as an indicator of visible injury and of the potential for air pollutants to affect photosynthesis via depletion of light-trapping pigments (4). Concentrations of chlorophyll pigments have been especially useful in quantifying ozone injury to leaves (15). Leaf sulfite, thiol, and total sulfur concentrations have been shown to increase with exposure of plants to SO_2 (2, 7, 19). Leaf buffering capacity has been shown to increase in response to SO_2 (2), but decrease in response to O_3 exposure (32). Leaf pH was determined as part of the buffering capacity measurements and may provide an additional tool for indicating air pollution stress to leaves. Total leaf starch concentration was an indicator of carbohydrate reserves which are available for allocation to different plant organs (16).

The most detailed biochemical analyses were carried out for leaf samples collected in January and October 1986 and January, April, and July 1987. Approximately 50 dark green, healthy leaves were selected per tree for leaf pigment, sulfite, thiol and pH assays. The leaves were frozen immediately in liquid nitrogen. Samples were stored on dry ice until all plots were sampled. Other methods of leaf selection were used for determination of leaf sulfur and starch concentrations. Leaf starch and total sulfur were measured for leaves sampled once during the study.

Detailed methods for the analyses are described below:

Chlorophyll and Carotenoid. One to four subsamples per tree of each treatment were homogenized in 80% acetone at 4°C. Chlorophyll and carotenoid concentrations were determined on a leaf area basis by spectrophotometric analysis of the acetone extracts (18). Absorption at 663, 646, and 470 nm were measured with a Beckman DB spectrophotometer.

Sulfite. One sample per tree of the SO_2 and filtered treatments was homogenized in 0.1 M sodium tetrachloromercurate at 4°C. Sulfite concen-

trations were determined by measuring the formation of the pararosaniline-sulfite complex at 560 nm with a Beckman DB spectrophotometer (38).

Thiol. One or three subsamples per tree of the SO_2 and filtered treatments were homogenized in 0.15% (w/v) ascorbic acid at 4°C. Thiol concentrations were determined by measuring the formation of paranitro-thiobenzoic acid at 412 nm with a Beckman DB spectrophotometer (7).

Leaf pH. One sample was measured per tree. Interveinal leaf material was ground in distilled water for 60 s in a Omni-mixer (Sorvall, Norwalk, CT). After measuring the pH of the aqueous leaf extract, changes in pH were monitored following six 0.5 ml additions of 0.01 N HCl following the general procedure described for titration by Pylypec and Redmann (25).

Specific Area. One sample containing discs from 12 leaves per tree of all treatments were dried to constant weight at 80°C and weighed (Ainsworth Type 10 N balance).

Total Sulfur. One sample each of 20 old and 20 recent flush leaves were taken from SO_2 and filtered treatment trees on November 16, 1987. Samples were sent to the University of California Cooperative Extension Laboratory at UCR where they were analyzed by a gravimetric procedure following digestion by nitric perchlorate.

Starch. Leaf starch was determined once during the study as a pilot measurement to determine whether leaf starch reserves may be related to potential flowering and fruit set capability of trees. Leaves were collected on February 17, 1988, during the late winter just before flowering. The sampling procedure was based on advice from Dr. Carol Lovatt of the UCR Department of Botany and Plant Sciences. Forty leaves were collected per tree and pooled into one sample. Leaves from the most recent flush of growth (late fall of 1987) were taken at chest height (approximately 1 m) from around the perimeter of each tree. The samples were put into plastic bags and transported on ice to the Department of Botany and Plant Sciences. The leaves were then frozen in liquid nitrogen for short-term storage. The frozen leaves were then lypophilized (freeze dried) over a period of two days. The freeze-dried samples were then stored until analyzed for starch.

Starch concentrations in the leaves were determined by assaying the glucose content of samples subjected to chemical and enzymatic digestion

(9). Subsamples (50 to 100 mg) were extracted with 80% ethanol (v/v) four times in a 80°C water bath until the samples were pigment-free. After the supernatant ethanol was removed, 1 M of 0.2 M KOH was added to the leaf brie, and the mixture was incubated at 100°C for 30 minutes (to facilitate the chemical digestion process). The sample was allowed to cool for 10 minutes, and the mixture then neutralized by adding 0.1 ml of 1 M acetic acid. The mixture was stirred and placed in a 55°C water bath for five minutes before adding 1 ml of amyloglucisidase from Rhizopus sp. (400 units ml^{-1}, Sigma Chemical Co., St. Louis, MO) in 50 mm sodium acetate buffer (pH 4.5). Enzymatic digestion was allowed to continue for 90 minutes, after which the samples were placed in a 100°C water bath for one minute to stop the enzyme digestion process. The samples were cooled to room temperature, centrifuged, and the supernatant adjusted to 10 ml with distilled water and kept frozen until the time of assay. Aliquots (0.2 to 0.5 ml) were analyzed for glucose content by measuring the production of oxidized o-dianisidine (Glucose Diagnostic Kit, Sigma Chemical Co., St. Louis, MO) at 450 nm with a spectrophotometer. Starch content (as glucose equivalents) was calculated on a dry weight basis.

H. Statistical Analysis

A number of different types of statistical analyses were carried out on the data following the general procedures described by Steel and Torrie (30). The specific type of analysis for each parameter depended on what was appropriate for the experimental design of the study, and what was logical, based on knowledge of the physiological, growth, and yield characteristics of orange trees. The basic experimental design was a completely randomized design with split-plots over time to consider repeated measurements on the same tree for the different response variables. In reporting results from the statistical analysis, "significant" indicates that the probability that the reported difference between treatments is "false" is <0.05%, i.e., less than one out of twenty times the statement is made.

The basic analysis of variance shown in Table 1 was used for a complete set of data on any one measurement period, e.g., yield in 1986. There were four degrees of freedom (df) for treatments which were divided among four contrasts. The linear and quadratic contrasts tested whether

Table 1. Analysis of Variance for Single Measurements

Source of Variation	Degrees of Freedom
Treatments	4
Linear Oxidant (filtered, half, ambient)	(1)
Quadratic Oxidant	(1)
Filtered vs. SO_2	(1)
Ambient vs. Outside	(1)
Error	30
TOTAL	34

whether: 1) plant responses decreased in magnitude with more oxidants, i.e., linear effect; or 2) plant responses with the half-ambient treatment were significantly different from the average of the responses from the filtered and ambient treatments, i.e., a nonlinear or quadratic effect. The linear and quadratic contrasts were determined using the following polynomial coefficients:

Effect	Ambient	Half-Ambient	Filtered
Linear	-1	0	+1
Quadratic	+1	-2	+1

The error 30 error df is the difference between the 34 total df and 4 treatment df. The 34 total df were calculated by the formula [(5 treatments x 7 replicate)-1 df for the grand mean of the data.]

The analysis of variance shown in Table 2 was used for evaluating yield and quality data across both years (1986, 1987) when all treatments were present. Similar analyses, but without the SO_2 treatment, were used for 1988 alone and 1988 plus the other years. The analysis of variance across all three years is shown in Table 3. Whenever the 1986 yield data were included in the analysis, the Error b degrees of freedom and total were reduced due to estimation of data from trees in chambers damaged by wind in January 1985. The chambers were partially missing from approximately January through April. These trees immediately changed their

Table 2. Analysis of Variance for Two Years of Data (1986, 1987)[a]

Source of Variation	Degrees of Freedom
Treatments	4
Linear Oxidant	(1)
Quadratic Oxidant	(1)
Filtered x SO_2	(1)
Ambient vs. Outside	(1)
Treatment Error a	30
Year	1
Treatment x Year	4
Linear x Year	(1)
Quadratic x Year	(1)
Filtered x SO_2 vs. Year	(1)
Ambient x Outside vs. Year	(1)
Year x Treatment Error b	30
TOTAL	59

[a]Note: comparisons under treatment x year not specifically evaluated.

growth and physiological responses and behaved like outside trees. The response means for the treatments without the affected trees were used for the responses of these trees to balance the analysis of variance. Thus, the number of degrees of freedom had to be reduced to account for the number of means replaced (i.e., five for the three years of data and four for the two years of data).

While the linear and quadratic terms from the analysis of variance gave some indication of the oxidant treatment effect, additional regression analysis was necessary to determine the quantitative relationship between yield responses and oxidant exposure (as indicated by ozone concentration). The ozone concentrations corresponded to filtered air, half-ambient and half-filtered air, and ambient air. They were based primarily on 12-hour averages from the summer two years before the harvest (e.g., 1986 growing season ozone data vs. May 1988 fruit harvest).

Table 3. Analysis of Variance for Three Years of Data (1986, 1987, 1988)[a]

Source of Variation	Degrees of Freedom
Treatment	3
Linear Oxidant	(1)
Quadratic Oxidant	(1)
Ambient vs. Outside	(1)
Treatment Error a	24
Year	2
Linear	(1)
Quadratic	(1)
Treatment x Year	6
Linear Oxidant x Linear Year	(1)
Linear Oxidant x Quadratic Year	(1)
Quadratic Air x Linear Year	(1)
Quadratic Air x Quadratic Year	(1)
Ambient x Outside vs. Linear Year	(1)
Ambient x Outside vs. Quadratic Year	(1)
Year x Treatment Error b	39
TOTAL	74

[a]Note: Comparisons under year and treatment x year not specifically evaluated

Growth and physiological data were also pooled to determine whether significant oxidant or SO_2 treatment effects could be determined over a long period of time. The data were grouped into winter (October-March) and summer (April-September) periods for analysis. A sample analysis of variance across 45 dates is shown in Table 4. The analysis of variance for multiple measurements is a split-plot over time, similar to those shown in Tables 2 and 3 for analyses over years, but with dates instead of years as a factor. The number of degrees of freedom (df) for day, day x treatment, Error b, and total differed with the total number of days available to pool measurements.

Table 4. Sample Analysis of Variance with Multiple Dates of Measurement[a]

Source of Variation	Degrees of Freedom
Treatments	4
Linear Oxidant	(1)
Quadratic Oxidant	(1)
Filtered vs. SO_2	(1)
Ambient vs. Outside	(1)
Rep (Treatment) Error a	30
Date	22
Date x Treatment	88
Error b	660
TOTAL	804

[a]Example is for water potential data across 23 summer months (April-October 1984-1988).

Biochemical data were evaluated according to the analysis of variance design shown in Table 1, except that a sampling error was added to account for multiple observations per tree. For leaf starch and total sulfur the outside tree and ambient chamber, or filtered and SO_2 chamber treatments, were compared with a one-tailed, nonpaired t-test. One-way analysis of variance was used for the three oxidant treatments (ambient, half-ambient and filtered air).

III. RESULTS

A. Pollutant Treatments

The exposure chambers were quite effective in providing pollutant exposures near the target concentrations. Ozone concentrations (measured as an indicator of total oxidants) during the day and over the growing season averaged approximately 0.075 ppm during this study in outside air (Table 5). The chambers themselves removed a small amount of O_3, likely due to deposition on the blower and walls, as shown by the approximately 8% lower O_3 concentration for the ambient chamber vs. outside air treatment. The half-ambient chamber treatment was successful in providing an average O_3 concentration that was approximately 50% of that in outside air. The filtered chamber treatment was successful in removing O_3, with an average O_3 concentration 92% lower than in outside air.

The daylight growing season average concentrations do not fully characterize the O_3 exposures to which the trees were subjected in the different treatments. Diurnal patterns, peak concentrations and their frequency, respite times between peaks, and annual averages (for this evergreen species) may all be important. However, the daylight growing season averages have been considered to be the most important for determining plant response in the recent National Crop Loss Assessment Network (NCLAN) studies (8), and these averages were used in regression equations with the yield data in this study. All of the O_3 data for over four years have been entered into computer files and have been analyzed. Appendix A indicates average concentrations and cumulative doses (<0.10 ppm) for all treatments and years. These data are available on computer tape for any additional analyses.

In general, the O_3 exposures were similar for all four growing seasons. Figure 4 illustrates the general diurnal pattern for O_3 over growing seasons for 1984-1988. Ozone concentrations were normally close to zero until 0700, after which they rose rapidly to a peak at approximately 1500. Concentrations then decreased rapidly to below 0.02 ppm by 2000. Therefore, at Riverside the 12-hour daylight concentration from 0800 to 2000 includes nearly all of the daily O_3 exposure to the plant. Ozone concentrations were relatively uniform over the four years shown here, with 1986 having slightly higher concentrations than the other four years.

Table 5. Representative Ozone and Sulfur Dioxide Concentrations for the Valencia Orange Study. Ranges of Daylight (0800-2000 PST) Growing-Season (April-October) Averages for 1984-1988[a]

Treatment	SO_2 (ppm)	O_3 (ppm)
Outside	0-0.002	0.071-0.078
Ambient Chamber	0-0.002	0.065-0.073
Half-Ambient Chamber	0-0.002	0.032-0.047
Filtered Chamber	0-0.002	0.008-0.016
SO_2 Chamber	0.081-0.090	0.009-0.015

[a]Data collection for June 1984 to July 1988 for O_3, and June 1984 to October 1987 for SO_2.

Sulfur dioxide concentrations averaged 0.08-0.09 ppm over three years (Table 5). This range was for the daylight growing-season average, but the concentrations were approximately the same on a daily and yearly average basis (Appendix A). This was expected as the exposure system was constructed to deliver 0.10 ppm SO_2 continuously. The concentration of 0.08-0.09 ppm SO_2 was higher than the mean annual average of 0.01-0.02 ppm found in orange growing areas of California such as Kern County. Hourly averages of up to 0.07 ppm (which is close to the concentration used in our study) have occurred in some areas of the San Joaquin Valley. Nonetheless, the average SO_2 concentrations used in this study should be considered to represent the worst-case situation if SO_2 emission controls were to be relaxed and concentrations were to rise.

B. Environmental Measurements

Environmental conditions over the study were only slightly different in the open-top field chambers compared to outside air (14). Table 6 indicates average daylight (0800-2000 PST) levels of environmental conditions for approximately three years based on data gathered with the microcomputer system. These data are intended to give a broad overview of the changes in the tree environment due to the presence of the chambers.

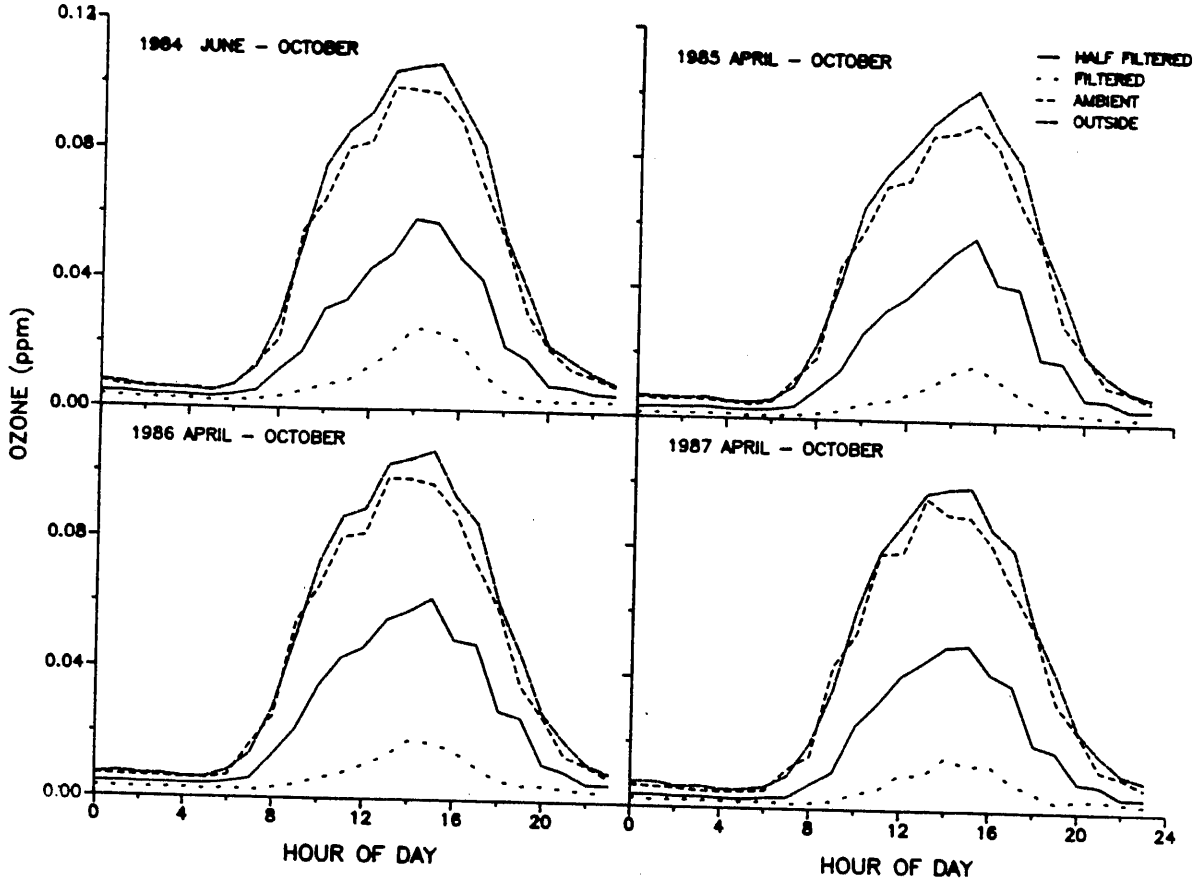

Figure 4. Diurnal variation in O_3 concentrations during growing season, 1984-1987. Data are from one or two trees per treatment per hour. Hours are in PST.

Table 6. Average Values for Daylight Environmental Parameters in Ambient Open-Top Chambers and Outside Over Three Years

Parameter	Open-Top[a] Chamber	Outside[a]	Chamber Minus Outside
Quantum Intensity (μmol m^{-2} s^{-1})	526	648	-122
Relative Humidity (%)	33.3	35.2	-1.9
Air Temperature (C)	24.7	23.8	+0.9
Leaf Temperature (C)	25.5	23.4	+2.1

[a]Values are means with number of weeks of measurement in parentheses. Each weekly value was for 0800-2000 from one or two trees.

The 19% lower quantum intensity for open-top chambers compared to outside air was somewhat misleading as the difference in intensity increased over time as the tree canopy filled the chambers (Table 6). By the end of the study there was no location within the chamber which was not shaded by the tree. Thus, the data could not indicate the overall intensity for the canopy, as is normally reported for herbaceous crops. Light transmission by the plastic at wavelengths from 350 to 800 nm was checked at the start of the study and was close to 100%.

The differences in air and leaf temperatures between the chambers and outside air were small based on porometer measurements (14). The small decrease in relative humidity in the chambers compared to outside air reflects the warmer air temperature, and, hence greater water vapor pressure deficit in the chamber air.

C. Oxidant Effects

1. Yield and Quality of Fruit

The study fulfilled its primary objective,i.e., to determine any effects of ambient oxidants on Valencia orange tree yield. Oxidants had a statistically significant effect on yield of Valencia oranges based on total weight of fruit per tree data for two "on" years, 1986 and 1987. There were more oranges per tree in the charcoal-filtered chambers than in half-ambient or ambient chambers, and ambient chambers had the lowest

yields (Figure 5). Based on analysis of variance (Table 7), the reduction in yield due to oxidants was statistically significant for 1986, but not 1987. When the yield data were compared to growing daylight season O_3 concentrations, however, there were significant linear regression equations for each across both years (Table 8). Quadratic regression equations were not determined as the quadratic oxidant effect was not significant for yield based on analysis of variance. Numerical data from the 1986, 1987, and 1988 harvests are shown in Appendix B. The large reduction in yield for Valencia orange trees exposed to oxidants was similar to the response observed previously for Navel oranges (36).

The reduced total weight of fruit per tree with oxidant exposure was associated both with reduced fruit number and with reduced individual fruit weight. There was a significant reduction in number across both 1986 and 1987, the "on" years of production (Figure 6, Table 7). This reduced number could be expressed by a significant linear regression equation for 1987, and across both years (Table 8).

The regression analysis considered orange tree yield as the dependent variable (y) and ozone concentration two years previous as the independent variable (x). For example, ozone data from April-October, 1985, was used with the regression for 1987 yields. Ozone data was used from two years before harvest because tree physiology prior to flowering and fruit set is highly dependent on growing conditions six months early during the previous summer (29). Since fruit set is the major factor affecting orange tree yields, the effects of the growing season and summer are reflected at the time of harvest 15 months after the fruit are set, or nearly two years later. Evidence for ozone effects on fruit number for tree and starch reserves in trees prior to flowering and fruit tree also suggested that the ozone concentrations two years before harvest should be included in the regression analysis. Nevertheless, results from the regression analysis would have been similar regardless of which year the ozone data were taken from. This is because average ozone concentrations varied only slightly between years (Appendix A).

Ambient oxidants had no affect on fruit yield in 1988, an "off" year for productivity as discussed later. The total weight per tree and number per tree were approximately the same across the filtered, half-ambient, and ambient treatments (Figures 5 and 6, Table 7). There were no significant regression equations for 1988 yields (Table 8.)

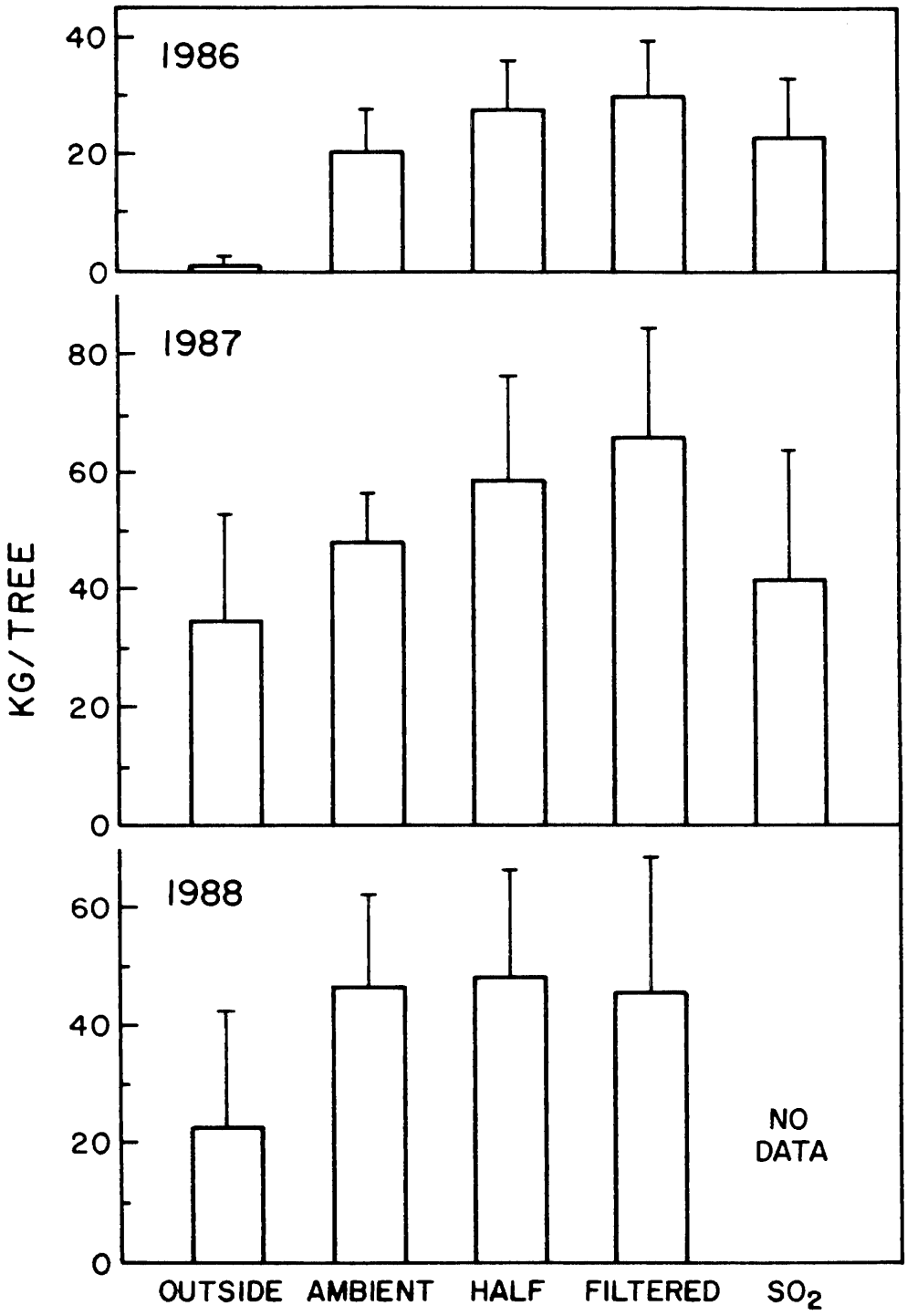

Figure 5. Weight of ripe oranges harvested per tree for the different air pollutant treatments in 1986, 1987, and 1988. Bars are means ± SD for seven trees; except for five, six, six and six for the ambient, half-ambient, filtered, and SO_2 treatments, respectively, in 1986.

Table 7. Results of Analysis of Variance for Ambient Oxidant Effects on Yield and Quality Per Tree for Valencia Orange Fruit[a]

Parameter	Years in Analysis						
	1986	1987	1988	1986, 1987	1986, 1988	1986 1987, 1988	1987, 1988
Total Fruit Weight	*[b]	ns	ns	*[b]	ns	ns	ns
Total Number	ns	ns	ns	*[b]	ns	ns	ns
% Sugar	ns	ns	ns	ns	ns	ns	ns
% Acid	ns	ns	ns	ns	ns	ns	ns
Weight/Fruit	ns	ns	*[b]	ns	ns	ns	ns
Color	ns	ns	ns	*[b]	*[b]	ns	*[b]
Fruit Height	**[c]	*[c]	ns	*[c]	ns	**[c]	**[c]
Fruit Width	*[d]	*[c]	ns	ns	ns	*[c]	ns
Rind Thickness	ns	*[c]	ns	ns	ns	*[c]	ns
Fruit Circum.	**[d]	*[c]	ns	*[c]	ns	**[c]	*[c]

[a] A 'ns' indicates no significant O_3 effect at $p < 0.05$. A *, **, or *** indicates a significant O_3 effect at $p < 0.05$, 0.01, or 0.005, respectively. There were seven replicates per treatment per year, except for five for the ambient and six for the filtered and half-ambient treatments in 1986. The analysis of variance models for one, two, or three years of data were used.

[b] Linear effect (i.e., decreased value with increasing O_3).

[c] Quadratic effect (i.e., highest value with half-ambient treatment).

[d] Quadratic effect (i.e., highest values with ambient and half-ambient treatments).

Several indicators of fruit quality was significantly affected by ambient oxidants (Table 7). Fruit had a less orange color with increasing oxidants across all three years and for 1986 plus 1987, and 1986 plus 1988. Average weight per fruit was decreased with increasing ambient oxidants in 1988. In 1986 fruit width and circumference also decreased with ambient and half-ambient air, which indicated that the oxidant-exposed fruit were larger but less dense than filtered air fruit. In 1987 the fruit were larger for the half-ambient treatment than for either the ambient or filtered air treatment. This half-ambient response was also

Table 8. Results From Regression Analysis of Ambient Ozone and Orange Yield Data from Three Harvests

Year(s)	df[a]	r[b,c]	Comparison of Years		Regression
			Intercept[d]	Slope[d]	Equation[e]
		Total Weight Per Tree			
1986	17	-0.489 *	N/A	N/A	Y = 34.2 - (188.9 x X)
1987	21	-0.459 *	N/A	N/A	Y = 69.3 - (317.3 x X)
1988	21	0.012 ns	N/A	N/A	N/A
1986,87	36	-0.452 **	**	*	Y = 53.7 - (261.1 x X)
1986,88	36	-0.121 ns	***	ns	N/A
1987,88	40	-0.207 ns	***	ns	N/A
1986,87,88	56	-0.248 ns	***	ns	N/A
		Total Number Per Tree			
1986	17	-0.252 nsz	N/A	N/A	N/A
1987	21	-0.477 *	N/A	N/A	Y = 372 - (1769 x X)
1988	21	0.108 ns	N/A	N/A	N/A
1986,87	36	-0.394 **	***	*	Y = 283 - (1245 x X)
1986,88	36	-0.015 ns	***	ns	N/A
1987,88	40	-0.197 ns	***	ns	N/A
1986,87,88	56	-0.205 ns	***	ns	N/A

[a]Degrees of freedom for regression line.
[b]Simple correlation coefficient.
[c]A 'ns' indicates nonsignificant regression. A *, or **, indicates a significant r value at $p < 0.05$, 0.01, or 0.001, respectively.
[d]The intercepts and slopes for different years were compared with an F test. A 'ns' indicates to significant difference between lines, an * indicates a significant difference between lines at $p < 0.05$.
[d]Equation where Y is the projected yield, and X is the O_3 exposure in ppm. Ozone exposure is 0800-2000 PST daylight, April-October growing season average for year, two years before harvest (June-October 1984, for 1986 harvest).

statistically significant when data for 1987 and 1988 were combined. The large fruit size for the half-ambient treatment likely was associated with the increased rind thickness. Fruit height and circumference were significantly greater for half ambient than for ambient and charcoal filtered trees across all three years.

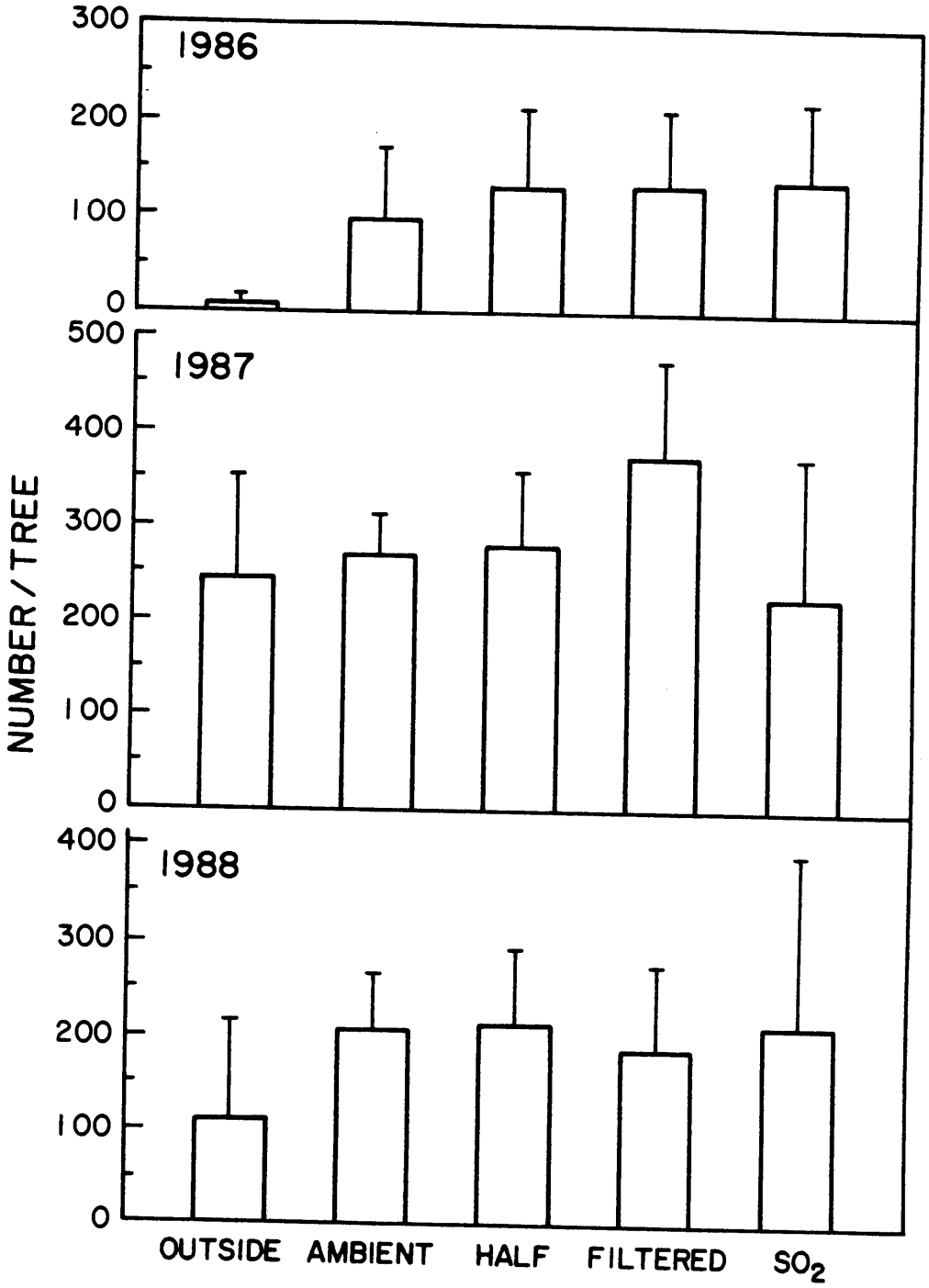

Figure 6. Number of ripe oranges harvested per tree for the different air pollutant treatments in 1986, 1987, and 1988. Bars are means ± SD for seven trees, except for five, six, six, and six, for the ambient, half-ambient, filtered, and SO_2 treatments, respectively, in 1986.

The half-ambient effect was difficult to explain. Two plant responses to ambient oxidants may have been responsible. First, oxidants apparently had an effect on the fruit carrying capacity of the tree as shown by the decrease in number with increasing oxidants. Decreased number of fruit would have been expected to result in increased fruit size as the available tree reserves are allocated to fewer fruit. At the same time, oxidants appeared to decrease availability of reserves for increased fruit growth. Thus, the larger half-ambient fruit may be due to fewer fruit but still adequate availability of reserves to support an increased size. Another explanation for the significant half-ambient effects may simply be a case of statistically overfitting with too small a data set.

The importance of the changes in fruit quality would depend on the marketplace. The commercial desirability of fruit quality parameters for Valencia oranges can vary from year to year depending on the fruit supply and consumer demand. For example, larger oranges may bring a higher price only if most oranges are small. In contrast, large oranges would be less desirable if they were more common than small oranges. Color may not be very important for Valencia oranges as they normally regreen to some extent in the summer and the orange industry tries to convince consumers of the desirability of regreened oranges in order to extend the commercial season. For Valencia oranges, fruit with thicker rinds tend to be more desirable. In general, fruit with thicker rinds tend to come from the San Joaquin Valley vs. southern California. Thus, if oxidants indirectly resulted in thicker rinds, then these fruit may become more valuable even though there would be fewer of them.

In general, the Valencia orange tree yields in our experimental orchard were lower than those expected for commercial orchards of the same calendar age. For example, four- to five-year-old trees (similar to 1986 age) normally produce five to six boxes of oranges per tree. In 1986 we only had the equivalent of two to three boxes per tree. As trees mature, five to six boxes of oranges per tree should have been expected. In 1987 we still had only three to four boxes of fruit per tree for the highest yielding trees. The reason for the lower yields for the experimental trees compared to commercial orchard trees was likely due to a younger physiological age for the experimental trees. This was because the experimental trees probably suffered considerable root shock when they

were transplanted during the dry, hot month of July, 1984. Normally young citrus trees are planted in very early spring during periods of cooler, more humid weather which reduces the water stress associated with transplanting. This root shock would have been greater for the two-year-old trees transplanted in this study than for the younger trees usually planted in groves.

The lower yields for our test trees vs. expected commercial yields had the secondary effect of resulting in overall larger fruit sizes in our study. In 1988 all fruit from all treatments were run through the commercial-type orange sizing line in the Post Harvest Fruit Research Laboratory at UCR. The test tree fruit fell primarily into sizes five to seven (higher numbers indicate larger fruit), whereas commercial fruit from Riverside are normally in the four to six range. Our fruit were larger because of lower yields than normally expected for this size of tree. The lower yields for our trees were not due to pollination as there appeared to be as many bees inside chambers as outside.

The Valencia orange yields were highly variable, as expected for such young trees. Dr. Tom Embleton of UCR has indicated that there is normally 30-40% variability in yield between trees during the first four years of bearing (the stage of tree development found in our study). Only after about the fifth year does variability decrease to 25% between trees. The average production should be about 22.7 kg $tree^{-1}$ the first year, increasing to 114 kg $tree^{-1}$ after eight years.

Fruit also were harvested for a fourth time in June 1989, eleven months after the blowers had been turned off and intensive care for the trees was terminated. There were no effects at that time, however, the oxidant results from the harvest were difficult to interpret as described in Appendix C.

2. Growth

Oxidants had no overall effect on growth of the orange trees. None of the growth parameters were significantly different between the three oxidant treatments for 1984, 1986, or 1988 (Table 9) (Appendix B).

Ambient oxidants had no effect on the total weight of leaves dropped from the trees during the entire study (Table 10). The lack of oxidant effect occurred not only across all months of all years, but also across the winter and summer months separately. This indicated that the total

Table 9. Results from Statistical Analysis of the Effects of Ambient Oxidants on Orange Tree Canopies[a]

Parameter	Year of Analysis		
	1984	1986	1988
Trunk Circumference	ns	ns	ns
Tree Height	ns	ns	ns
E-W Diameter	ns	ns	ns
N-S Diameter	ns	ns	ns
Trunk Height	ns	ns	--
Crown Height	ns	ns	--
Canopy Volume	ns	ns	ns

[a]Based on analysis of variance for seven trees per treatment, except in 1986 there were five ambient, six half-ambient and six filtered air trees. A 'ns' indicates no significant oxidant effects.

production of leaf mass by the trees was not affected by oxidants in the chambers. In fact, the total production of leaves was within 4% among the filtered, half-ambient, and ambient treatments across all months. The average leaf production (recorded as dry weight of dropped leaves) for the chamber trees was 13 kg across all three oxidant treatments.

There was no clear pattern of early senescence of leaves due to oxidants in our study as reported earlier for Navel oranges (36).

Oxidants resulted in decreased individual leaf weights as shown by the average weights for 30 leaves, especially during summer months (Table 10). The oxidant effect was linear, with ambient air exposed leaves weighing approximately 5% less than filtered air exposed leaves. The reduced leaf weight was likely associated with smaller leaf size and not thinner or less dense leaves as specific leaf area (a measure of weight per unit of leaf surface) was not affected by oxidants (see Section III.C.4).

Table 10. Average Seasonal Leaf, Fruit, and Flower Drop Responses for Valencia Orange Trees Exposed to Oxidants

Parameter	Period[a]	n[b]	Filtered	Half-Ambient	Ambient	Significance[c]
Total Weight (g)	Winter	18	314	350	323	ns
	Summer	27	269	261	267	ns
	All Months	45	286	296	289	ns
Individual Leaf Weight (g)	Winter	18	8.8	8.6	8.6	ns
	Summer	27	10.1	9.5	9.6	*(L)[d]
	All Months	45	9.6	9.1	9.2	*(L)[d]
Fruit Drop (g)	1985-86	6	28	48	33	*(Q)[e]
	1986-87	11	208	256	225	ns
	1987-88	8	193	171	154	ns
Fruit Drop (#)	1985-86	7	23	16	19	ns
	1986-87	11	4	2	4	ns
	1987-88	8	3	3	4	ns
Flower Drop (g)	1985	4	111	102	114	ns
	1987	2	442	501	666	ns
	1988	2	278	318	253	ns
Flower Drop (#)	1985	4	105	108	114	ns

[a]For leaves data are based on analysis of variance separately for winter (November-March), and summer months (April-October). Fruit drop analysis based on pooled data for one-year periods from approximately June-May. Flower drop analysis based on pooled data from the spring (March-April) months of each year indicated.
[b]Indicates number of individual measurement dates included in the pooled data. The analysis of variance model for pooled data over multiple dates was used.
[c]A 'ns' indicates no significant difference at $p < 0.05$, a *, **, or *** indicates a significant difference at $p < 0.05$, 0.01, and 0.005, respectively.
[d](L) indicates a significant linear effect.
[e](Q) indicates a significant quadratic.

Oxidants had little effect on immature fruit drop in this study. There was a significant quadratic response for weight of dropped fruit across all months leading up to the 1986 harvest (Table 10). The greatest amount of fruit loss was for the half-ambient treatment, with only a little more fruit loss for the ambient treatment than for the filtered air

treatment. There were no significant oxidant effects for total fruit drop leading up to the 1987 or 1988 harvests or for number of dropped fruit across any year.

Oxidants had no significant effect on flower drop (Table 10). The total weight of dropped flower parts (including small fruit that did not set) was the same for filtered, half-ambient, and ambient trees across all three years when it was measured. Oxidants had no effect on numbers of dropped flowers as shown by the nonsignificant response across four collection dates in 1985. The only significant response for any collection date was the higher number of dropped flowers per 5 g subsample for the ambient oxidant trees in May 1985. This suggested the possibility that there were more small fruit and flower parts for the ambient chambers. This could have been due to either a delay in flowering, or increased flower part drop for the ambient compared to the half-ambient and filtered air. Reduced or delayed flower production could contribute to the smaller number of fruit on ambient trees. However, the effect in May 1985 may also be spurious as there were no other treatment effects for other flower parameters.

3. Physiology

Ambient oxidants had a dramatic effect on leaf water relations for the orange trees across all years of measurement. Stomatal conductance was lower, transpiration was higher, and water potential more negative with the highest oxidant treatment as shown by the significant linear responses (Table 11). The linear responses occurred only across the summer months when physiological activity was greatest. The responses were indicative of increased stress to leaves similar to water stress. These data reinforce the hypothesis that averaging physiological responses across growing seasons is necessary to detect significant air pollution effects (32). Previously, Matshushima et al. (23) had reported lowered stomatal conductance due to O_3 in Mandarin orange; however, they used an extremely acute exposure (1.2 ppm O_3 for three minutes) and not ambient conditions.

There was also a significant quadratic effect for transpiration and water potential in the winter when the half-ambient treatment had higher values than the filtered or ambient air treatments (Table 11). There were also quadratic effects along with the linear effects for conductance and

Table 11. Average Winter and Summer Physiological Responses for Valencia Orange Trees Exposed to Oxidants[a]

Parameter	Season	n	Filtered	Ambient	Half-Ambient	Significance[b]
Conductance ($cm\ s^{-1}$)	Winter	51	0.115	0.121	0.111	ns
	Summer	92	0.171	0.169	0.150	***(L),*(Q)
Transpiration ($mg\ m^{-2}\ s^{-1}$)	Winter	50	14.1	15.2	14.2	*(Q)
	Summer	78	34.5	34.8	36.0	**(L),*(Q)
Photosynthesis ($mol\ m^{-2}\ s^{-1}$)	Winter	17	3.51	3.72	4.09	ns
	Summer	32	4.59	4.58	4.82	ns
Water Potential (MPa)	Winter	17	-1.33	-1.44	-1.38	*(Q)
	Summer	27	-1.59	-1.62	-1.67	*(L)

[a]Data are based on analysis of variance separately for winter (November-March) and summer (April-October) months over three to four years. The analysis of variance model for pooled data over multiple dates was used.
[b]A 'ns' indicates no significant difference at $p < 0.05$, a *,**, or *** indicates a significant difference at $p < 0.05$, 0.01; and 0.005, respectively. A (L) indicates a significant linear effect, a (Q) indicates a significant quadratic effect.

transpiration in the summer, but the half-ambient treatments were only close to, and not greater than, the filtered air values. The reason for these quadratic physiological responses is not known.

Photosynthetic rates on unit leaf area bases were not affected by ambient oxidants in this study (Table 11). Evidently, the oxidant exposures were not adequate to affect carbon dioxide fixation in the orange trees as reported for other species (26).

4. Biochemistry

The most significant biochemical response for orange leaves was a greater starch concentration with increasing O_3 exposure. A summary of the results from analysis of variance for each biochemical response parameter is shown in Table 12. The data for the individual measurement dates are shown in Appendix D.

Table 12. Effect of Ambient Oxidants on Leaf Biochemistry for Valencia Orange Trees - Summary of Data from Individual Dates

Parameter[a]	Number of Measurement Dates	Type of Response[b]		
		ns	Increase	Decrease
Chlorophyll a	5	4	1	0
Chlorophyll b	5	4	1	0
Total Chlorophyll	5	4	1	0
Chloro. a/b Ratio	5	5	0	0
Carotenoids	5	5	0	0
Chloro./Car. Ratio	5	5	0	0
Specific Leaf Area	5	5	0	0
Sulfite	1	1	0	0
Thiols	1	1	0	0
pH	1	1	0	0
Starch	1	0	1	0

[a]Chlorophylls, carotenoids, and specific leaf area were measured in January and October 1986; and January, April, and July 1987. Sulfite, thiols, and leaf pH were measured in January 1986. Leaf starch was measured in February 1988.

[b]A 'ns' indicated no significant difference between treatments, and an increase indicated the highest value with ambient air at $p < 0.05$ based on one-way analysis of variance using the model for single measurements. All increases are linear.

Leaf starch was significantly lower with increasing oxidants. Average leaf percent starch by weight was 7.6 ± 2.2 for charcoal-filtered chambers, 8.5 ± 1.5 for half-ambient chambers, and 9.4 ± 1.4 for ambient chambers. The p value was 0.044 with seven trees per treatment. This 24% greater starch in ambient compared to filtered air exposed leaves is important because it indicates that O_3 changed the pattern of carbon allocation in the trees, since carbon fixation (photosynthesis) was not affected for the leaves, as described earlier.

Other significant biochemical effects were increases in leaf chlorophyll concentrations with increased oxidants in April 1987 (Table 12). The increased chlorophyll may have reflected the higher starch concentrations in the ambient O_3 leaves early in the year. However, the overall effect of oxidants on leaf chlorophyll is minimal as concentrations were not affected by O_3 on any other date, and no other parameter was affected by oxidants on any date.

D. Sulfur Dioxide Effects

1. Yield and Quality of Fruit

Added SO_2 reduced orange yields in 1986 and 1987, with the response statistically significant in 1987 and across both years (Table 13). Total fruit weight was 23% and 35% lower with SO_2 as compared to filtered air in 1986 and 1987 (Figure 5). Number of fruit per tree was the same and 39% lower with SO_2 as compared to filtered air in 1986 and 1987 (Figure 6). This indicated that the yield loss was due to reduced fruit size in 1986, but reduced fruit number in 1987. Numerical data for 1986, 1987 and 1988 harvests are shown in Appendix B.

The estimated 1988 orange yield for the SO_2 trees (based on the November 1987 count) was approximately the same as for the filtered air trees (Figure 6). The actual count was 214 ± 180 for the SO_2 vs. 187 ± 91 for the filtered air trees. Weight of fruit per tree also was determined for the SO_2 trees (36.1 ± 28.5 kg/tree). This weight was slightly less than the weight of fruit for the filtered air trees at the May 1988 harvest (46.6 ± 22.8 kg/tree). Neither the number nor weight of fruit per tree were significantly different between SO_2 and filtered air trees based on an unpaired t-test. The data for the SO_2 trees were not included in the analysis of variance for the 1988 harvest data, as changes in count and especially in weight would have been expected between November 1987 and May 1988. Thus, the t-test analysis was only for general comparison purposes. Nevertheless, the 1988 data for the SO_2 treatment do show the large variability and lower yield, which indicate an "off-year" in 1988 compared to 1986 and 1987.

Table 13. Results of Analysis of Variance for Sulfur Dioxide Effects on Yield and Quality Per Tree for Valencia Orange Fruit[a]

Parameter	Years in Analysis		
	1986	1987	1986, 1987
Fruit Weight	ns	*[b]	**[b]
Number	ns	*[b]	*[b]
Percent Sugar	ns	ns	ns
Percent Acid	ns	ns	ns
Weight/Fruit	ns	ns	ns
Color	ns	ns	ns
Fruit Height	ns	ns	***[c]
Fruit Width	ns	ns	**[c]
Rind Thickness	ns	ns	ns
Fruit Circumference	ns	ns	***[c]

[a]A 'ns' indicates no significant sulfur dioxide effect at $p < 0.05$. A *, **, or *** indicates a significant sulfur dioxide effect at $p < 0.05$, 0.01, or 0.005, respectively. There were six and seven trees per sulfur dioxide and filtered air treatment in 1986 and 1987, respectively. The analysis of variance models were for one or two years of data.
[b]Significantly lower value with sulfur dioxide than with filtered air.
[c]Significantly higher value with sulfur dioxide than with filtered air.

Sulfur dioxide exposed trees tended to have larger fruit compared to filtered air trees (Table 13). The response was noted for fruit height, width, and circumference across both years, but not for the individual years. The larger fruit was likely due to the greater availability of reserves to the smaller number of fruit with the sulfur dioxide exposure, as noted previously for the reduced number of fruit with the ozone exposure. No other quality parameters were affected by sulfur dioxide.

2. Growth

Sulfur dioxide had no affect on overall tree growth as shown by the canopy measurements (Table 14). None of the canopy parameters were significantly different between sulfur dioxide and filtered air trees. Sulfur dioxide had no effect on total weight of leaves dropped from the

Table 14. Results from Statistical Analysis of the Effects of Sulfur Dioxide on Orange Tree Canopies[a]

Parameter	Years of Analysis	
	1984	1986
Trunk Circumference	ns	ns
Tree Height	ns	ns
E-W Diameter	ns	ns
N-S Diameter	ns	ns
Trunk Height	ns	ns
Crown Height	ns	ns
Canopy volume	ns	ns

[a]Based on analysis of variance for six and seven trees in 1986 and 1987, respectively, per SO_2 and filtered air treatment. The analysis of variance model was for a single set of measurements.

trees (Table 15). The total weight of dropped leaves was the same across all months, indicating that sulfur dioxide had no effect on overall leaf production by the trees. Numerical data for 1984 and 1986 canopy measurements are shown in Appendix 13.

Sulfur dioxide exposure decreased individual leaf weights when the data were averaged across all months (Table 15). The reduced leaf weight with sulfur dioxide exposure was likely associated with smaller leaf size, as specific area was actually greater for sulfur dioxide leaves on one biochemistry sampling date (see Section III.D.4).

There were no significant fruit or flower drop responses across collection periods for trees exposed to sulfur dioxide (Table 15).

3. Physiology

Sulfur dioxide had no effect on most physiological parameters. Only transpiration was affected, with a significant increase across summer months (Table 16). This rather small (7%) increase in water loss was probably physiologically insignificant.

4. Biochemistry

Sulfur dioxide had little affect on leaf biochemistry. The total leaf sulfur concentration was higher for sulfur dioxide compared to

Table 15. Average Seasonal Leaf, Fruit, and Flower Drop Responses for Valencia Orange Trees Exposed to Sulfur Dioxide

Parameter	Period[a]	n[b]	SO_2	Filtered	Significance[c]
Total Weight (g)	Winter	13	310	267	ns
	Summer	20	194	216	ns
	All Months	33	240	236	ns
Individual Leaf Weight (g)	Winter	13	7.7	8.1	ns
	Summer	20	9.3	9.8	ns
	All Months	33	8.7	9.1	*
Fruit Drop (g)	1985-86	6	28	28	ns
	1986-87	10	209	208	ns
Fruit Drop (#)	1985-86	7	32	23	ns
	1986-87	10	3	4	ns
Flower Drop (g)	1985	4	118	111	ns
	1987	2	580	442	ns
Flower Drop (#)	1985	4	95	105	ns

[a]Leaf data are based on analysis of variance separately for winter (November-March) and summer months (April-October). Fruit drop analysis based on pooled data for one-year periods from approximately June-May. Flower drop analysis based on pooled data from the spring (March-April) months of each year indicated.

[b]Indicates number of individual measurement dates included in the pooled data. The analysis of variance model for pooled data over multiple dates was used.

[c]A 'ns' indicates no significant difference at $p < 0.05$, a * indicates a significant difference at $p < 0.05$.

filtered air trees (Table 17). However, this was expected given the large amount of gaseous sulfur to which the trees were exposed. Specific leaf area was increased with sulfur dioxide exposure on one date. No other parameters were affected by sulfur dioxide, not even thiol or sulfite concentrations, both of which were likely indicators of sulfur dioxide exposure. Numerical data for the biochemical measurements are shown in Appendix D.

Table 16. Average Winter and Summer Physiological Responses for Valencia Orange Trees Exposed to Sulfur Dioxide[a]

Parameter	Season	n	Filtered	SO_2	Significance[b]
Conductance ($cm\ s^{-1}$)	Winter	46	0.123	0.118	ns
	Summer	88	0.171	0.173	ns
Transpiration ($mg\ m^{-2}\ s^{-1}$)	Winter	45	14.7	15.1	ns
	Summer	74	34.3	36.8	**
Photosynthesis ($mol\ m^{-2}\ s^{-1}$)	Winter	12	4.14	4.78	ns
	Summer	28	4.70	5.06	ns
Water Potential (MPa)	Winter	12	-1.50	-1.55	ns
	Summer	23	-1.57	-1.64	ns

[a]Data are based on analysis of variance separately for winter (November-March) and summer (April-October) months. The analysis of variance model for pooled data over multiple dates was used.
[b]A 'ns' indicates no significant difference at $p < 0.05$; a ** indicates a significant difference at $p < 0.01$.

E. Chamber Effects

1. Yield and Quality of Fruit

Yields were much higher in the ambient open-top field chambers than in outside air. The greater total fruit weight for ambient chamber trees was statistically higher than for outside trees in 1986 and 1988, as well as across all combinations of years (Table 18). In 1986 only three of the seven outside trees had oranges, and the weight and number of fruit per tree were very low (Figures 5 and 6). In 1987 and 1988, the outside trees produced oranges, but the amount was still much less for ambient chamber trees. There was 39% and 51% higher orange weight for ambient chamber trees compared to outside trees in 1987 and 1988, respectively. The number of fruit per tree was significantly greater in ambient chambers than for outside trees in 1986, and for data polled across 1986 and 1987, 1986 and 1988, and all three years. There were 10% and 84% more oranges in the ambient chambers than in outside air in 1987 and 1988, respectively. Numerical data for the yield measurements are shown in Appendix B.

Table 17. Effect of Sulfur Dioxide on Leaf Biochemistry for Valencia Orange Trees - Summary of Data from Individual Dates

Parameter[a]	Number of Measurement Date	Type of Response[b]		
		ns	Increase	Decrease
Chlorophyll a	5	5	0	0
Chlorophyll b	5	5	0	0
Total Chlorophyll	5	5	0	0
Chlorophyll a/b Ratio	5	5	0	0
Carotenoids	5	5	0	0
Chlorophyll/Car. Ratio	5	5	0	0
Specific Leaf Area	5	4	1	0
Sulfite	5	5	0	0
Thiols	5	5	0	0
pH	1	1	0	0
Total Sulfur - New	1	0	1	0
Total Sulfur - Old	1	0	1	0

[a]Chlorophylls, carotenoids, sulfite, thiols, and specific leaf area were measured for January and October 1986; and January, April, and July 1987. Leaf pH was measured for January 1986. Total sulfur was measured for November 1987.

[b]A 'ns' indicated no significant difference between treatments, and an increase indicated the highest value with ambient air at $p < 0.05$ based on analysis of variance using the model for single measurements, and an unpaired t-test between sulfur dioxide and filtered air treatments for some parameters.

The 34% decrease in yield between 1987 and 1988 was the prime indicator that 1988 was an "off" year for productivity compared to 1987. It had been expected that these young orange trees would increase in yield every year until they reached a more mature size.

The chambers had a dramatic effect on fruit quality. The chamber tree fruit were generally larger and heavier than outside tree fruit (Table 18). Rind thickness was greater across 1986 and 1987 for ambient chamber compared to outside fruit. The ambient chamber fruit also were less acidic than outside fruit. This may have been due to a dilution of

Table 18. Results of Analysis of Variance for Chamber Effects on Yield and Quality Per Tree for Valencia Orange Fruit[a]

Parameter	Years of Analysis						
	1986	1987	1988	1986, 1987	1986, 1988	1987, 1988	1986 1987, 1988
Fruit Weight	***[b]	ns	*[b]	**[b]	**[b]	**[b]	**[b]
Number	***[b]	ns	ns	*[b]	***[b]	ns	**[b]
% Sugar	ns	ns	ns	ns	ns	ns	ns
% Acid	ns	ns	ns	**[c]	ns	ns	**[c]
Weight/Fruit	ns	ns	ns	**[b]	***[b]	*[b]	**[b]
Color	ns	ns	ns	ns	ns	ns	ns
Fruit Height	*[b]	ns	ns	**[b]	***[b]	**[b]	***[b]
Fruit Width	*[b]	ns	ns	*[b]	*[b]	ns	**[b]
Rind Thickness	ns	ns	ns	**[b]	ns	ns	ns
Fruit Circum.	**[b]	ns	ns	**[b]	***[b]	*[b]	***[b]

[a]A 'ns' indicates no significant chamber effect at $p < 0.05$. A *, **, or *** indicates a significant chamber effect at $p < 0.05$, 0.01, or 0.005, respectively. There were seven replicate trees per treatment except for five for the ambient chamber in 1986. There were only three replicates per treatment for outside trees (quality parameters in 1986) as only three trees had fruit. The analysis of variance models were for one, two or three years of data.
[b]Significantly higher value in ambient chamber than outside.
[c]Significantly lower value in ambient chamber than outside.

the amount of acid substances in fruit of the ambient chamber trees, but not the outside trees, assuming that the available acids per fruit was the same for trees in both treatments. The larger size of the fruit in the chambers may have been related to the larger amount of available canopy per fruit compared to outside trees.

2. Growth

The open-top chamber environment greatly stimulated the growth of the trees compared to growth in outside air. This began to happen immediately after placement of the chambers over the trees, so that by December 1984 the canopy was much larger for the ambient chamber trees

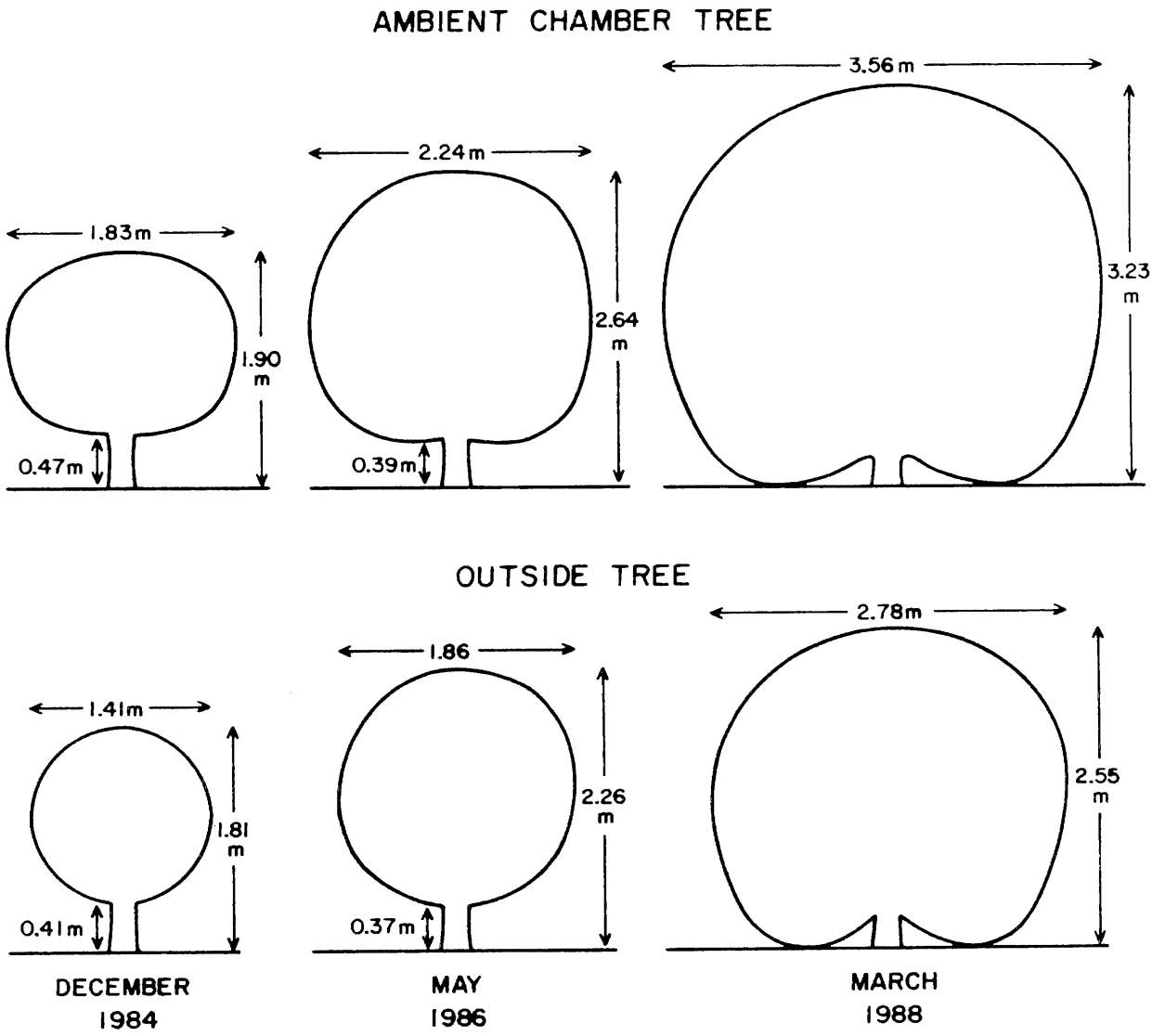

Figure 7. Diagram of relative growth of Valencia orange trees in ambient chambers compared to outside air. Values are averages of seven trees.

Table 19. Results from Statistical Analysis of Chamber Effects on Orange Tree Canopies[a]

Parameter	Year of Analysis		
	1984	1986	1988
Trunk Circumference	ns	ns	ns
Tree Height	ns	*	***
E-W Crown Diameter	***	**	***
N-S Crown Diameter	***	***	***
Trunk Height	*	ns	--[b]
Crown Height	ns	*	--[c]
Canopy volume	**	***	***

[a]A 'ns' indicates no significant chamber effect at $p < 0.05$. A *, **, or *** indicates a significant chamber effect at $p < 0.05$, 0.01, or 0.005, respectively. There were seven replicate trees per treatment except for five for the ambient chamber in 1986. There were only three replicates per treatment for outside trees (quality parameters in 1986) as only three trees had fruit. The analysis of variance model was for a single set of measurements.

[b]In 1988 no separate trunk and crown height measurements were made because the lower branches of the tree touched the ground, and thus the crown height was equal to the tree height.

compared to outside trees (Figure 7, Table 19). This increased growth is graphically illustrated in Figure 11 which shows the heights and diameters of the tree canopies in December 1984, May 1986, and March 1988. The relative canopy volumes for the two treatments were 2.53 ± 0.45 m^3 for ambient chamber trees vs. 1.47 ± 0.56 m^3 for outside trees for 1984; 5.81 ± 0.89 m^3 for ambient chamber trees vs. 3.46 ± 1.07 m^3 for outside trees for 1986; and 21.02 ± 1.67 m^3 for ambient chamber trees vs. 10.31 ± 2.04 m^3 for outside trees for 1988. The difference in canopy volume was statistically significant at $p < 0.001$ for each year. By 1988 the ambient chamber tree canopy volume was over twice the volume for outside trees. The canopies of the ambient chamber trees appeared to be more open than the canopies of the outside trees; however, the chamber trees still appeared to produce more leaves. Numerical data for the measurements are shown in Appendix B.

The total weight of dropped leaves was much higher for ambient chamber trees than for outside trees both over winter and summer months as well as across all months (Table 20). The leaf loss for ambient chamber trees was over twice that of outside trees and was statistically significant at $p < 0.001$ across all time periods.

Individual leaf weights were greater for ambient chamber trees than for outside trees across the summer months, but not across all months nor across winter months (Table 20). The greater leaf weight may have occurred because of greater density as well as increased size of leaves, as shown by the greater specific leaf area for three of the area sampling dates (see Section III.E.4).

There was a pronounced difference in fruit and flower drop between the ambient chamber trees and outside trees. The total weight and number of dropped immature fruit were lower for the ambient chamber than for the outside trees from May 1986 to April 1987 (Table 20). This difference was due primarily to a large number of fruit blown off outside trees (but not ambient chamber trees) by high winds in December 1986. If these fruit had reached maturity in May 1987, the yields of the outside trees would have been greater than the yields of ambient chamber trees. This is because there was an average of 378 and 319 fruit/tree, respectively, for outside and ambient chamber trees if the total number of fruit dropped between 1986-1987 was added to the number harvested in May 1987. There was also a significantly greater lower number of fruit dropped from ambient chamber trees compared to outside trees from June 1985 to April 1986 leading up to the May 1986 harvest. There was no difference in immature fruit drop between the ambient chamber and outside trees from May 1987 to April 1988 leading up to the May 1988 harvest. The number of dropped fruit for both ambient chamber and outside trees leading up to the 1988 harvest was less than half that of the number leading up to the 1987 harvest. This was a further indication that 1988 was an "off" production year.

Ambient chamber trees had greater flower drop than outside trees in the spring of 1987 (Table 20). The difference between the two treatments was based on total weight of flowers dropped from ambient vs. outside trees. This likely indicated that more flowers were produced on chamber trees than on outside trees. It was also possible that the slightly later

Table 20. Average Seasonal Fruit and Flower Drop Responses for Valencia Orange Trees in Ambient Chambers and Outside[a]

Parameter	Season	n[b]	Ambient Chamber	Outside	Significance[c]
Total Weight (g)	Winter	18	323	123	***
	Summer	27	267	132	***
	All Months	45	289	128	***
Individual Leaf Weight (g)	Winter	18	8.6	9.0	ns
	Summer	27	9.6	8.8	***
	All Months	45	9.2	8.9	ns
Fruit Drop (g)	1985-1986	6	33	45	ns
	1986-1987	11	225	457	*
	1987-1988	8	154	123	ns
Fruit Drop (#)	1985-1986	7	19	45	**
	1986-1987	11	4	11	**
	1987-1988	8	4	6	ns
Flower Drop (g)	1985	4	114	75	ns
	1987	2	666	175	*
	1988	2	253	285	ns
Flower Drop (#)	1985	4	114	160	***

[a]Data are based on analysis of variance separately for winter (November-March), and summer months (April-October for leaves). Fruit drop analysis based on pooled data for one-year periods from approximately June-May. Flower drop analysis based on pooled data from the spring (March-April) months of each year indicated. The analysis of variance model for pooled data over multiple dates was used.

[b]Indicates number of individual measurement dates included in the pooled data.

[c]A 'ns' indicates no significant difference at $p < 0.05$; a *, **, or *** indicates a significant difference at $p < 0.05$, 0.01, and 0.005, respectively.

flowering period for the outside trees resulted in fewer flowers being collected for the outside trees on the set dates. The number of flowers (per weight of subsample) was greater for outside trees than ambient chamber trees in 1985. This indicated that the flowers were smaller for outside trees compared to chamber trees at the time of sampling, which was probably due to later flowering for the outside trees.

Though the flower collection data indicated interesting effects for chamber vs. outside trees, the results should be approached with caution.

The flower collection method was relatively imprecise, as flower parts had to be swept up from beneath the trees, dried, floated on water to separate flowers (lighter) from dirt and pebbles (heavier), redried, and weighed. This long process allowed for loss of flower parts and error. Also, there were many small young fruit mixed in with the flower parts which complicated the weight data considerably. Young small fruit would be much lighter than old small fruit; thus, if treatments had more old small fruit, their total "flower" weight would be overestimated.

3. Physiology

The chambers themselves had a greater impact on orange tree leaf physiology than either pollutant. Stomatal conductance and transpiration rates were lower, photosynthetic rates were higher, and water potential was more negative for ambient chamber leaves compared to outside leaves across all years (Table 21). The effects occurred primarily in the summer when the trees were most physiologically active; however, water potential also was more negative for ambient chamber leaves compared to outside leaves in the winter when physiological activity was lowest.

The changes in conductance, transpiration, and water potential were all indicative of increased water stress to leaves within the chambers compared to leaves outside. This increased water stress may have been related to the higher air temperature, higher relative humidity, and constant air flow in the chambers. All of these would tend to increase water deficits in the chambers compared to outside air. The decreased conductance in the chambers may represent a decreased sensitivity of chamber trees to pollutants compared to outside, as the higher the conductance rate the greater the amount of pollutants taken up into leaves (37).

4. Biochemistry

There were many changes in leaf biochemistry for the chambers compared to outside trees (Table 22). Specific leaf area was greater for ambient chamber leaves than for outside leaves on three of the five measurement days. Leaf chlorophyll "b" was greater for ambient chamber trees on two of the measurement days; chlorophyll "a", total chlorophyll, and pH were all greater for ambient chamber trees on one day. Leaf carotenoid concentration was lower for ambient chamber trees than for outside trees on one day. The fact that each "day" actually occurred during a different season as shown in Appendix D indicated that these

Table 21. Average Winter and Summer Physiological Responses for Valencia Orange Trees in Ambient Open-Top Chambers vs. Outside Air[a]

Parameter	Season	n	Ambient	Outside	Significance[b]
Conductance ($cm\ s^{-1}$)	Winter	51	0.111	0.105	ns
	Summer	92	0.150	0.174	***
Transpiration ($mg\ m^{-2}\ s^{-1}$)	Winter	50	14.2	13.6	ns
	Summer	78	31.7	36.0	***
Photosynthesis ($mol\ m^{-2}\ s^{-1}$)	Winter	16	4.09	3.60	ns
	Summer	32	4.82	4.27	*
Water Potential (MPa)	Winter	17	-1.38	-1.21	***
	Summer	27	-1.67	-1.31	***

[a]Data are based on analysis of variance separately for winter (November-March) and summer (April-October) months. The analysis of variance model for pooled data over multiple dates was used.
[b]A 'ns' indicates no significant difference at $p < 0.05$, a * or *** indicates a significant difference at $p < 0.05$ or 0.005, respectively.

chamber effects occurred over differing environmental conditions. Thus, the chamber effects on leaf biochemistry may represent a long-term impact of the chambers on tree growth.

The greater specific leaf area for ambient chambers indicated that the leaves were thinner on chamber trees than on outside trees. This provided further evidence that chamber tree leaves were larger than outside tree leaves in addition to the data indicating that chamber tree leaves were also heavier (Table 20), and visibly larger than outside tree leaves. The fact that chamber leaves were thinner, but still had more negative water potentials emphasized that the chamber leaves were under water stress, as thinner leaves normally have less negative water potentials (31).

There was a statistically significant increase in leaf starch concentration for nonfiltered chamber vs. outside trees (Table 22). Leaf starch averaged 9.4 ± 1.4% for nonfiltered chamber vs. 7.2 ± 1.3% for outside trees, with the difference between the two treatments significant at p =

Table 22. Effect of Open-Top Field Chamber on Leaf Biochemistry for Valencia Orange Trees - Summary of Data from Individual Dates[a]

Parameter	Number of Measurement Dates	Type of Response[b]		
		ns	Increase	Decrease
Chlorophyll a	5	4	1	0
Chlorophyll b	5	3	2	0
Total Chlorophyll	5	4	1	0
Chlorophyll a/b Ratio	5	5	0	0
Carotenoids	5	4	0	1
Chlorophyll/Car. Ratio	5	5	0	0
Specific Leaf Area	5	2	3	0
Sulfite	1	1	0	0
Thiols	1	1	0	0
pH	1	1	1	0
Starch	1	0	1	0

[a]Chlorophylls, carotenoids, and specific leaf area were measured in January and October 1986 and January, April, and July 1987. Sulfite, thiols, and leaf pH were measured in January 1986. Leaf starch was measured in February 1988.

[b]A 'ns' indicated no significant difference; an increase indicated the highest value with ambient chamber, and a decrease indicated the lowest value with ambient chamber at $p < 0.05$ based on analysis of variance using the model for single measurements, and an unpaired t-test between ambient chamber and outside treatments for single measurements.

0.016. As for the O_3 treatment, this could indicate that carbon allocation in the trees was affected by the chambers. It is possible that the chamber environment somehow resulted in increased storage due to reduced transport of starch out from leaves. However, the higher photosynthetic rate for chamber leaves also likely played a role in the higher starch concentration in leaves of chamber trees compared to leaves on outside trees.

IV. DISCUSSION

A. Crop Loss Estimates Based on These Results

This study has documented the potential effects of O_3 on Valencia orange trees under the open-top chamber conditions necessary to control the concentrations of pollutant in the air. The effects were based on three years of data. While this was very little data with which to predict the response of trees in commercial orchards, it was still the best available data with which to estimate orange yield losses from ambient O_3 in California.

These estimated orange yield losses were calculated using an O_3 exposure-orange yield equation, as described for the ARB-sponsored crop loss assessment project (24). The equation used ambient O_3 data and crop productivity data from each county in the State to estimate percentage yield losses form O_3 on a county-by-county and statewide basis. The equation provided a % yield loss according to the formula:

$$\% \text{ loss} = 1 - [(a + (b \cdot X))]/[(a + (b \cdot X'))] \times 100$$

where a was the intercept from the yield loss equation, b was the slope of the equation, X is the ambient O_3 concentration, and X' is a "background" O_3 concentration used to estimate potential yield in clean air. Normally, an 0800-2000 growing season average concentration of 0.025 ppm has been used to represent clean air (24).

For oranges, the question of which yield loss equation to use was complicated by the fact that Valencia oranges exhibit the alternate year pattern of fruit bearing. From the data shown in Table 8, it was evident that there was a substantial yield loss from O_3 during the "on" years of bearing vs. no effect ("0 % loss") on yield loss during the "off" year.

The two "on" years had similar loss equations in terms of slope (the main determinant of % loss) (Table 8), and thus the data from the two "on" years could be used together to produce the equation shown in Figure 8. This resulted in an "a" of 53.7 and "b" of 261.1 in the "on" year. However, any general loss equation also has to consider the alternate year bearing pattern which varies between orchards across the state. Therefore, for our loss equation, we have assumed that the O_3-induced losses

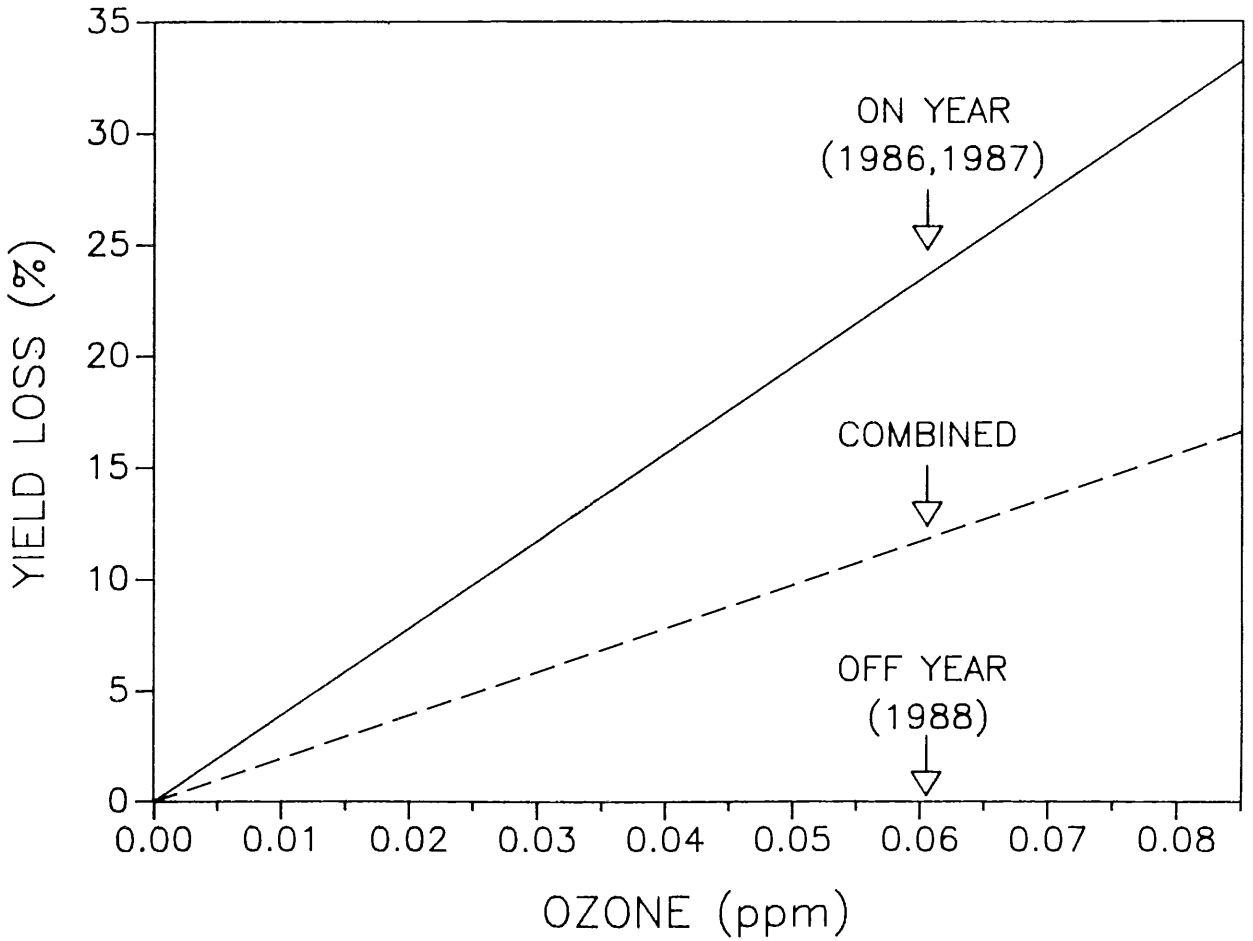

Figure 8. Ozone exposure vs. percent yield loss equations for Valencia oranges. Based on data in Table 8.

in oranges are an average of the potential losses of "on" year vs. no losses for "off" year orchards. The assumption had to be made that the "off" and "on" year orchards were distributed equally across the state, as the pattern for orange yields varies between orchards, counties, and years. Thus, the percent loss equation was modified so that it was multiplied by 50 and not 100, to indicate that the estimated losses were only one-half those expected if all orchards were having an "on" year. This resulted in the "combined" equation shown in Figure 8.

This combined equation approach to estimating orange yield losses from ozone is probably most valid for estimating long-term losses across the entire state. However, because annual losses must be predicted for crops as part of the crop loss assessment project, we also used the combined equation approach to estimate losses in single counties and for individual harvest years, recognizing that these annual losses may be inaccurate for some areas.

The orange yield loss equation was used to estimate losses due to ozone on a county-by-county basis for 1986 as shown in Table 23. The estimated losses were for all types of oranges, with the equation generated for Valencia oranges from this study also used for navels and all other cultivars. The yield losses in 1986 were based on growing season ambient ozone concentrations in 1984. The greatest losses were in Riverside and San Bernardino counties, which are part of the heavily polluted South Coast Air Basin. Counties in the southern San Joaquin Valley also had relatively large estimated losses. Losses were lowest on the west side of the San Joaquin Valley and in the Sacramento Valley counties where a small acreage of oranges is grown.

The losses would, of course, be higher if the whole county were assumed to have orange orchards that were experiencing an "on" production year. In contrast, if all orchards were experiencing an "off" year, no losses would be expected. Thus, a more accurate estimate of losses would be mid-way, with an average statewide yield loss of 9% for oranges if "on" and "off" year orchards were equally represented across the state. However, it must be remembered that this estimate was a loss in "potential" yield which could be attained if the ozone concentrations across the state could be reduced to the 0.025 ppm growing season average estimated for "clean" air. The estimated losses do not take into account losses due to any pests or other environmental factors besides ozone air pollution.

Table 23. Estimated Percentage Yield Losses for 1986 for Oranges Based on Ozone Exposure-Yield Loss Data in This Report[a]

County	Actual Tons	12-Hour Average[b]	% Yield Loss			Potential Tons[c]
			On Year	Combined	Off Year	
Butte	2,033	0.030	2.8	1.4	0	2,091
Fresno-West	3,698	0.042	9.1	4.6	0	4,082
Fresno-East	227,402	0.057	17.7	8.9	0	276,349
Glenn	9,636	0.043	10.0	5.0	0	10,702
Imperial	4,598	0.040	8.3	4.2	0	5,014
Kern	200,600	0.054	16.1	8.0	0	238,956
Placer	275	0.042	9.4	4.7	0	304
Riverside	167,937	0.072	26.0	13.0	0	226,986
San Bernardino	60,091	0.079	29.9	15.0	0	85,708
San Diego	134,227	0.052	15.0	7.5	0	157,811
Tulare	941,200	0.057	17.7	8.9	0	1,143,788
Ventura	159,415	0.057	17.7	8.9	0	193,728
Statewide	2,007,287		18.3	9.2	0	2,457,908

[a]Losses calculated using the data from Table 8, and are for ambient air compared to "clean" air (i.e., a 12-hour growing season average of 0.025 ppm).
[b]The 12-hour average is for 0800-2000 PST from April through October 1984.
[c]Assuming all orchards were experiencing "on" years.

B. Do Results from Chambers Represent Responses to Air Pollution in Commercial Orchards?

The question persisted whether or not the projected yield decrease from the pollutants accurately represents field growing conditions without chambers. Data taken over three years of weekly to biweekly stomatal conductance indicated that the uptake of O_3 into leaves likely was actually less for chamber vs. outside trees. This would indicate that the chamber trees would be less sensitive to pollutants than outside trees. Hence the projected yield losses based on chambers would be conservative, underestimating losses in the field. In contrast, the individual leaf weight-specific leaf area data suggested that chamber tree leaves were thinner and possibly more sensitive to air pollutants than outside tree leaves. The higher photosynthetic rate and greater leaf chlorophyll concentrations for ambient chamber vs. outside tress indicated potentially greater carbon fixation rates for chamber than outside trees. Plants with such higher metabolic activity such as in chambers may be more sensitive to pollutants than plants with lower rates such as the outside trees (26).

The net effect of the changes in physiological and biochemical responses for chamber vs. outside trees was not known. For example, the relative importance of stomatal conductance vs. photosynthetic rate in determining plant response to air pollutants would have to be determined to definitively say whether the yield losses found in the chambers are representative of the conditions in commercial fields.

C. Mechanistic Basis for Air Pollution Effects on Orange Trees

The reduced number of fruit, especially with O_3, may be due to a number of factors affecting either flower bud formation or fruit set on a whole tree basis. These factors may not be reflected in the instantaneous measurements of photosynthetic rates or stomatal conductance for leaves at similar stages of development as measured in the study. Thus, these instantaneous measurements show no general O_3 or SO_2 treatment effects. As described by Sinclair (29), fruit development from flowering to maturity is a complex process dependent on environmental factors, the interplay between vegetative and reproductive growth, and the effects of previous year's productivity on current year's productivity.

In terms of relationship between flowering and productivity, Valencia oranges normally produce many more flowers than needed to produce fruit, with only about 1% of the flower buds becoming mature fruit (6). Carbohydrate reserves increase in leaves in late winter prior to flowering (5), and are especially important in determining the subsequent fruit production of the trees (11,13). These reserves and the amount of fruit produced are affected by the size of the crop and time of picking the previous year (10,12).

The interplay of vegetative growth and fruit production is even more complex than the flowering-fruit production relationship. Saurer (27) reported that fruit production in Valencia oranges is affected by the proportion of leafless to leafy shoots, with leafy shoots producing more fruit. In general, vegetative growth is needed to produce a large orange crop, yet the presence of fruit also tends to reduce vegetative growth (29). The relationship between leaf abscission and fruit production is also very important, as defoliation reduces fruit size (17) and would also tend to result in less carbohydrate reserves available for flower production and fruit set. Selective defoliation at different times of the year would also encourage vegetative growth which also would have a complicated effect on subsequent flowering and fruit production.

Thus, a number of factors may be involved in the mechanism by which air pollutants affect fruit number, including amount and time of defoliation, proportion of leafy to leafless shoots, number of flowers, and amount and timing of fruit set. All of these processes are tied to the carbohydrate reserves of the tree. Thus, the fact that leaf starch (an important carbohydrate) is retained in leaves prior to flowering for oxidant exposed trees indicates that oxidants can affect carbohydrate allocation in oranges. Additional research is needed in this area.

V. REFERENCES

1. Adams, J. A., H. B. Johnson, F. T. Bingham, and D. M. Yermanos. 1977. Gaseous exchange of Simmondsig chinensis (Jojoba) measured with a double isotope porometer and related to water stress, salt stress, and nitrogen deficiency. Crop Science 17:11-15.

2. Bytnerowicz, A., D. M. Olszyk, G. Kats, P. J. Dawson, J. Wolf and C. R. Thompson. 1987. Effects of SO_2 on physiology, elemental content and injury development of winter wheat. Agric. Ecos. Environ. 20:37-47.

3. California Department of Food and Agriculture. 1988. California Agriculture, A Statistical Review, 1987.

4. Darrall, N. M. and H. J. Jager. 1984. Biochemical diagnostic tests for the effect of air pollution on plants. pp. 333-350 In: M. J. Koziol and F. R. Whatley, Gaseous Air Pollutants and Plant Metabolism, Butterworths:London.

5. Dugger, W. M. and R. L. Palmer. 1969. Seasonal changes in lemon leaf carbohydrates. Proc. First. Intl. Citrus Symp. 1:339-343.

6. Erickson, L. C. and B. L. Brannaman. 1960. Abscission of reproductive structures and leaves of orange trees. Proc. Amer. Soc. Hortic. Sci. 75:222-229

7. Grill, D., H. Esterbauer, and U. Klosch. 1979. Effect of sulphur dioxide on glutathione in leaves of plants. Environ. Pollut. 18:187-194.

8. Heck, W. W., W. W. Cure, J. O. Rawlings, L. J. Zaragosa, A. S. Heagle, H. E. Heggestad, R. J. Kohut, L. W. Kress, and P. J. Temple. 1984. Assessing impacts of ozone on agricultural crops: I. Overview. J. Air Pollut. Control Assoc. 34:729-735.

9. Huber, S. C. and D. W. Israel. 1982. Biochemical basis for portioning of photosynthetically fixed carbon between starch and sucrose in soybean (Glycine max Merr.) leaves. Plant Physiol. 69:691-696.

10. Jones, W. W. and C. B. Cree. 1965. Environmental factors relating to fruiting of Washington Navel oranges over a 38-year period. Proc. Amer. Soc. Hortic. Sci. 86:267-271.

11. Jones, W. W. and T. W. Embleton. 1971. Effects of delayed harvest on California Valencias. Cal. Citrograph. 56:102,114-115.

12. Jones, W. W., T. W. Embleton, M. L. Steinacker, and C. B. Cree. 1964. The effect of time of fruit harvest on fruiting and carbohydrate supply in the Valencia orange. Proc. Amer. Soc. Hortic. Sci. 84:152-157.

13. Jones, W. W., T. W. Embleton, M. L. Steinacker, and C. B. Cree. 1970. Carbohydrates and fruiting of Valencia orange trees. J. Amer. Soc. Hortic. Sci. 95:380-381.

14. Kats, G., D. M. Olszyk, and C. R. Thompson. 1985. Open-top experimental chambers for trees. J. Air Pollut. Control Assoc. 35:1298-1301.

15. Knudson, L. L., T. W. Tibbitts, and G. E. Edwards. 1977. Measurement of ozone injury by determination of leaf chlorophyll concentration. Plant Physiol. 60:606-608.

16. Koziol, M. J. 1984. Interactions of gaseous pollutants with carbohydrate metabolism. pp. 251-274. In: M. J. Koziol and F. R. Whatley, Gaseous Air Pollutants and Plant Metabolism, Butterworths:London.

17. Lenz, F. 1967. Relationships between the vegetative and reproductive growth of Washington Navel orange cuttings (Citrus sinensis L. Osbeck). J. Hort. Sci. 43:31-39.

18. Lichtenthaler, H. K. and A. R. Wellburn. 1983. Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 603:591-592.

19. Maas, F. 1987. Responses of Plants to Sulfur Containing Air Pollutants. University of Groningen, Netherlands.

20. Matsushima, J. and M. Harada. 1964. Sulfur dioxide gas injury to fruit trees. Bull. Fac. Agric. Mie University 30:11-32.

21. Matsushima, J. and M. Harada. 1966. Sulfur dioxide gas injury to fruit trees. V. Absorption of sulfur dioxide by citrus trees and its relation to leaf fall and mineral content of leaves. J. Japan. Soc. Hortic. Sci. 35:3.

22. Matsushima, J. and M. Harada. 1965. Sulfur dioxide gas injury to fruit trees. III. Leaf fall of citrus trees by Bordeaux spray before sulfur dioxide fumigation. J. Japan. Soc. Hortic. Sci. 34:4.

23. Matsushima, J., K. Yonemori, and K. Kwao. 1985. Sensitivity of Mandarin orange to ozone as related to stomatal function indicated by transpiration rate, change in stem diameter, and leaf temperature. J. Am. Soc. Hortic. Sci. 110:106-108.

24. Olszyk, D. M., H. Cabrera, and C. R. Thompson. 1988. California statewide assessment of the effects of ozone on crop productivity. J. Air Pollut. Control Assoc. 38:928-931.

25. Pylypec, B. and R. E. Redmann. 1984. Acid-buffering capacity of foliage from boreal forest species. Can. J. Bot. 62:2650-2653.

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

27. Saurer, M. R. 1954. Flowering in sweet orange. Aust. J. Agr. Res. 5:649-657.

28. Scholander, P. F., H. T. Hammel, E. D. Bradstreet, and E. A. Hemmingsen. 1965. Sap pressure in vascular plants. Science 148:339-346.

29. Sinclair, W. B. 1984. The Biochemistry and Physiology of the Lemon and Other Citrus Fruits. University of California Press: Oakland.

30. Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics, 1st ed., McGraw-Hill: New York.

31. Syvertsen J. P. and Y. Levy. 1982. Diurnal changes in citrus leaf thickness leaf water potential and leaf to air temperature difference. J. Exp. Bot. 33:783-789.

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

33. Thomas, M. D. 1961. Effects of air pollution on plants. In: Air Pollution, pp. 233-278. World Health Organization Monograph Series, No. 46.

34. Thompson, C. R., G. Kats, and E. Hensel. 1971. Effects of ambient levels of NO_2 on Navel oranges. Environ. Sci. Technol. 5:1017-1019.

35. Thompson, C. R., O. C. Taylor, M. D. Thomas, and J. O. Ivie. 1967. Effects of air pollutants on apparent photosynthesis and water use by citrus trees. Environ. Sci. Technol. 1:644-650.

36. Thompson, C. R. and O. C. Taylor. 1969. Effects of air pollutants on growth, leaf drop, fruit drop, and yield of citrus trees. Environ. Sci. Technol. 3:934-940.

37. Tingey, D. T. and G. E. Taylor, Jr. 1982. Variation in plant response to ozone: A conceptual model of physiological events. In: Effects of Gaseous Air Pollution in Agriculture and Horticulture, pp. 113-138. Unsworth, M. H. and D. P. Ormrod, eds., Butterworth Scientific: London.

38. West, P. W. and G. C. Gaeke. 1956. Fixation of sulfur dioxide as sulfito-mercurate III and subsequent colorimetric determination. Analyt. Chem. 28:1816-1823.

APPENDIX A

Average Pollutant Concentrations for 1984-1988

Table A-1. Average O_3 Concentrations for Valencia Orange Treatments, April-October

Year	O_3 Concentration (ppm)			
	Filtered	Half-Ambient	Ambient	Outside
	12-Hour (0800-2000 PST) Average			
1984[a]	0.013	0.036	0.070	0.077
1985	0.008	0.033	0.065	0.071
1986	0.010	0.040	0.071	0.077
1987	0.008	0.032	0.066	0.071
1988[b]	0.016	0.047	0.073	0.078
	24-Hour Average			
1984[a]	0.008	0.020	0.040	0.043
1985	0.005	0.019	0.037	0.040
1986	0.006	0.023	0.041	0.044
1987	0.005	0.018	0.038	0.041
1988[b]	0.012	0.029	0.044	0.047
	Hours x pphm > 10 pphm Dose			
1984[a]	2	89	1049	1407
1985	0	70	1215	1762
1986	0	81	1360	1879
1987	9	35	1235	1749
1988[b]	0	84	727	983

[a]June-October 1984.
[b]April-July 1988.

Table A-2. Average O_3 Concentrations for Valencia Orange Treatments, Year Long

Year	O_3 Concentration (ppm)			
	Filtered	Half-Ambient	Ambient	Outside
		12-Hour (0800-2000 PST) Average		
1984[a]	0.010	0.027	0.054	0.058
1985	0.006	0.026	0.049	0.054
1986	0.008	0.029	0.051	0.055
1987	0.007	0.024	0.047	0.051
1988[b]	0.013	0.034	0.055	0.058
		24-Hour Average		
1984[a]	0.006	0.016	0.031	0.033
1985	0.004	0.015	0.028	0.031
1986	0.006	0.017	0.030	0.032
1987	0.005	0.014	0.028	0.030
1988[b]	0.010	0.022	0.034	0.035
		Hours x pphm > 10 pphm Dose		
1984[a]	4	89	1053	1413
1985	0	75	1272	1450
1986	0	82	1396	1935
1987	9	35	1235	1749
1988[b]	0	84	734	997

[a]June-December 1984.
[b]January-July 1988.

Table A-3. Average SO_2 and O_3 Concentrations for the SO_2 Treatment of the Valencia Orange Study

Year	SO_2 (ppm)		O_3 (ppm)	
	April-October	Annual	April-October	Annual
	12-Hour (0800-2000 PST) Average			
1984	0.081[a]	0.082[b]	0.015[a]	0.012[b]
1985	0.082	0.078	0.009	0.008
1986	0.089	0.086	0.012	0.010
1987	0.090	0.073[c]	0.011	0.010[c]
	24-Hour Average			
1984	0.090[a]	0.086[b]	0.010[a]	0.008
1985	0.082	0.073	0.006	0.006
1986	0.084	0.074	0.009	0.007
1987	0.084	0.068[c]	0.007	0.007[c]

[a]June-October 1984.
[b]June-December 1984.
[c]January-December 1987 (even though treatment ended in October).

Table A-4. Average SO_2 in Filtered Air Treatment

Year	April-October	Annual
	12-Hour (0800-2000 PST) Average	
1984	0.002[a]	0.002[b]
1985	0[c]	0
1986	0	0.001
1987	0	0[c]
	24-Hour Average	
1984	0.002[a]	0.002[b]
1985	0	0
1986	0	0.001
1987	0	0

[a]June-October 1984.
[b]June-December 1984.
[c]An "0" indicates below the level of detection.
[d]January-December 1987 (even though treatment ended in October).

APPENDIX B

Yield, Quality, and Growth Data for 1986, 1987, 1988

Table B-1. Treatment Means for Fruit Yield and Quality in 1986[a]

Parameter	SO_2	Filtered	Half-Ambient	Ambient	Outside
Weight (kg)	24.3 ± 9.9	31.4 ± 9.4	28.1 ± 8.3	20.7 ± 7.5	1.0 ± 2.3
Number (±)	140 ± 79	139 ± 72	134 ± 78	97 ± 70	6 ± 13
Weight/Fruit	167 ± 33	193 ± 10	195 ± 30	166 ± 20	175 ± 1
Acidity (%)	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.1
Sugar (%)	11.3 ± 0.9	11.3 ± 0.6	11.4 ± 0.7	11.0 ± 0.6	10.6 ± 1.6
Color (3-13)	11 ± 0.3	11 ± 0.3	11 ± 0.3	10 ± 2	11 ± 1
Height (mm)	73 ± 4	76 ± 3	77 ± 4	79 ± 4	71 ± 3
Width (mm)	69 ± 4	72 ± 3	73 ± 5	74 ± 4	69 ± 7
Rind (mm thick)	4 ± 1	5 ± 1	5 ± 1	6 ± 1	5 ± 1
Circumf. (mm)	222 ± 12	232 ± 9	234 ± 13	239 ± 13	232 ± 4

[a]Values are means ± standard deviation for five (ambient), six (half-ambient, filtered, SO_2), or seven (outside) trees for weight and number and three (outside) trees for other parameters. For other parameters, values are means ± standard deviation for 20-60 observations, 10 from each of the trees, except for three to six observations for acidity and sugar based on the pooled juice from the 10 fruit per tree.

Table B-2. Treatment Means for Fruit Yield and Quality in 1987[a]

Parameter	SO_2	Filtered	Half-Ambient	Ambient	Outside
Weight (kg)	42.9 ± 21.4	66.3 ± 18.3	59.6 ± 17.3	48.3 ± 9.0	34.8 ± 18.5
Number (±)	229 ± 150	376 ± 72	279 ± 81	271 ± 40	246 ± 113
Wt./Fruit (g)	220 ± 64	179 ± 29	215 ± 16	179 ± 32	141 ± 30
Acidity (%)	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.2	1.4 ± 0.1	1.8 ± 0.2
Sugar (%)	12.3 ± 1.2	13.0 ± 1.1	12.3 ± 0.8	12.2 ± 0.7	13.3 ± 0.7
Color (3-13)	11 ± 1	12 ± 0	12 ± 0	12 ± 0	11 ± 1
Height (mm)	78 ± 11	72 ± 7	78 ± 3	70 ± 3	65 ± 4
Width (mm)	72 ± 8	67 ± 6	72 ± 4	65 ± 4	62 ± 3
Rind (mm thick)	5 ± 1	5 ± 1	5 ± 1	4 ± 1	4 ± 1
Circumf. (mm)	236 ± 31	219 ± 20	236 ± 10	212 ± 11	201 ± 12

[a]Values are means ± standard deviation for seven trees. For other parameters, values are means ± standard deviation for 210 observations, 30 from each of seven trees, except for seven observations for acidity and sugar based on the pooled juice from 30 fruit.

Table B-3. Treatment Means for Fruit Yield and Quality in 1988[a]

Parameter	Filtered	Half-Ambient	Ambient	Outside
Weight (kg)	46.6 ± 22.8	49.0 ± 17.6	47.1 ± 15.3	22.9 ± 20.2
Number (±)[b]	187 ± 91	213 ± 82	206 ± 62	112 ± 101
Wt./Fruit (g)	256 ± 32	236 ± 19	228 ± 19	215 ± 26
Acidity (%)	0.7 ± 0.04	0.8 ± 0.1	0.8 ± 0.04	0.9 ± 0.1
Sugar (%)	10.9 ± 0.5	10.9 ± 0.8	11.0 ± 0.06	10.8 ± 0.6
Color (3-13)	11 ± 2	10 ± 2	11 ± 2	11 ± 2
Height (mm)	82 ± 9	82 ± 9	80 ± 17	76 ± 80
Width (mm)	76 ± 6	74 ± 7	73 ± 6	72 ± 6
Rind (mm thick)	5 ± 2	6 ± 2	5 ± 1	5 ± 1
Circumf. (mm)	248 ± 23	246 ± 24	240 ± 21	233 ± 22

[a]Values are means ± standard deviation for seven trees. For other parameters, values are means ± standard deviation for 210 observations, 30 from each of seven trees, except for seven observations for acidity and sugar based on the pooled juice from 30 fruit.
[b]There was an average of 214 ± 180 fruit per tree for SO_2 trees in November 1987.

Table B-4. Treatment Means for Canopy Size Measured in December 1984[a]

Parameter	SO_2	Filtered	Half-Ambient	Ambient	Outside
Trunk circumf. (mm)	161 ± 9	166 ± 14	169 ± 14	165 ± 6	157 ± 20
Tree Height (m)	1.92 ± 0.11	1.91 ± 0.10	1.87 ± 0.13	1.90 ± 0.81	1.81 ± 0.14
E-W diam. (m)	1.69 ± 0.20	1.74 ± 0.16	1.68 ± 0.23	1.79 ± 0.17	1.45 ± 0.25
N-S diam. (m)	1.77 ± 0.18	1.77 ± 0.15	1.74 ± 0.29	1.87 ± 0.18	1.37 ± 0.17
Trunk Height (m)	0.44 ± 0.06	0.47 ± 0.06	0.43 ± 0.28	0.47 ± 0.05	0.41 ± 0.07
Crown Height (m)	1.49 ± 0.12	1.44 ± 0.15	1.44 ± 0.11	1.43 ± 0.80	1.40 ± 0.13
Volume (m^3)	2.32 ± 0.55	2.34 ± 0.55	2.26 ± 0.71	2.53 ± 0.45	1.47 ± 0.56

[a]Values are means ± standard deviation for seven observations.

Table B-5. Treatment Means for Canopy Size Measured in May 1986[a]

Parameter	SO_2	Filtered	Half-Ambient	Ambient	Outside
Trunk circumf. (mm)	234 ± 16	236 ± 19	239 ± 19	234 ± 11	227 ± 28
Tree Height (m)	2.66 ± 0.22	2.57 ± 0.24	2.53 ± 0.28	2.64 ± 0.38	2.26 ± 0.25
E-W diam. (m)	2.20 ± 0.18	2.27 ± 0.25	2.26 ± 0.13	2.18 ± 0.15	1.85 ± 0.32
N-S diam. (m)	2.18 ± 0.18	2.24 ± 0.17	2.24 ± 0.13	2.29 ± 0.08	1.87 ± 0.13
Trunk Height (m)	0.36 ± 0.02	0.41 ± 0.03	0.40 ± 0.02	0.39 ± 0.09	0.37 ± 0.05
Crown Height (m)	2.28 ± 0.24	2.16 ± 0.22	2.13 ± 0.26	2.25 ± 0.32	1.89 ± 0.25
Volume (m^3)	5.68 ± 1.24	5.71 ± 1.21	5.59 ± 0.88	5.81 ± 0.89	3.46 ± 1.07

[a]Values are means ± standard deviation for seven observations.

Table B-6. Treatment Means for Canopy Size Measured in March 1988[a]

Parameter	Filtered	Half-Ambient	Ambient	Outside
Trunk circumf. (%)	309 ± 24	313 ± 26	301 ± 22	309 ± 35
Height (m)	3.24 ± 0.09	3.21 ± 0.09	3.23 ± 0.07	2.55 ± 0.23
E-W diam. (m)	3.62 ± 0.22	3.55 ± 0.23	3.51 ± 0.24	2.81 ± 0.28
N-S diam. (m)	3.54 ± 0.28	3.60 ± 0.11	3.58 ± 0.11	2.76 ± 0.19
Volume (m^3)	21.43 ± 2.87	20.48 ± 1.93	21.02 ± 1.67	10.31 ± 2.04

[a]Values are means ± standard deviation for seven observations.

APPENDIX C

Results from 1989 Orange Harvest

Fruit were harvested for a fourth time over June 14-28, 1989. This harvests was designed to indicate whether a) yields increased substantially over 1988 which would help substantiate the hypothesis that 1988 was an "off" production year, and b) and oxidant effect on yields was still present despite the fact that the chambers had been turned off in July, 1988. The fruit were harvested, counted, and weighed as described in the Methods section for 1986, 1987, and 1988. Only data for number and weight of fruit on trees was obtained. No fruit quality parameters were measured as we assumed that the lack of ventilation through the chambers after July, 1988, would have a severe effect on fruit quality. The same statistical analysis procedures were used as for previous harvests.

The number and weight of fruit harvested per tree was much higher in 1989 than in any of the preceding years (Table C-1). There were over twice as many fruit in 1989 as in the previous high yield year, 1987; and nearly three times as many fruit as in the previous low yield year of 1988. The fact that yields increased so much in 1989 vs. 1988 for both chamber and outside trees indicated that 1988 was indeed a true "off" bearing year as described earlier in this report.

There was no oxidant effect on either number or weight of fruit harvested from the trees (Table C-1). The number and weight of fruit per tree were nearly the same for the filtered and ambient air treatments and very far from being significantly different according to statistical analysis. This lack of an oxidant effect was somewhat surprising. We had hypothesized that fruit set was the determining factor for oxidant effects on yield. The blowers were still on and the oxidant treatments still in effect during the 1987 growing season which established the tree carbohydrate reserves for flowering and fruit set in early 1988. In addition, the chambers were still on during fruit set and initial drop through mid-July, 1988. Thus, the previous oxidant treatments were hypothesized to affect yield in 1989. The fact that they did not raises the question of whether oxidants do not affect orange tree yields even during some good production years.

However, a likely reason that no oxidant effect was found in 1989 was a possible lack of normal air flow through the tree canopy during at least the last year that the blowers were on. The trees filled the entire volume of most chambers and the chamber effect on growth became substantially greater as time went on. Branches and oranges were pressed against the dome vinyl for most trees by the time the 1989 orange crop was set early in 1988. Therefore, any oxidant effect on yield may have been overshadowed by the chamber effect on tree growth. The chamber effect was not nearly as great on tree growth prior to the 1986 harvests when yields were increasing and an oxidant effect was found. However, this theory that the chamber effect may have overshadowed any oxidant effect could also hold for 1987, the "off" production year. Thus, the lack of an ozone effect in 1988 may have been due to the same factors that resulted in no effect in 1989 and not the presence of an "off" year.

The termination of the exposures in 1989 may have also had an effect on tree yields in 1989. To determine whether an oxidant effect on yields was masked by loss of extra fruit after the chambers were turned off, the total number of larger fruit set on the trees was determined. Total number was obtained by adding all of the fruit dropped from each tree in May, June, and July, 1988; large fruit dropped between August 1988 and May 1989 and picked up in May, 1989; and the fruit harvested from the trees in June 1989. The total number of fruit produced per treatment is shown in Table C-1. There was still no oxidant effect on fruit number based on this analysis. However, the data did show that the number of fruit per outside tree was closer to the number on ambient chamber trees when all fruit produced were taken into consideration. In other words, outside trees had 55% fewer fruit than outside trees based on harvest data, but only 37% fewer fruit based on total fruit produced. This provided further evidence that fruit drop was less for chamber compared to outside trees.

Table C-1. Results from 1989 Harvest of Valencia Orange Trees

Parameter	Treatment			
	Half-Filtered	Ambient	Ambient	Outside[b]
Harvested Fruit on Tree (#)	631 ± 199	583 ± 116	610 ± 89	272 ± 84***
Total Fruit Produced (#)	800 ± 289	687 ± 137	767 ± 169	484 ± 287*
Weight of Fruit on tree (g)	137 ± 30	134 ± 24	130 ± 14	58 ± 17***

[a]Average ± SD for seven trees per treatment.
[b]Significantly more fruit for outside than chamber trees at $p < 0.005$ (***) or $p < 0.05$ (*).

APPENDIX D

Leaf Biochemistry Data for Individual Measurement Dates

Table D-1. Results from Biochemical Analysis of Valencia Orange Leaves-Leaf Chlorophyll and Carotenoid Concentrations (mg g dry weight^{-1}), 1986-1987[a]

Treatment	Month				
	January 1986	October 1986	January 1987	April 1987	July 1987
			Total Chlorophyll		
Outside	1.84 ± 0.30	2.19 ± 0.40	1.98 ± 0.24	2.14 ± 0.42	2.56 ± 0.74
Ambient Chamber	2.60 ± 0.82	2.50 ± 0.62	2.65 ± 0.90	3.06 ± 0.95*	2.99 ± 1.16
Half Ambient Chamber	2.11 ± 1.15	2.07 ± 0.39	2.39 ± 0.43	2.35 ± 0.34	2.96 ± 1.20
Filtered Chamber	2.33 ± 1.33	2.09 ± 0.53	2.42 ± 0.63	2.00 ± 0.62	2.66 ± 0.83
SO_2 Chamber	3.10 ± 0.15	2.30 ± 0.62	2.52 ± 0.63	2.33 ± 0.53	2.61 ± 0.91
			Total Carotenoids		
Outside	0.49 ± 0.09	0.73 ± 0.10	0.45 ± 0.06	0.20 ± 0.07	0.14 ± 0.08
Ambient Chamber	0.58 ± 0.19	0.78 ± 0.16	0.50 ± 0.11	0.19 ± 0.10	0.07 ± 0.04*
Half Ambient Chamber	0.56 ± 0.21	0.68 ± 0.11	0.50 ± 0.08	0.21 ± 0.06	0.10 ± 0.07
Filtered Chamber	0.52 ± 0.25	0.67 ± 0.13	0.51 ± 0.09	0.20 ± 0.07	0.09 ± 0.05
SO_2 Chamber	0.66 ± 0.08	0.73 ± 0.13	0.52 ± 0.18	0.20 ± 0.08	0.09 ± 0.07

[a]For Oct. 1986-July 1987, values are means ± SD three or four observations from seven trees, and four leaves per tree. For January 1986, values are means ± SD for two trees, four observations per tree. The pair of outside and ambient chamber means followed by an * are significantly different at $p<0.05$ according to an unpaired t-test. There also was a significant ozone effect on total chlorophyll for the April sampling at $p<0.05$ according to analysis of variance.

Table D-2. Results from Biochemical Analysis of Valencia Orange Leaves-Chlorophyll a and b (mg g dry weight^{-1}), 1986-1987[a]

Treatment	Month				
	January 1986	October 1986	January 1987	April 1987	July 1987
			Chlorophyll[a]		
Outside	1.44 ± 0.25	1.79 ± 0.35	1.41 ± 0.16	1.19 ± 0.20	1.42 ± 0.40
Ambient Chamber	2.00 ± 0.63	2.05 ± 0.49	1.85 ± 0.62	1.63 ± 0.49*	1.60 ± 0.62
Half Ambient Chamber	1.64 ± 0.92	1.68 ± 0.30	1.69 ± 0.30	1.31 ± 0.14	1.60 ± 0.70
Filtered Chamber	1.79 ± 1.01	1.69 ± 0.43	1.72 ± 0.44	1.11 ± 0.36	1.43 ± 0.45
SO_2 Chamber	2.31 ± 0.12	1.87 ± 0.36	1.76 ± 0.49	1.33 ± 0.39	1.39 ± 0.48
			Chlorophyll[b]		
Outside	0.41 ± 0.06	0.40 ± 0.11	0.56 ± 0.10	0.94 ± 0.27	1.14 ± 0.36
Ambient Chamber	0.60 ± 0.19	0.45 ± 0.14	0.81 ± 0.28*	1.43 ± 0.58**	1.39 ± 0.54
Half Ambient Chamber	0.47 ± 0.23	0.39 ± 0.13	0.70 ± 0.14	1.04 ± 0.28	1.37 ± 0.52
Filtered Chamber	0.54 ± 0.33	0.41 ± 0.12	0.70 ± 0.21	0.90 ± 0.33	1.23 ± 0.39
SO_2 Chamber	0.79 ± 0.09	0.43 ± 0.13	0.75 ± 0.26	1.01 ± 0.24	1.22 ± 0.43

[a]For Oct. 1986-July 1987, values are means ± SD for seven trees, three or four observations per tree. For January 1986, values are means ± SD for two trees, four observations per tree. The pair of outside and ambient chamber means followed by an * are significantly different at $p<0.05$ according to an unpaired t-test. There also was a significant ozone effect on total chlorophyll for the April sampling at $p<0.05$ according to analysis of variance.

Table D-3. Results from Biochemical Analysis of Valencia Orange Leaves - Chlorophyll a/b and Chlorophyll/Carotenoid Ratios

Treatment	Month				
	January[b] 1986	October 1986	January 1987	April[c] 1987	July[c] 1987
			Chlorophyll/Carotenoids		
Outside	0.26 ± 0.02	2.98 ± 0.33	4.47 ± 0.78	14 ± 13	30 ± 38
Ambient Chamber	0.22 ± 0.03	3.18 ± 0.27	5.21 ± 1.33	476 ± 1230	180 ± 658
Half Ambient Chamber	0.29 ± 0.07	3.06 ± 0.41	4.80 ± 0.51	15 ± 17	107 ± 293
Filtered Chamber	0.23 ± 0.03	3.09 ± 0.29	4.70 ± 0.79	103 ± 490	50 ± 65
SO_2 Chamber	0.21 ± 0.03	3.19 ± 0.58	5.03 ± 1.12	87 ± 394	310 ± 1003
			Chlorophyll a/b		
Outside	3.53 ± 0.35	4.79 ± 1.71	2.55 ± 0.24	1.32 ± 0.23	1.26 ± 0.14
Ambient Chamber	3.39 ± 0.32	4.75 ± 0.82	2.33 ± 0.22	1.21 ± 0.27	1.16 ± 0.11
Half Ambient Chamber	3.45 ± 0.52	4.80 ± 1.98	2.45 ± 0.34	1.30 ± 0.22	1.15 ± 0.18
Filtered Chamber	3.45 ± 0.53	4.26 ± 0.68	2.54 ± 0.39	1.29 ± 0.26	1.18 ± 0.20
SO_2 Chamber	2.96 ± 0.34	4.75 ± 0.82	2.18 ± 0.21	1.36 ± 0.44	1.15 ± 0.09

[a]For Oct. 1986-July 1987, values are means ± SD for seven trees, three or four observations, per tree. For January 1986, values are means ± SD for two trees, four observations per tree The pair of outside and ambient chamber means followed by an * are significantly different at $p<0.05$ according to an unpaired t-test. There also was a significant ozone effect on total chlorophyll for the April sampling at $p<0.05$ according to analysis of variance.
[b]For January 1986, chlorophyll/carotenoids was originally calculated as carotenoids/chlorophyll.
[c]Data for chlorophyll/carotenoids questionalbe due to some very low carotenoid values.

Table D-4. Results from Biochemical Analysis of Valencia Orange Leaves-Specific Leaf Area (cm^2 g dry wt^{-1}), 1986-1987[a]

Treatment	Month				
	January 1986	October 1986	January 1987	April 1987	July 1987
Outside	81 ± 10	76 ± 4	72 ± 7	95 ± 28	86 ± 7
Ambient Chamber	91 ± 7	83 ± 4*	82 ± 5**	89 ± 6	97 ± 5**
Half Ambient Chamber	83 ± 32	83 ± 7	81 ± 4	94 ± 8	98 ± 7
Filtered Chamber	89 ± 6	82 ± 6	78 ± 7	98 ± 13	98 ± 8
SO_2 Chamber	92 ± 12	81 ± 4	86 ± 3*	100 ± 5	91 ± 3

[a]Values are means ± SD for seven trees, and one observation per tree. The pair of outside and ambient chamber means followed by an * are significantly different at $p<0.05$ according to analysis of variance.

Table D-5. Results from Biochemical Analysis of Valencia Orange Leaves-Leaf Thiol and Sulfite Concentrations (μmoles per g dry wt^{-1}), 1986-1987[a]

Treatment	Month			
	October 1986	January 1987	April 1987	July 1987
		Thiols		
Filtered Chamber	1.22 ± 0.55	1.22 ± 0.31	1.35 ± 0.56	1.43 ± 0.59
SO_2 Chamber	0.99 ± 0.31	1.25 ± 0.28	1.46 ± 0.49	1.34 ± 0.38
		Sulfite		
Filtered Chamber	1.39 ± 0.46	1.52 ± 0.44	1.95 ± 0.31	1.62 ± 0.68
SO_2 Chamber	1.52 ± 0.45	1.59 ± 0.51	2.03 ± 0.59	1.62 ± 0.57

[a]Values are means ± SD for seven trees, and two or three leaves per tree. No treatment differences are statistically significant at $p<0.05$.

Table D-6. Results from Biochemical Analysis of Valencia Orange Leaves - Leaf pH, Sulfite, and Thiols in January, 1986[a]

Treatment	Leaf pH	Sulfite (nmoles cm^{-2})	Thiol (μmoles cm^{-2})
Outside	5.87 ± 0.03	60.7 ± 10.8	8.55 ± 2.64
Ambient Chamber	5.98 ± 0.06***	54.2 ± 6.34	9.76 ± 3.76
Half Ambient Chamber	6.00 ± 0.04	54.6 ± 9.46	9.67 ± 3.87
Filtered Chamber	5.99 ± 0.02	56.7 ± 6.01	9.39 ± 2.93
SO_2 Chamber	5.96 ± 0.06	45.8 ± 16.4	10.60 ± 3.99

[a]Values are means ± SD for six trees for leaf pH, seven for sulfite and thiols, one measurement per tree. The pair of outside and ambient chamber means follwed by *** are significantly different at $p < 0.005$ according to analysis of variance.

APPENDIX E

Results from the Pilot Leaf and Fruit Tagging Study in 1988

New leaves and small fruit were tagged in the late winter and spring of 1988 in order to determine the effects of ozone and the chamber on leaf and fruit retention over time.

Leaf Retention. Approximately 50 young leaves per tree were tagged on March 9-10, 1988. The leaves were from the first flush of new growth which occurred in late February 1988. White twist ties were used for tags. The leaves were tagged at a height of approximately 1 to 2 m around the perimeter of the entire tree. Flowering shoots were used wherever possible, with tags put around leaves near terminal flowers. At the time of tagging, the leaves on outside trees were smaller than leaves on chamber trees, likely due to a two- to three-week delay in leafing out for outside vs. chamber trees. Leaf retention (or conversely, drop) was determined by counting both the tags remaining on the tree and tags that had fallen to the ground approximately monthly from March through August as shown in Table E-1. Leaf retention was calculated as a percentage of the leaves tagged.

The outside trees began to drop new leaves in March, as indicated by casual observations soon after tagging. Therefore, a collection and count of all dropped tags was made on March 26, 1988. It was obvious that only outside trees were losing tags this time, with an average of approximately 10% of the tags missing per tree. The loss of tags was definitely due to loss of leaves, as none of the tag loops were open that had been tied around the petioles of the leaves. In addition, one tag actually was found still on the petiole with the leaf blade missing. This pattern of loss of new leaves from outside accelerated until there was only a small percentage of the original leaves remaining on the outside trees (Table E-1). The chamber trees lost very few new leaves during the entire tagging period as shown by the lack of tags found on the ground. The count of tagged leaves on the trees decreased over time, but probably due to a decreased ability to find the tags which were obscured by later leaf flushes. Casual observations indicated that the leaf loss seemed to be more prevalent on the northeast sides of the outside trees.

The loss of young leaves for outside, but not for chamber trees, was likely related to occasional high gusts of wind outside but not inside the chambers.

Table E-1. Orange Tree Leaf Retention with Ambient Ozone in 1988 Based on Tagging on March 9-10, 1988[a]

Collection Date	Treatment			
	Filtered	Half-Ambient	Ambient	Outside[b]
	Percentages			
4/7/88	90 ± 9	92 ± 6	91 ± 5	54 ± 10
5/9/88	87 ± 9	83 ± 5	84 ± 5	41 ± 6
5/20/88	78 ± 6	76 ± 8	82 ± 8	44 ± 9
6/8/88	72 ± 12	75 ± 11	70 ± 12	38 ± 7
7/8/88	60 ± 18	65 ± 13	56 ± 9	24 ± 5
8/8/88	58 ± 20	60 ± 10	54 ± 12	26 ± 6

[a]Average ± SD for seven trees per treatment with 48-50 leaves tagged per tree.
[b]Significantly greater retention for ambient than outside trees for each date at $p < 0.05$.

The greater winds outside may have blown already abscissed leaves off the trees more readily outside than in chambers. However, the persistence of leaf loss over several months also suggested that the leaves on outside trees were inherently more susceptible to leaf abscission than leaves on chamber trees.

Ambient O_3 had no effect on the leaf retention as there were no differences between the filtered, half-ambient, and ambient chamber treatments on any measuring date (Table E-1). This lack of O_3 effect on leaf loss reinforced the lack of ozone effect also observed with the monthly total leaf weight data described in Section III.C.2 of this report.

Fruit Drop. Approximately 50 small fruit per tree were tagged on April 7, 1988, following fruit set. The size of the fruit ranged from pea to marble size. Fruit were generally larger on the chamber trees than outside trees, as their development was more advanced due to earlier flowering for the chamber than outside trees. White twist ties which were spray painted red were used for tags. The fruit were tagged at a height

of approximately 1 to 2 m around the perimeter of the entire tree. Wherever possible, the terminal fruit adjacent to a leaf tag was used. Fruit retention was determined from May through August as shown in Table E-2. Fruit drop was calculated on a percentage basis (i.e., [(Fruit tagged - fruit with tags)/fruit tagged] x 100), to correct for the fact that some trees only received 47 to 49 tags.

The first fruit count on May 9, 1988 indicated that more fruit were lost for the ambient than filtered chamber trees (Table E-2). This resulted in approximately 44% of the fruit remaining on the filtered trees vs. 33% remaining on the ambient trees. Approximately 39% of the fruit remained on the half-ambient trees. This 33% higher number of fruit was approximately the same as the 34% higher yield (by weight) for filtered vs. ambient trees in 1987. The outside trees still had 29% of their fruit remaining, which was similar to the amount for ambient chamber trees.

However, by the second count on May 20, 1988, enough extra fruit had dropped from filtered chamber trees so that the number of fruit remaining was the same for the filtered and ambient chambers (Table E-2). This lack of difference in fruit loss due to O_3 persisted through the rest of the counts. Thus, while the fruit tag data at first provided a possible mechanism for yield losses due to O_3, the effect was not verified with further counts. Tagging of only 50 fruit per tree was likely not sufficient to detect the real effect of O_3 on fruit retention over time.

On May 20, 1988 and succeeding dates, there were fewer fruit retained (i.e., fewer tags remaining) for the outside vs. ambient trees (Table E-2). This may have been due to metabolic changes in the trees which resulted in decreased fruit abscission for chamber vs. outside trees, or the physical enhancement of fruit loss due to higher wind gusts outside than inside chambers. However, a key reason for the apparently decreased fruit retention for the outside trees may have been the earlier stage of development for the outside compared to chamber tree fruit at the time of tagging. It is well known that most of the young fruit drop from orange trees soon after set. Thus, there were more fruit at a susceptible stage for abscission on outside than on chamber trees. In the chambers the fruit that could be set by the trees likely had already been determined. Thus, future fruit tagging studies would have to consider the physiological age of the fruit, which would result in tagging of chamber fruit on an earlier date than tagging of outside fruit.

Table E-2. Orange Tree Fruit Retention with Ambient Ozone in 1988 Based on Tagging on March 9-10, 1988[a]

Collection	Treatment			
	Filtered	Half-Ambient	Ambient	Outside
	Percentages			
5/9/88	44 ± 7	39 ± 10	33 ± 7[b]	29 ± 12
5/20/88	26 ± 7	25 ± 7	25 ± 4	11 ± 4[c]
6/8/88	20 ± 8	17 ± 6	17 ± 7	4 ± 2[c]
7/8/88	12 ± 3	13 ± 4	14 ± 4	3 ± 3[c]
8/8/88	11 ± 4	12 ± 3	12 ± 3	3 ± 2[c]

[a]Average ±SD for seven trees per treatment, with 48-50 fruit tagged per tree.
[b]Significant linear ozone effect at $p < 0.05$.
[c]Significantly greater retention for ambient than outside trees at $p < 0.005$.